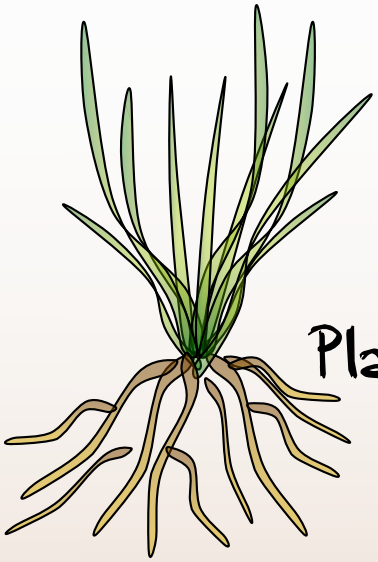
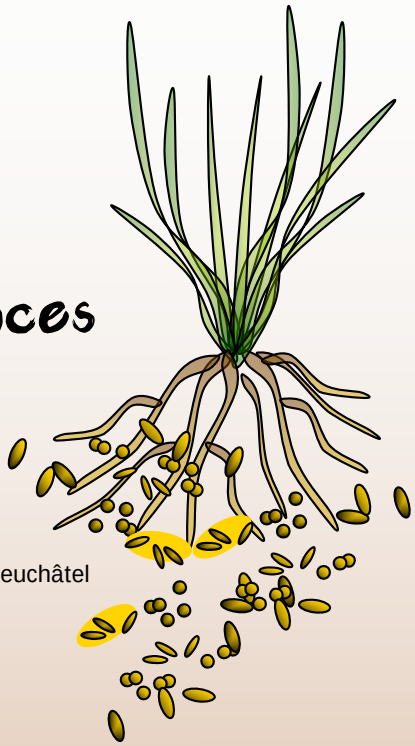
UNIVERSITE DE NEUCHATEL (SUISSE)
FACULTE DES SCIENCES
INSTITUT DE BIOLOGIE
DEPARTEMENT MICROBIOLOGIE

Rhizosphere Bacterial Communities Associated with *Lolium perenne* :



Structuration and Plant-mediated Influences



Thèse présentée à la Faculté des Sciences de l'Université de Neuchâtel
pour l'obtention du grade de Docteur ès Sciences

par

Maryline Jossi

Membres du jury :

Michel Aragno	Professeur (Directeur de thèse) Laboratoire de Microbiologie, Université de Neuchâtel (CH)
Elena Havlicek	Collaboratrice Scientifique, Chargée d'enseignement Laboratoire Sol & Végétation, Université de Neuchâtel (CH)
Mauro Tonolla	Collaborateur Scientifique, Chargé d'enseignement Instituto Cantonale di Microbiologia, Bellinzona (CH), Université de Genève (CH)
Alain Brauman	Directeur de recherches IRD, UR 179 SeqBio, Montpellier (FR)
Claude Plassard	Chargée de recherches INRA, UMR Agro Rhizosphère et Symbiose, Montpellier (FR)

- 2008 -

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IMPRIMATUR POUR LA THESE

Rhizosphere bacterial communities associated with Lolium perenne: structuration and plant-mediated influences

Maryline JOSSI

UNIVERSITE DE NEUCHATEL

FACULTE DES SCIENCES

La Faculté des sciences de l'Université de Neuchâtel,
sur le rapport des membres du jury

Mme E. Havlicek,
MM. M. Aragno (directeur de thèse), C. Plassard (INRA Montpellier F),
A. Braumann (IRD Montpellier F) et M. Tonella (Genève)

autorise l'impression de la présente thèse.

Neuchâtel, le 20 mai 2008

Le doyen :
F. Kessler

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Rue Emile-Argand 11 - CP 158
CH-2009 Neuchâtel
felix Kessler

REMERCIEMENTS

Ce travail de thèse a été réalisé au Laboratoire de Microbiologie de l'Université de Neuchâtel (LAMUN) sous la direction scientifique du Prof. Michel Aragno, que je tiens à remercier tout particulièrement pour m'avoir ouvert les portes de l'univers fascinant des microorganismes, et pour la confiance et l'intérêt qu'il m'a accordés.
Un grand merci également à Sonia Tarnawski qui a contribué de façon importante à l'encadrement de ce travail, et qui a été bien plus qu'une encadrante, une amie, un univers..
Merci à Ludovic Roussel-Delif avec qui j'ai partagé les aléas de la science.. Et qui, tout en réalisant son propre travail de thèse a trouvé le temps de suivre celui-ci avec beaucoup d'attention.
Merci à Vanessa Di Marzo (alias « Poussin ») et Nicole Jeannerét pour leur soutien moral, technique, leur disponibilité et leur dynamisme.
Merci également à Claire Le Bayon, Laure Weisskopf et Nadia Khammar pour leurs conseils avisés et leur amitié.

Merci à tous les membres (actifs et passifs) des laboratoires de Microbiologie, Sol et végétation de l'Université de Neuchâtel, pour la bonne ambiance qu'ils ont su créer autour d'eux. Merci à toutes les personnes qui ont collaboré de près ou de loin à ce projet: Nathalie Fromin et Jérôme Hamelin, prédécesseurs, piliers du groupe FACE du LAMUN, qui ont activement participé à la mise en place de ce travail de recherche. Jaqueline Moret, Florian Kohler et François Gillet pour leur expertise dans le domaine des analyses statistiques et leur compréhension de la « bio-logique ». Armelle Vallat pour son aide concernant les analyses HPLC. Yves Dessaux pour m'avoir fourni les souches de référence pour l'étude de la production d'homosérine lactones, et Michael Kertesz pour sa collaboration dans l'étude des producteurs de sulfatases.

Je remercie le Fond National de la Recherche Scientifique (FNRS, Grant number 3100-055899.98 and 31-68208.02) et le projet COST 631 pour leur soutien financier, ainsi que le National Centre of Competence in Research (NCCR) « Plant survival », et son école doctorale, pour leur soutien financier et pour la qualité de leurs cours de perfectionnement.
J'adresse également mes remerciements aux organisateurs le l'Université d'été MICROTROP, pour m'avoir donné l'opportunité de participer à cet événement particulièrement enrichissant tant du point de vue humain que scientifique.
Ma gratitude va bien sûr à Claude Plassard, Elena Havlicek, Alain Brauman et Mauro Tonolla pour avoir accepté le rôle de rapporteur et de membre du jury.

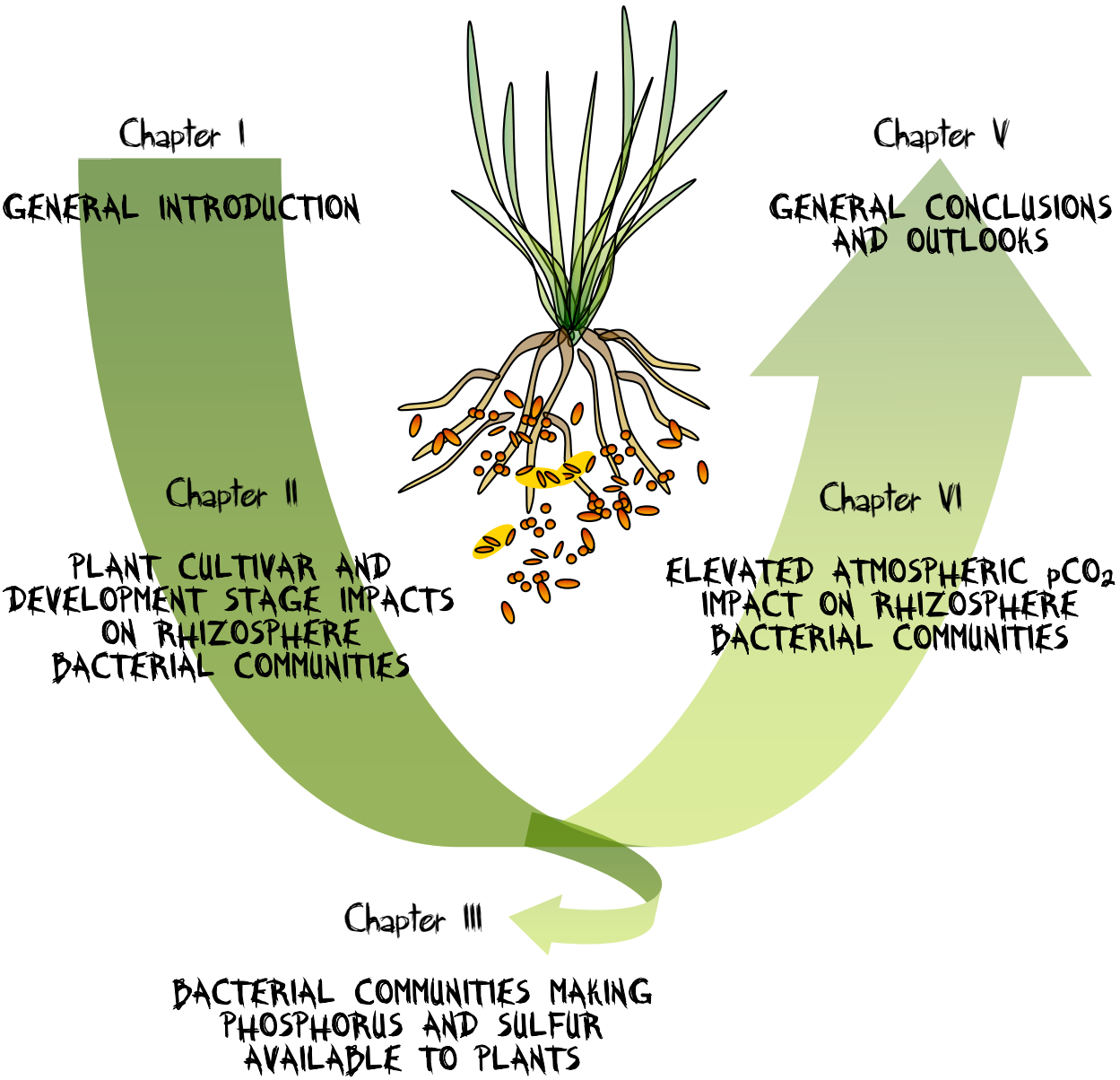
Merci enfin aux trois Jossi 's pour leur amour, et leur précieux appui en toutes circonstances et à tous les niveaux.

Last.. but not least.. Merci à Cyril, l'Homme, pour son énergie, sa sagesse et son amour..

On résout les problèmes qu'on se pose et non les problèmes qui se posent.
[Henri Poincaré]

RHIZOSPHERE BACTERIAL COMMUNITIES ASSOCIATED WITH *LOLIUM PERENNE* : STRUCTURATION AND PLANT-MEDIATED INFLUENCES

RHIZOSPHERE BACTERIAL COMMUNITIES ASSOCIATED WITH *LOLIUM PERENNE* : STRUCTURATION AND PLANT-MEDIATED INFLUENCES



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MOTS CLES

- Rhizosphère, Rhizobactéries
- Sol
- Exsudats racinaires, acides organiques, composés phénoliques
- *Lolium perenne*, *Molinia coerulea*, Graminées pérennes
- Communautés bactériennes, 16S rDNA, 16S rRNA
- Fonctions bactériennes, Protéases, Exopolysaccharides, Réduction du nitrate, Dénitrification, Sidérophores, Phytases, Solubilisation du Ca-phosphate, Arylsulfatases, N-acyl-homosérines lactones, auxine, Acide cyanhydrique
- Actinobacteria

KEYWORDS

- Rhizosphere, Rhizobactria
- Soil
- Root exudates, organic acids, phenolic compounds
- *Lolium perenne*, *Molinia coerulea*, Perennial grasses
- Bacterial communities, 16S rDNA, 16S rRNA
- Bacterial functions, Proteases, Exopolysaccharides, Nitrate reduction, Denitrification, Siderophores, Phytases, Ca-phosphate solubilisation, Arylsulfatases, N-acyl-homoserines lactones, Auxin, Cyanhydric acid
- Actinobacteria

RESUME

Du fait de leur présence, leur activité et leur physiologie, les racines des plantes influencent physiquement, chimiquement et biologiquement leur sol environnant. Certains microorganismes du sol vont être favorisés, ou au contraire inhibés par les racines. La rhizosphère, à la jonction entre le sol et la plante, favorise de plus une activité intense en raison de la stimulation de la microflore par la rhizodéposition et de la variété des micro-habitats.

Un lien étroit existe entre les microorganismes du sol et la plante, en particulier lorsque l'on considère les plantes pérennes qui, se maintenant d'année en année au même endroit, présentent des communautés particulièrement adaptées à leur environnement. Parmi ces plantes, la graminée nitrophile *Lolium perenne* a été choisie dans cette étude comme plante modèle pour son abondance et son importance dans le domaine agricole en tant que plante fourragère.

Les activités des microorganismes sont extrêmement diversifiées et ont un impact important au niveau de la fertilité des sols (ex : cycles des éléments nutritifs, formation et décomposition de la matière organique) et de la santé des plantes. Ces organismes constituent de ce fait le principal champ d'investigation pour le développement de bio-fertilisants et de bio-pesticides. L'impact des microorganismes et en particulier des bactéries, sur la croissance et la résistance de la plante est couramment reconnu. Les connaissances concernant l'importance des différents groupes fonctionnels bactériens de la rhizosphère, leur écologie et leur structuration, doivent cependant être enrichies afin d'améliorer la compréhension des interactions entre plantes et microorganismes dans la rhizosphère, et d'augmenter le potentiel des bio-fertilisants.

Les objectifs principaux des expériences menées dans cette étude consistent à élargir les connaissances du fonctionnement de la rhizosphère, et de la structuration des communautés bactériennes. Les communautés bactériennes totales, actives et cultivables de la rhizosphère sont caractérisées en relation avec différentes perturbations (variations au niveau du génotype de la plante, modifications dues au développement de la plante, changements liés au climat global). Ceci afin de cibler les modifications spécifiques aux différentes conditions et d'identifier les populations susceptibles de tenir une place importante dans le fonctionnement de la rhizosphère et la promotion de la croissance des plantes. Toutes les approches de communautés sont réalisées dans le même sol agricole afin de conserver la contribution du sol dans le fonctionnement rhizosphérique.

Les approches génotypiques des communautés bactériennes totales et actives, au champ et en serre, montrent que les influences liées à la racine affectent peu la diversité globale, et que les communautés métaboliquement actives se révèlent être plus sensibles aux perturbations liées à la plante que les communautés totales. Ce qui indique que l'influence des racines se manifeste par la prolifération, ou au contraire la mise en dormance, de populations spécifiques au sein du réservoir bactérien représenté par les communautés du sol.

L'établissement, *in* vitro, du profil fonctionnel des populations bactériennes associées à différents cultivars (diploïdes: Cavia, Lipresso ; tétraploïdes: Anaconda, Bastion) et stades de développement de *L. perenne*, permet de mettre en évidence les fonctions bactériennes dont la fréquence est affectée par ces deux facteurs liés à la plante. Le cultivar Anaconda semble héberger des communautés particulièrement spécifiques. De plus, quel que soit le cultivar, le passage de la floraison de la plante semble être un stade critique, à partir duquel l'influence de la plante s'estompe et les caractéristiques des communautés rhizosphériques tendent à rejoindre celles du sol nu. Cette approche fonctionnelle est également employée pour comparer, dans l'environnement racinaire et dans le sol nu, les fréquences de certaines capacités bactériennes connues comme étant impliquées dans les interactions entre plantes et bactéries, ainsi que pour mettre en évidence les corrélations entre ces différentes capacités.
La caractérisation du sol rhizosphérique des différents cultivars, ainsi que l'analyse de leurs exudats racinaires (acides organiques, composés phénoliques), mettent en évidence des différences cohérentes avec les celles observées au niveau du profil fonctionnel de leurs communautés bactériennes rhizosphériques.

Une approche génotypique (au champ) des communautés bactériennes associées à *L. perenne*, effectuée précédemment au LAMUN, a révélé l'importance du groupe des *Pseudomonas*. Dans l'étude présentée ici, l'approche génotypique (au champ) ainsi que l'approche fonctionnelle (*in vitro*) de ces communautés mettent toutes les deux en évidence le groupe des Actinobacteria. Connu pour être particulièrement résistant aux perturbations et adapté au statut nutritionnel limité du sol. Ce groupe s'avère tenir une place importante au sein des populations actives de la rhizosphère. Il est également le principal groupe de minéralisateurs potentiel de phytate dans des conditions limitantes en P inorganique et en présence de C soluble; deux conditions fréquemment rencontrées dans la rhizosphère. Les capacités des Actinobacteria semblent être essentielles pour le maintien à long terme des environnements changeants et devraient être étudiés dans la rhizosphère avec beaucoup plus d'attention.

SUMMARY

Due to their presence, activity and physiology, plant roots influence physically, chemically and biologically their surrounding soil. Some microorganisms will be favoured, or on the contrary inhibited by roots. As the junction between soil and plant, the rhizosphere presents an intense activity because of the stimulation of microflora by rhizodeposition and of the existence of various micro-habitats.

A close link exists between soil microorganisms and plants, in particular when considering perennial plants which, growing at the same place from year to year, present particularly adapted associated microbial communities. Among these plants, the nitrophilic perennial grass *Lolium perenne*, was choosen in this study as model plant because of its abundance, and its agricultural importance as forage plant.

Microorganisms' activities are extremely diversified and strongly implicated in soil fertility (nutrient cycling, organic matter formation and decomposition) and plant health. These organisms are therefore the main investigation field for development of bio-fertilisers and bio-pesticides. The impact of microorganisms, in particular of bacteria, on plant growth and resistance is currently well recognised. Knowledge about the importance of the different functional groups of bacteria in the rhizosphere, their ecology, and their structuration, have nevertheless to be enriched to improve the understanding of plant-bacteria interactions in the rhizosphere, and to increase the potential of biofertilisers.

Throughout the experiments conducted in this work, the main aims are to gain additionnal knowledge about rhizosphere functionning and structuration of bacterial communities. Total, active and culturable rhizosphere bacterial communities are characterised in relation with different perturbations (plant genotype variations, modifications due to plant development, and to global climate changes), in order to target the modifications, which are specific of the different conditions, and to identify bacterial groups likely to take an important place in rhizosphere functionning and plant growth promotion. All the community approaches were conducted in the same agricultural soil in order to conserve the soil contributions in the rhizosphere functionning.

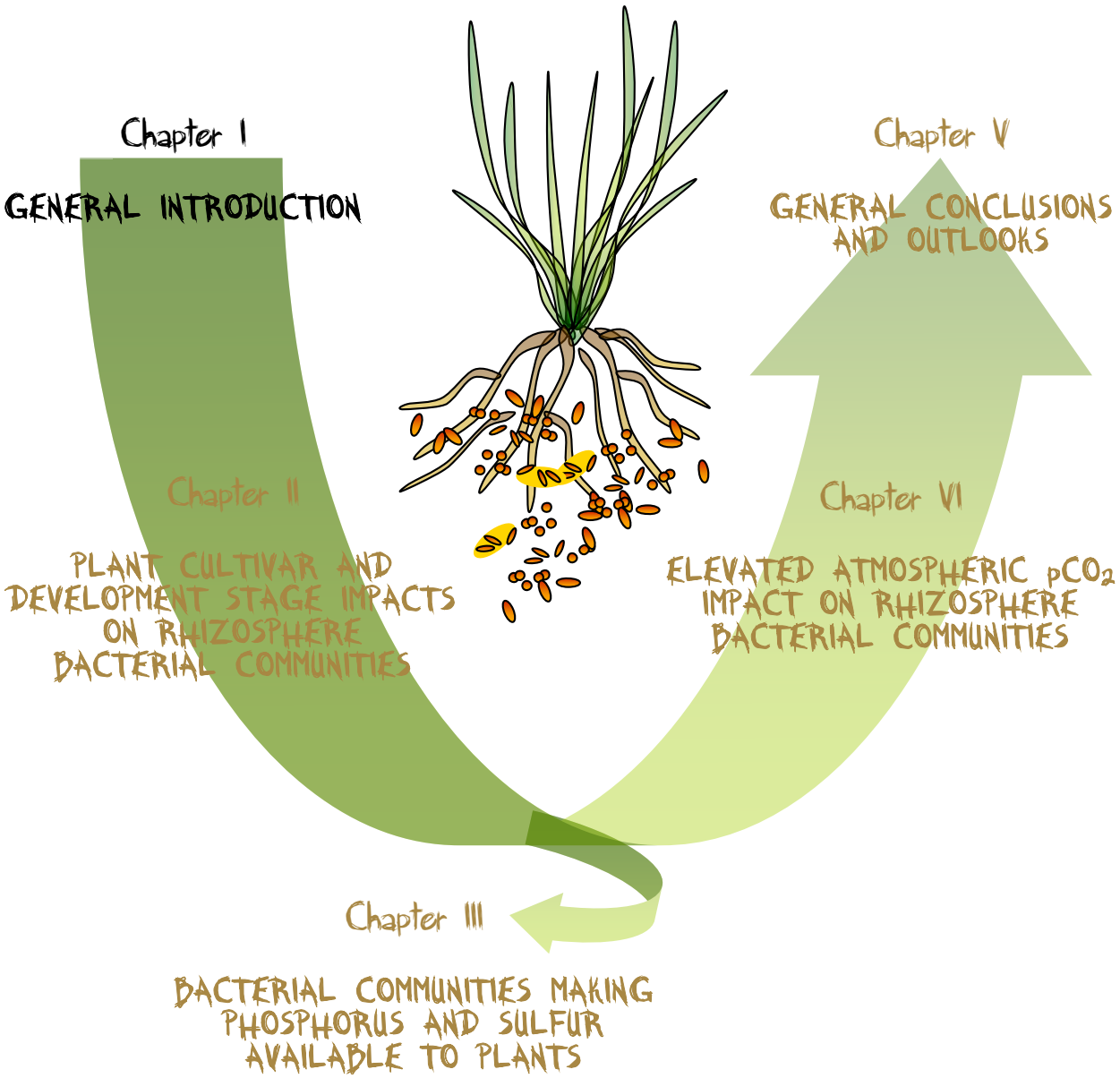
Genomic approaches of total and active bacterial communities, performed in field and in greenhouse conditions in the same agricultural soil, revealed that root-mediated perturbations affect only slightly the global divesity, and that the metabolically active part of the communities was more sensitive to plant-mediated influences than the total communities. Indicating that root influence lead to the proliferation or, on the contrary, to the dormance, of specific populations among the bacterial reservoir represented by soil bacterial communities.

The establishment, in vitro, of the functional profiles of bacterial populations associated with different cultivars (diploïdes: Cavia, Lipresso ; tetraploïdes: Anaconda, Bastion) and development stages of *L. perenne*, allows to highlight the bacterial functions presenting frequencies which are affected by these two plant-related factors. Anaconda cultivar seems to harbour particularly specific bacterial communities. Furthermore, whatever the cultivar, plant flowering appears to be a critical stage beyond which plant influence is attenuated, and characteristics of rhizosphere communities tend to gather with those of bulk soil communities. This functional approach is also used to compare, in the root environment and in the bulk soil, the frequencies of some bacterial abilities known to be implicated in plant-bacteria interactions, and to highlight the existence of correlations between these different abilities.
Furthermore, the characterisation of the rhizosphere soil of the different cultivars, and the analysis of their root exudates (organic acids, phenolics), allows to highlight differences coherent with those observed on the functional profiles of their bacterial communities.

A genomic approach (field conditions) of bacterial communities associated to *L. perenne*, performed in previous experiments, revealed the importance of the *Pseudomonas* group. In the present study, the genomic approach (field conditions), as well as the functional approach (in vitro) of these communities, both highlighted the Actinobacteria group. Known to be particularly resitant to perturbations and to be adapted to the poor nutrient status of the soil, this group take an important place in the key active rhizosphere populations. It is also the main group of potential phytate mineralisers under limiting inorganic P conditions and in presence of soluble C sources; two frequent characteristics of the rhizosphere environment. The abilities of Actinobacteria are thought to be essential for long term maintaning of changing environments and have to be investigated in the rhizosphere whith more attention.

CHAPTER 1 :

GENERAL INTRODUCTION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GENERAL INTRODUCTION
Chapter II
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CHAPTER I : GENERAL INTRODUCTION

Overview

I.1. MICROORGANISMS AND DURABLE AGRICULTURAL DEVELOPMENT

The world population has grown by about four billion since the beginning of the Green Revolution. The intensification of agricultural production, with the excessive use of chemical fertilizers or pesticides, has led to a reduction in soil fertility and to environmental degradation, and affects the natural soil biology (see chap. I.2). In order to avoid the worsening of these problems, and to save the living soil, agricultural production has to be optimised. The technological development of agriculture is important to the future but has to be directed in an ecological way to be durable. New technologies such as selection of high yielding plant varieties (maize, wheat, rice), biological fertilisers and bio-pesticides have to be developped. For these purposes, fundamental knowledges of the rhizosphere (see chap. I.3) have to be improved.
Growing at the same location from year to year, perennial grasses, such as *Lolium perenne* (see chap. I.5) are a suitable model for the investigation of rhizosphere functionning. *L. perenne* present also both advantages to be frequently used for forage and to account for a large part of terrestrial natural ecosystems.

Microorganisms' abilities are extremely diversified and strongly implicated in soil fertility (nutrient cycling, organic matter formation and decomposition, see chap. I.2) and plant health (see chap. I.4). They are therefore the main investigation field for development of bio-fertilisers and bio-pesticides. Among various types of biofertilizers, bacterial inoculant is one of the major tool. It includes rhizobia, nitrogen fixing rhizobacteria, plant growth promoting rhizobacteria, phosphate-solubilizing bacteria, and so on. Most of bacteria included in biofertilizer have close relationship with plant roots (Kostov and Lynch, 1998; Daza *et al.,* 2000; Reban *et al.*, 2002; Abd-Alla and Omar, 2001).
Implication of microorganisms in plant growth and resistance is currently well recognised (Bashan and Holguin, 1998), but knowledges about the ecology of rhizosphere microorganisms and the processes involved in rhizosphere communities selection have to be improved to predict the occurence rate, and the evolution of microbially mediated functions which are of agronomic and environmental importance. Sustainable agricultural development thus depends on the fundamental investigation of the rhizosphere functionning.

This work aims to improve the knowledges about the structuration of rhizosphere bacterial communities and their implication in nutrient availability, and plant growth promotion (for details see chap. I.6 and I.7).

I.2. THE SOIL SYSTEM

I.2.1. SOIL STRUCTURE AND COMPOSITION

Soil is composed of a solid (inorganic and organic matter, living organisms), a liquid (soil solution), and a gazeous (carbon dioxyde, oxygen, nitrogen) phase (Gobat *et al.*, 2004). The soil organic matter is mainly derived from plants, and composed of non or partially degraded litter (glucids, lignins, lipids, nitrogen compounds) and of the humus fraction (humic acids).

The spatial arrangement of the solid particles results in a complex and discontinuous pattern of pore spaces of various size and shapes that are more or less filled with water or air, forming a multitude of different soil micro-habitats for microorganisms (figure I.1, Chenu and Stotzky, 2002). Soil properties, defining environmental conditions of these habitats, such as mineral composition, nutrient availability, pH, redox potential, temperature, water content, and organic inputs depends on the fauna and vegetation, but also on the geographical, the geological, the climatic and the anthropogenic influences (Gobat *et al.*, 2004).

Although many aspects have still to be investigated, the mechanisms of bacterial communities establishment in soils, which are induced by soil structure and properties, have been well studied (Wieland *et al.*, 2001; Marschner *et al.*, 2001; Dalmastri *et al.*, 1999; Latour *et al.*, 1996). The variety of soil microhabitats allows the growth of a high bacterial diversity. For instance, the variety of oxygen concentration and redox potential encountered in soils results in an enhancement of the metabolic diversity of micoorganisms. Aerobic respiration is favoured in presence of oxygen, whereas activities such as nitrate, iron and sulfate reductions take place at lower redox potential conditions, in anaerobic microhabitats (Gobat *et al.*, 2004). The soil argilo-humic complex, a central actor in soil functionning, strongly impacts nutrient availability and is thus determinant for the soil food chain establishment (Gobat *et al.*, 2004).

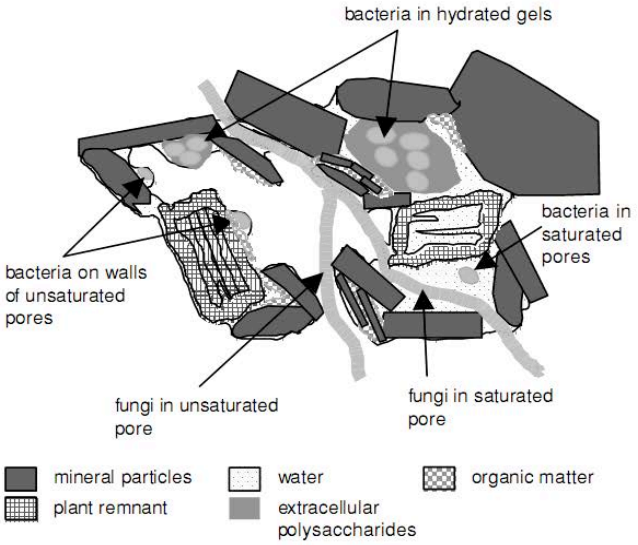


Figure I.1. Soil micro-habitats.

Schematic representation of the various habitats of microorganisms in soils (Chenu and Stotsky, 2002)

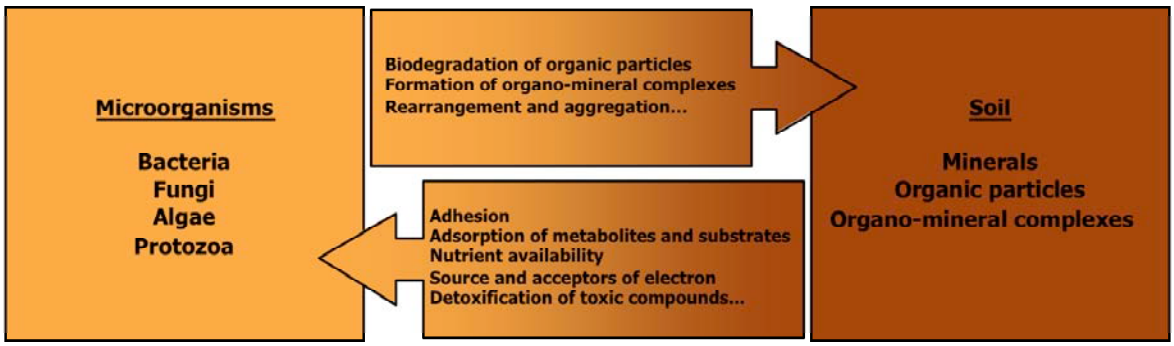
I.2.2. BACTERIA AND THE LIVING SOIL

Soil is an heterogenous system generally poor in nutrients (table I.1) and energy sources (Nannipieri *et al.* 2003, Gobat *et al.*, 2004). The living populations inhabiting soil includes macrofauna (i.e. Earthworms, Mice), mesofauna (i.e. Acari, Collembola and nematoda), microfauna (i.e. protozoa) and microflora (i.e. bacteria, fungi). Plant derived organic input in soil is the main source of organic compounds, providing nutrients sources throught decomposition and mineralisation by soil microorganisms. However, nutrient availability (fertility of the soil) is also closely linked to soil properties. Nutrients are therefore at the junction of soil, plants and microorganisms interactions (figure I.2, figure I.3).

Table I.1. Element concentrations in soils and plants (Gobat *et al.*, 2004).

	Concentration ($^0/_{00}$ of dried matter)			Concentration (ppm of dried matter)	
Element	Soils	Plant	Element	Soils	Plant
N	0.3-3	5-50	Fe	40 000	50-1000
P	0.1-1	1-5	Mn	200-4000	20-200
S	0.1-1	0.5-5	Cu	5-100	2-200
K	2-30	5-50	Zn	10-300	10-100
Ca	2-15	0.5-50	Mo	0.5-5	0.2-10
Mg	1-10	1-10	B	5-100	2-100
			Al	50-200	2-3

Figure I.2. Simplified representation of the interactions between microorganisms (orange) and soil (brown).



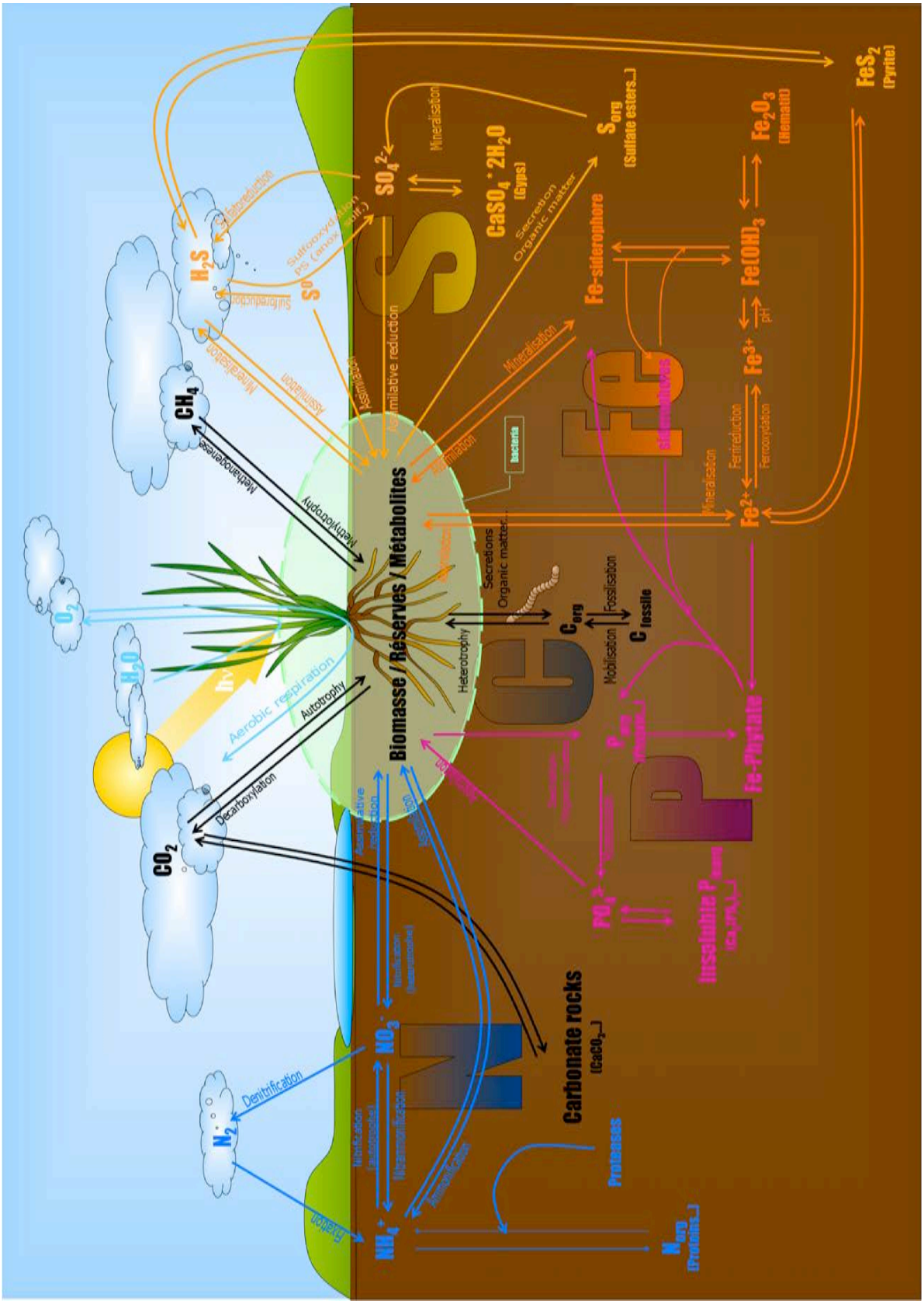
Considering that 80-90 % of the processes in soil are reactions mediated by microbes (Nannipieri *et al.*, 2003), microorganisms could be considered as key players in element cyclings (Gobat *et al.*, 2004, figure I.3). Indeed, bacteria and fungi are highly versatile and can carry out almost all known biological reactions. In this study, emphasis was mainly put on bacterial abilities related to the N cycle (nitrate mineralisation and reduction), the P cycle (organic P mineralisation and inorganic P solubilisation), the Fe cycle (siderophores production), the S cycle (mineralisation of organic S) and the C cycle (secretion of exopolysaccharide, and of signal molecules).

Soil is characterised by a redundancy of functions (Nannipieri *et al.*, 2003; Hamelin, 2003). Some minimum number of species is essential for ecosystem functioning under steady conditions and a large number of species is probably essential for the maintaining of stable processes in changing environments (Loreau *et al.,* 2001). The presence and activity of microorganisms depends on the number and the characteristics of the soil microhabitats, on the amounts of available metabolic substrates, and the interactions with other organisms. In soils, the rhizososphere (see chap. I.3) present the highest microhabitat diversity (Kuzyakov, 2002), and it seems to be the greatest microbial activity hot spot (Aragno, 2005). Marilley *et al.* (1999) however reported a lower bacterial diversity in the rhizosphere compared to bulk soil.

Bacteria are the most abundant microorganisms in soils, one gramm of dried soil could contain from 10^7 to 10^{10} bacteria corresponding to several thousand different species (Gobat *et al.*, 2004). In a temperate grassland soil, the bacterial and fungal biomass amounted to 1-2 and 2-5 t. ha^{-1}, respectively (Killham, 1994).

Figure I.3. Nutrient cycles in the soil-plant environment.

Schematic representation of the nutrient cycles in the rhizosphere (based on Gobat *et al.*, 2004; Paul and Clark, 1996). Carbon : black, Nitrogen : blue, Phosphore : purple, Iron : orange, Sulfur : yellow.



I.3. THE RHIZOSPHERE

I.3.1. DEFINITION OF THE RHIZOSPHERE

Root respiration, water and nutrient uptake, as well as secretion of organic coumpounds influence the physico-chemical properties of the surrounding soil. The volume of soil influenced by root, as well as the root itself, is called the rhizosphere (Hiltner, 1904; Gobat *et al.*, 2004;) and corresponds to the soil-plant interface. The rhizosphere is constituted by the root surface (rhizoplan) and interior (endorhizosphere), and the rhizospheric soil, corresponding to the surrounding soil submited to root influence (exorhizosphere).

I.3.2. ROOT-INDUCED CHANGES OF THE SURROUNDING SOIL

Roots affect soil structure and aeration, and soil biological activities as they are the major source of organic inputs and the major consumer of inorganic compounds in the rhizosphere (Lynch and Whipps, 1990; Paterson, 2003; Bertin *et al.*, 2003). Due to the diffusion of root productions in the surrounding soil, a gradual rhizosphere effect is created from the root surface to the bulk soil. In addition, the differentiation of root productions along the root growth axis (figure I.4) induce changes at another level.

Although the presence of roots induce an increase of the oxygen concentration due to drainage of water, a decreasing oxygen gradient is nevertheless observed from bulk soil to root, as aerobic microorganisms respiration, improved by exsudates consumption, is added to root respiration. Microaerophilic conditions are thus created at root proximity (Hojberg *et al.*, 1999; Gobat *et al.*, 2004).

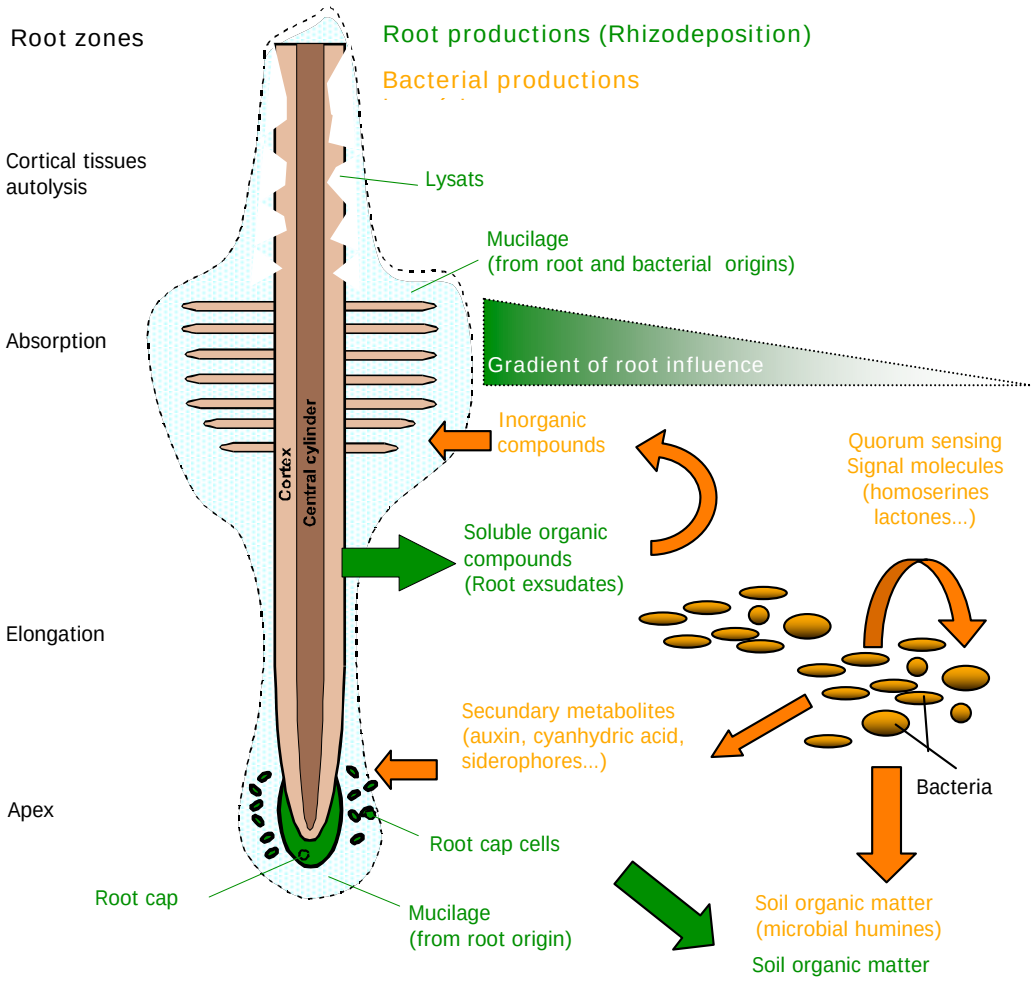
Root organic input in soil (rhizodepostion, figure I.4) provides carbon and energy sources, which leads to an increased availability of substrate in the rhizosphere compared to bulk soil. Thus the root-soil interface, or rhizosphere, is the site of intense activities and complex interactions. However, nutrient availability is decreased by the absorption for plant and microbial biomass production (Gobat *et al.*, 2004).
As shown in several studies, nutrient-limiting conditions could lead to competition or to beneficial interactions between plant and rhizosphere microorganisms (Bashan and Holguin, 1998, Lugtenberg *et al.*, 2002; Bais *et al.*, 2006).

Root symbiotic and non-symbiotic association with fungi or bacteria (Roesti, 2005; Hamelin, 2003), root density modulation (Forde and Lorenzo, 2001) or proteoid root development (Neumann *et al.*, 2000, Weisskopf, 2005), are some exemples of the nutrition improvement strategies of plants.

The main part of root deleterious and beneficial interactions with other plant roots, soil fauna and microflora, is mediated by root exudation. For a complete and recent review on root exudate-mediated interactions in the rhizosphere see Bais *et al.* (2006).

Figure I.4. Root morphology and productions.

Root morphology, rhizodepostion (green), and main bacterial productions (orange) implicated in plant growth and resistance. (Modified from Gobat *et al.*, 2004).



I.3.3. THE RHIZODEPOSITION

Rhizodeposition (figure I.4) constitute a complex mixture of sugars, organic acids, amino acids, phytosiderophores, phenolics, vitamins, purines, nucleosides, inorganic ions, gaseous molecules, enzymes and root border cells which have direct or indirect effects on the acquisition of mineral nutrients required for plant and microbial growth (Dakora and Phillips, 2002, Jones *et al.*, 2004; Bais *et al.*, 2006). Root exudation, the main part of rhizodeposition, involves the release of a wide variety of organic compounds, usually separated into low-molecular weight compounds (e.g. amino acids, organic acids, sugars, phenolics) and high-molecular weight compounds (e.g. mucilage and extracellular enzymes) (Marschner, 1995). It mainly consists of carbon-containing compounds (Uren, 2000).
In the case of meadow plants, up to 30-50% of total assimilated carbon can be translocated to the soil (Kuzyakov, 2001). The amount of root exudates varies with the plant species, cultivar, age, root zone considered and stress factors (Jones *et al.*, 2004).

Functional roles of the root exudates components in the rhizosphere have been investigated and many of them have been shown to be determinant for bacterial community structure and diversity (Jones *et al.*, 2004). Carbohydrates, organic acids, amino acids and purines provide nutrient and energy sources and promote the growth of microorganisms (Deubel *et al.*, 2000). Organic substances released from roots not only provide an energy and nutrient source for rhizosphere microorganisms, but also promote the chemotaxis of organisms towards roots (de Weert *et al.*, 2002; Bauer and Caetano-Anolles, 1990). Furthermore, phytosiderophores, organic acids and phenolics are chelatators of poorly soluble nutrients.

Microbial activities in the rhizosphere are stimulated by the exceptional growth conditions due to plant inputs in soil. However, microbial diversity is restricted by numerous factors depending on soil, plant, predation, and competition (Gobat *et al.*, 2004; see chap. I.4). Some microorganisms will thus be favoured, or on the contrary inhibited by roots.

Immediate consumption of soluble organic compounds by microorganisms (Hodge *et al.*, 1998; Münch, 2007) is a major difficulty associated with exudates caracterisation experiments conducted in soil. Therefore, the exact composition of root exudates in natural conditions and the influence of these compounds on soil fauna and microflora are at this day still largely unknown (Bais *et al.*, 2006).

I.4. RHIZOSPHERE BACTERIAL COMMUNITIES

I.4.1. ROOT SELECTION PROCESSES : THE RHIZOSPHERE EFFECT

Broadly speaking, a bacterial community corresponds to the whole of the bacterial populations in a given habitat. Each member of the community takes part in a complex interaction system, existing in the rhizosphere between soil, plant and soil biota. To keep the dynamic balance of the system, this set of species will be able to react to abiotic and biotic environmental modifications by adjustment of the activity and abundance of the different populations (species proportions), or by modification of their abilities (genetic plasticity).

Root-induced changes in the surrounding soil therefore leads to the adaptation of soil bacterial communities and to the selection of specific communities mainly constituted by the more competent populations (Gobat *et al.*, 2004).

A large amount of organic compounds is released by the roots in their surrounding soil, and can be used by microorganisms as carbon and energy source. In addition to the elective effect due to the enrichment in root-exudated organic compounds, plants exert a selective effect on bacterial communities by secretion of specific compounds inhibiting microbial growth (Latour *et al.,* 1996; Lemanceau *et al.,* 1995) or by chemico-physical modification of the rhizosphere environment (see chap. I.3.2).

Selective bacterial-grazing by Protozoa is another determinant factor for bacterial communities structuration and population turnover. For a recent study on the impact of Protozoa in the rhizosphere see Roussel-Delif (2008).

The rhizosphere effect was reported to increase bacterial density, and to reduce diversity of bacteria in the rhizosphere compared to the bulk soil (Marilley *et al.,* 1998; Kuzyakov, 2002; Tarnawski *et al.*, 2003).

I.4.2. IMPACT OF MICROORGANISMS IN THE RHIZOSPHERE

Modifications in the structure of root-associated bacterial communities at the genotypic level as well as at the phenotypic level, affect bacterial productions (figure I.4) and plant-bacteria interactions, and may generate a beneficial or deleterious feedback effect on plant.

Soil microorganisms play a key role in the plant-soil system, in particular in plant nutrition, as many microbial activities (i.e. reduction, oxydation, solubilisation activities) are strongly implicated in nutrient cycles, and availability (figure I.3).

Both plant and micoorganisms depend on the soil mineral nutrient content. However, due to their enzymatic activities and their high element-concentrating capacity, microorganisms may be more efficient than plants in the uptake of these nutrients (Gobat *et al.*, 2004). This could lead to plant-bacteria competition (i.e. for nitrate, see Fromin *et al.,* 2005) or to the improvment of plant nutrition. For instance, the ability of microorganisms to produce extracellular sulfatases (Kertesz and Mirleau, 2004) and phytases (Richardson and Hadobas, 1997) directly influence plant nutrition by the release of soluble phosphate and sulfate from soil organic compounds poorly available to plants. As it is the only biological way for incorporation of N in the ecosystem, nitrogen fixing symbiotic or non-symbiotic association is another example of direct impact of bacteria on plant growth (Gobat *et al.,* 2004; Squartini *et al.,* 2000; Brimecombe *et al.,* 2000).

Mycorrhizal fungi, as well as bacteria, in addition to filling their own needs, can thus improve plant growth by the supply of mineral nutrients. Most of plants rely on mycorrhizal fungi (arbuscular or ectomycorrhizal) for their mineral nutrient acquisition, particularly for phosphorus uptake, thus creating a preferential flux of nutrients through mycorrhize and modifying functional abilities of soil microflora (Ouahmane *et al.,* 2007; Frey-Klett *et al.,* 2005). Bacteria could therefore indirectly intervene as mycorrizal helper bacteria (Roesti, 2005; Frey-Klett and Garbaye, 2005).

In addition to their roles in plant nutrition, microbial activities affect plant growth and resistance in many ways (Glick, 1995; Lugtenberg *et al.*, 2002), either directly by producing plant hormones such as auxin (Patten and Glick, 2002), or indirectly by protection against pathogens for instance through the degradation of the fungal cell-wall (Fogliano *et al.*, 2002), the production of antibiotics (Haas and Defago, 2005), or the production of cyanhydric acid (Castric, 1977; Blumer and Haas, 2000).

In the rhizosphere, plant growth promoting (PGP) microorganisms are divided in two categories, the endophytic (symbiotic) and the root associated free-living soil microorganisms. Among rhizosphere bacteria, some have a negative impact on plant (i.e. denitrifying, parasitic or pathogenic bacteria), however the main part of plant deleterious microorganisms belong to pathogenic fungi, like *Fusarium* or *Pythium* species (Crowley, 2000).

I.4.3. BACTERIAL COMMUNITIES INVESTIGATIONS : METHODS AND LIMITATIONS

The central challenge encountered in studies of microbial ecology is the understanding of the relations between genetic diversity and community structure, and between community structure and function (Nannipieri *et al.,* 2003). The problem is posed by the functional redundancy observed in soil (Nannipieri *et al.*, 2003; Hamelin, 2003), the methodological biases in microbial diversity and functions measuring, and the limitation of the meanning of these measurements. For a complete review of methods for the study of microbial communities see Morgan and Whipps (2000) and Fromin et al. (2002).

On one hand, the use of current molecular techniques allows to know which populations are present (i.e. 16S rDNA- or PLFA based community profiles), actives (i.e. 16S rRNA-based community profiles, stable isotope probing approaches), and which functions are represented among communities (functional genes and mRNA based approaches). However, these molecular approaches generally do not allow the correlation of the taxonomic and the functional informations obtained. Molecular approaches based on functional gene or mRNA, generally do not to allow the investigation of the broad bacterial diversity. Indeed, due to the variable number of genes implicated in a given function and the genomic variability encountered among bacterial populations, the design of primers targeting the overall community is very difficult.
The use of molecular fingerprinting approaches based on 16S rDNA gives a snapshot of the communtiy displayed as patterns related to the presence of dominant population (Fromin *et al.*, 2002). While allowing the differentiation between inactive and active microbial cells (Felske and Akkermans, 1998; Koizumi *et al.*, 2003) and the identification of microorganisms, the use of both 16S rDNA and 16S rRNA molecular fingerprinting approaches is therefore a first step toward a better understanding of the relation between microbial diversity and soil activities. Recently developed molecular approaches such as stable isotope probing (Radajewski *et al.*, 2000; Manfield *et al.,* 2002) may also be appropriate to investigate the active part of communities.

On the other hand, the use of classical culture-mediated approach implicates the restriction of the investigations to the culturable bacterial community. Although representing only 1-10 % of the overall microflora (Nannipieri *et al.*, 2003), this part of the community has an ecological significiance in soil (Bakken, 1997). Moreover, in the rhizosphere, most of the active bacterial populations are r-strategists adapted to the increased carbon and energy flux, the proportion of culturable populations is then likely to be higher than the 1-10 % currently obtained in the bare soil (Benizri *et al.*, 2007).

Cultivable communities approach allows not only to identify the studied microorganisms, but also to differentiate the populations able or not to perform a given function, and to link these informations. The difference between *in situ* and *in vitro* activity of bacterial populations is therefore an important inconvenient related to culture-mediated approaches. *In vitro* studies gives only information on the putative functions of the studied microorganisms. However, considering the high genomic plasticity of bacteria, it could be hypothetised that bacteria do not harbour useless abilities. Moreover, *in situ* assays actually do not allow the correlation of taxonomic and functional informations.

Taking into account all these informations, the employment, in parallel, of both molecular and culture-mediated approaches of bacterial communities could give complementary informations and appears to be a good compromise to better understand the selection processes of rhizosphere bacterial communities.

I.5. THE MODEL PLANT : RYEGRASS (*Lolium perenne*)

I.5.1. IMPORTANCE AND OCCURENCE OF *Lolium perenne*

Plants are known to influence physically and chemically their surrounding soil (see chap. I.3.2). Perennial plants are growing at the same location from year to year, and the persistence in soil of vegetative roots during winter leads to the enhancement of root influence on the surrounding soil. They thus appear to be a suitable model for rhizosphere investigations.

Perennial meadows account for a large part of land cover and have a high agricultural importance. Perennial ryegrass, which was probably the first to be cultivated as a pasture grass in Europe, is one of the more closely studied grasses. It also presents the advantage to grow fast and was used as model plant in many other studies, including previous researches on rhizosphere bacterial communities conducted in our laboratory (see chap. I.6).

Plant type and species are commonly known to influence bacterial community structure (Westover *et al.*, 1997; Smalla *et al.*, 2001; Marschner *et al.*, 2005; Patra *et al.*, 2006; Costa *et al.*, 2006), however, cultivar influence is currently less studied (Dalmastri *et al.*, 1999; Varma *et al.*, 2004). In our study, we choose to investigate different *L. perenne* cultivars to determine the impact of minor plant variability on bacterial communities and to evaluate the response sensitivity of bacterial communities. Management, nutritive value, and genetic of *L. perenne* is well documented, many diploids (2n) and tetraploids (4n) cultivars are available, conferring various characteristics of pest and/or disease resistance and particular growth properties (Frakes, 1973).

I.5.2. TAXONOMY AND PLANT MORPHOLOGY

Taxonomy

Subkingdom	Tracheobionta	(Vascular plants)
Superdivision	Spermatophyta	(Seed plants)
Division	Magnoliophyta	(Flowering plants)
Class	Liliopsida	(Monocotyledons)
Subclass	Commelinidae	
Order	Cyperales	
Family	Poaceae	(Grass family)
Genus	*Lolium* L.	(Ryegrass)
Species	*Lolium perenne* L.	
Subspecies	*Lolium perenne* L. ssp. perenne	(Perennial ryegrass)

Morphological description

(Source : USDA, NRCS. 2007. The PLANTS Database. National Plant Data Center, Baton Rouge, LA 70874-4490 USA)

Perennial ryegrass (figure II.5) belongs to the C3 photosynthetic plant categorie and grows from 30-60 cm tall with a bunchy form. There are numerous long, narrow, stiff leaves near the base of the plant. The under surfaces of leaves are bright, glossy, and smooth. Inflorescence stems are nearly naked. Seedheads are spikes with spikelets growing edgewise to the seedhead stem. Seeds do not have awns (bristles). There are approximately 500 seeds per gram.
L. perenne harbours an homorhize root system. It is usually formed by thin, moderately branching roots growing from the stem. The primary root, originating in the radicle of the seedling, is not dominant. The whole root system is fibrous and branches in all directions.

Figure I.5. *Lolium perenne* : Plant morphology

Picture © Texas A&M Bioinformatics Working Group

I.5.3. ECOLOGY AND PLANT DEVELOPMENT

Distribution and ecological requirements

This plant is adapted to a wide range of nitrophilic (or fertilized) soil types and drainage conditions, and is found in lowland and mountains until about 1200 m of altitude. Common in Switzerland (figure I.6) and native to Eurasia, perennial ryegrass is also widely planted in North America.

Figure I.6. *Lolium perenne* : Distribution (Switzerland) Lauber and Wagner. Flora Helvetica, 2000

Plant development

L. perenne is an hemicryptophytic grass (root system maintained during winter period) which begins growth early in spring and have high N requirements. The optimum of biomass production occurs during cool moist conditions in spring and again in autumn (Grime, 1979). Flowering occurs from April or May to August, depending on environmental conditions (Frakes, 1973; Radford *et al.*, 1968). Seeds are released in late spring and summer and germination takes place from August to September or as soon as moisture conditions allow it (Grime, 1979; Thompson and Grime, 1979).

The maximum stem carbohydrate reserve levels occur in early summer, just after flowering begins, dropping off through summer to a minimum in October. This is in contrast to many other grasses that have two peaks of carbohydrate reserves, one in early spring which is depleted by flowering, then replenished by late summer. Perennial ryegrass is more efficient at maintaining carbohydrate reserves through the flowering period (Waite and Boyd, 1953).

I.5.4. AGRICULTURAL USE AND INFECTION BY DISEASES AND PESTS

Agricultural use

L. perenne is the predominant forage grass in Europe, and has also been used in the United States for forage and lawns. The tetraploid cultivars are generally used for forage, and diploid cultivars for lawns and conservation plantings. Many cultivars are available for turf and forage application.

This species forms vesicular-arbuscular endomycorrhizal associations (Berch *et al.*, 1988), and newer turf-type cultivars are often intentionally infected with an endophytic fungus to improve stress-tolerance.

Perennial ryegrass grows rapidly and is easily established; it is often used for stabilization of soils (Gross *et al.*, 1989; Hafenrichter *et al.*, 1980). Ryegrass is often seeded in mixtures with slower growing, longer lived species to provide a quick cover (Vogel, 1981).

Diseases and pests

Crown rust (*Puccinia coronata*) may severely reduce forage value in wet areas. Other fungal infections include brown rust (*Puccinia dispersa*), and red thread (*Corticum fuciforme*). Occasional attacks by *Helminthosporum* species also occur. Ergot (*Claviceps purpurea*) and blind-seed disease (*Phialea temulenta*) reduce seed yield and quality (Frakes, 1973). Leaf blotch (*Helminthosporium spiciferum*), ergot and blind seed disease can be substantially controlled by burning perennial ryegrass fields in the spring (Hardison, 1980).

I.6. CONTEXT OF THE STUDY

The previous researches on rhizosphere microbial ecology at the Laboratory of Microbiology, University of Neuchâtel (LAMUN) was mainly dealing with rhizosphere bacterial communities associated to perennial grasslands (*Trifolium repens, L. perenne* and *Molinia coerulea*). These researches focused on the impact on these communities of an increase of the armospheric CO_2 content, and benefited from the Swiss FACE (Free Air CO_2 Enrichment) system in Eschikon (Zurich), in collaboration with the Institute of Plant Science (ETHZ).

During his PhD, Laurent Marilley showed that plants harboured a reduced bacterial diversity with increasing root proximity (Marilley *et al.*, 1998), and present more cultivable heterotrophic bacteria in their rhizosphere and rhizoplane when grown under elevated pCO_2 (Marilley *et al.*, 1999). An increasing proportion of 16S rDNA sequences related to the genus *Pseudomonas* was observed with root proximity. This proportion was enhanced, in root-associated fractions, for plants grown under elevated pCO_2. (Marilley *et al.*, 1999).

Ludovic Roussel-Delif, Sonia Tarnawski (PhD), Jérôme Hamelin (PhD) and Nathalie Fromin (post-doc fellow) investigated different aspects of bacterial ecology in the rhizosphere of the nitrophilic *L. perenne* and the oligonitrophilic *M. coerulea* grown in the Swiss FACE system. Communities were approached at different levels, including: (i) investigation of total communities, (ii) and (iii) of functionnal communities, and (iv) of the guild of *Pseudomonas* populations.

(i) The bacterial communities were characterised by cloning-sequencing approaches (Marilley *et al.*, 1998; Marilley *et al.*, 1999), and by PCR-DGGE of 16S rDNA. The relationships between DGGE community profiles and environmental descriptors of the analysed samples, as well as the use for the analysis of DGGE fingerprinting profiles, of statistical tools available in ecology (in particular ordination methods), were investigated (Fromin *et al.*, 2002).

(ii) The metabolic capabilities of bacterial communities to oxidise 31 substrates was determined using the Biolog™ ECOplates in the rhizosphere of *M. coerulea* grown under ambient and elevated pCO_2. The root-associated bacterial community metabolised better some of the carbon sources (especially carbohydrates) for plants grown under elevated pCO_2. No direct effect of pCO_2 could be evidenced on the bulk soil community, suggesting that this effect is mediated by the rhizodeposition. When characterizing (by DGGE profiling of 16S rDNA) the bacterial guilds

responsible for the oxidation of the different substrates, a trend to metabolic redundancy (low similarity of 16S rDNA profiles with similar oxidation pattern) was observed.

(iii) The diversity of nitrogen fixing bacteria (NFB) was investigated in the rhizosphere of *M. coerulea* using the *nifH* gene as target sequence for a molecular approach. It has been shown that the rhizosphere of *M. coerulea* harboured a large proportion of *nifH* sequences that were only distantly related to the *nifH* sequences of known, culturable NFB. 56% of all retrieved sequences were closely related and grouped in cluster A (Hamelin *et al.,* 2001; Hamelin *et al.,* 2002). A few related sequences had been evidenced in other environments, including rice root endophytes.

(iv) Bacteria belonging to the genus *Pseudomonas*, which are abundant in the rhizosphere of plant and have interesting properties regarding the plant growth, were investigated in the rhizosphere of *L. perenne* and *M. coerulea*. More than 1000 *Pseudomonas* strains were isolated from the rhizosphere of *L. perenne* and *M. coerulea* grown under ambient and elevated pCO_2.
A molecular approach based on the specific amplification of the 16S – 23S rDNA internal transcripted spacer (ITS1) was developed for the detection and characterisation of environmental *Pseudomonas* strains (Locatelli *et al.,* 2002). *Pseudomonas* diversity was shown to be different in the rhizosphere of *M. coerulea* and in surrounding soil. It was also shown that the use of a selective culture medium (mS1) for the isolation of environmental *Pseudomonas* lead to underestimate *Pseudomonas* counts and diversity, especially in soil environment (Tarnawski *et al.*, 2003).
The genus *Pseudomonas* is also recognized as one of the main (cultivable) denitrifying group in soil environment. Phenotypic characterisation of the *Pseudomonas* population in the rhizosphere has been performed (Tarnawski *et al.*, 2006). The frequency as well as the diversity of nitrate-dissimilating Pseudomonas in the rhizosphere of *L. perenne* and *M. coerulea* grown under ambient and elevated pCO_2 in the FACE conditions has been also investigated. The frequency of nitrate-dissimilating *Pseudomonas* decreased with root proximity. Additional data suggest that this may be due to limited nitrate availability in the rhizosphere, due to N plant uptake (Fromin *et al.,* 2005). Moreover, the frequency of nitrate-dissimilating strains was enhanced under elevated pCO_2. The membrane-bound form of nitrate reductase (NAR) was more frequently detected than the periplasmic form (NAP) among root- compared to soil-associated strains and for strains isolated from CO_2-enriched plots. Anyway, the diversity of corresponding genes (narG and napA) was not influenced by the root proximity nor by the CO_2 treatment (Roussel-Delif *et al.*, 2005).

I.7. GENERAL AIMS AND OVERVIEW OF THE THESIS

Durable agricultural development, which is one of the main current environmental challenge, require the improvement of fundamental knowledges of soil and rhizosphere functioning. Studies thus have to relate bacterial diversity with functions. In addition to the difficulties posed by methological biases, which limits all community investigations, the direct correlation of genomic and functional diversity of the broad bacterial communities is nowadays not possible. In this context, we tried in this work to gain additional informations about bacterial community structuration, and plant-bacteria interactions by the combination of different approaches. The questions assessed are presented in figure I.7.

Considering that bacterial density is higher in rhizosphere than in bulk soil (Gobat *et al.*, 2004) and that bacterial diversity might be reduced in the rhizosphere environment (Marilley *et al.,* 1998), there is an evidence that plant presence and activities induce the selection of bacterial populations, resulting in the dominance of a restricted number of populations from the initial soil community. Differences in the structure of bacterial communities associated to different plant species (Westover *et al.,* 1997; Smalla *et al.,* 2001; Marschner *et al.,* 2005; Costa *et al.,* 2006; Patra *et al.,* 2006), cultivars (Dalmastri *et al.,* 1999), development stages (Wieland *et al.,* 2001), and pCO_2 conditions (Jossi *et al.,* 2006; Tarnawski *et al.,* 2006; Marilley *et al.,* 1999) have been already reported. However, the mecanisms involved in these selection processes are still largelly unknown.
The present work is going on with the research interest on rhizosphere functionning, on the structuration of bacterial communities, and on their implication in nutrient availability and plant growth. *Lolium perenne* was choosen as model plant. Growing fast, it also present both advantages to be frequently used for forage and to recover a large part of terrestrial environment.

After the general introduction (CHAPTER I), which describes briefly the soil-plant-microorganism system, a first experimental part (CHAPTER II) is devoted to the investigation of plant genotype influence on bacterial communities structure in the rhizosphere of *L. perenne* grown in greenhouse conditions. The main aim is to determine the degree to which the rhizosphere effect is plant dependant, to determine if different plant cultivars differs significantly in their rhiozodeposition, and if they harbour different bacterial community structures (at the functional or genotypic level). This will be done by characterisation and comparison of bacterial communities associated with four cultivars possessing different ecology

requirement and degree of ploïdy, and by determination of soil nutrient content and comparison of root exudates of the different cultivars.

Bacterial community structures and functions will be characterised in relation with root proximity, plant cultivar and development stage. Methodological biases and restrictions in microbial ecology are important (Morgan and Whipps, 2000; Fromin *et al.*, 2002), the combination of different investigation approaches seems thus to be necessary to obtain a more accurate vision of the overall bacterial community. That's why bacterial communities will be investigated here using molecular genotypic approach (total and active communities, see CHAPTER II.3.1) and cultivation-based functionnal approach (see CHAPTER II.3.2). For functionnal approach, emphasis will be put on some bacterial abilities which present the advantages to be wildly distributed among rhizosphere bacterial communities, to be recognised as root competence or PGP traits, and to be useful for further development of biofertilizers. Additionnaly, a broad bacterial diversity was investigated in order to improve the knowleges about the diversity and ecology of root-associated functional communities.

It is a well-known fact that plant species differ in their impact on the structure of rhizosphere microbial communities (Westover *et al.,* 1997; Smalla *et al.,* 2001; Marschner *et al.,* 2004, 2005; Costa *et al.,* 2006; Patra *et al.,* 2006), but until now, the extent to which this effect might be linked to the differential exudation behaviour of the various plant species has remained an open question.
Plant impact has been shown as determinant of the structure of bacterial communities in the rhizosphere, in particular by the mean of root exudates (Hodge *et al.,* 1998; Bais *et al.,* 2006). Soil however has an important impact on bacterial communities (Wieland *et al.,* 2001; Latour *et al.,* 1996). In order to keep the soil contribution in our system, experiments for the investigation of bacterial communities were conducted in a natural soil. Soil nutrient content was assessed after growth of each cultivar (see CHAPTER II.4.1). However, taking into account the difficulties encountered for root exsudates characterisation in soils, experiments were conducted, for this purpose, in steril microcosms with an artificial growth substrate. Out of the large panel of root-secreted compounds, we focused our attention on organic acids (see CHAPTER II.4.2), as readily available carbon source for most heterotrophic soil bacteria, and on phenolics (see CHAPTER II.4.3), as biocontrol compounds.

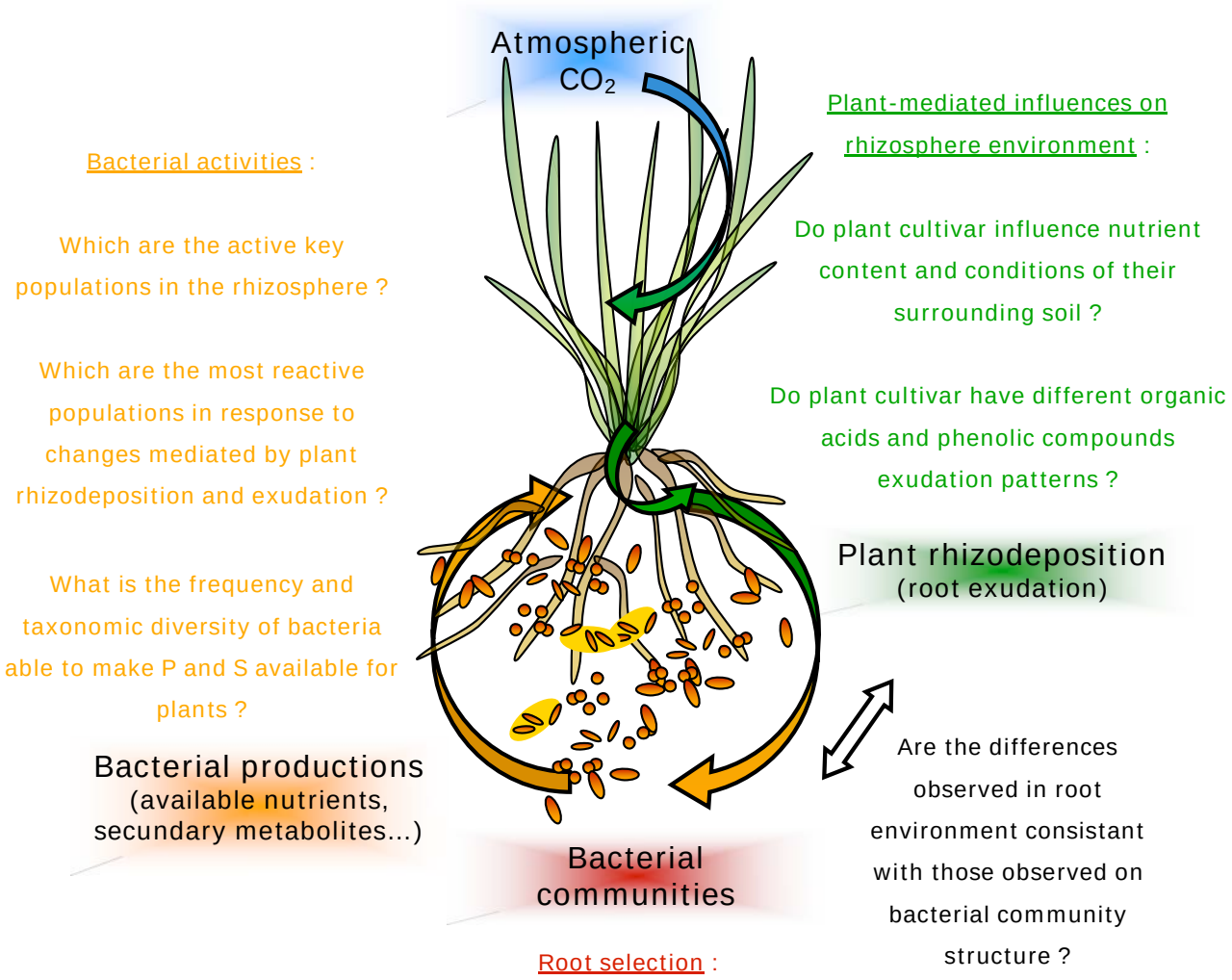
The characterisation of functional groups which are closely linked with plant root could be useful for biofertilizer development. In this context, this part of the work will also allow to determine which bacterial functions are strongly associated with root and to evidence correlations between these functions.

The second experimental part (CHAPTER III) focuses on the frequency and diversity of two bacterial groups implicated in rhizosphere competence and plant nutrition. The aims are to evaluate the occurence and the structure of bacterial populations able to mineralise organic phosphate or organic sulfate, and likely to be able to improve plant growth by these P and S nutrient supply.

The last experimental part of this work (CHAPTER IV) deals with bacterial communities structure in the rhizosphere of two perennial grasses grown in field conditions under ambiant and elevated atmospheric pCO_2. Under enriched CO_2 atmosphere, the rhizodeposition is enhanced and its composition is altered (Hodge *et al.*, 1998). Moreover, the availability of nutrients is decreased, because of higher plant demand and of enhanced microbial activities (Nösberger et al., 2006). Rhizosphere bacterial communities selection processes are therefore likely to be enhanced under elevated pCO_2. The objectives are here to determine the impact of atmospheric pCO_2 elevation on bacterial communities structuration, and to identify indicative populations responsive to these plant-mediated changes.

Due to the variety of the investigated topics, each chapter is preceded by a specific introduction and ends up with a synthesis, and a conclusion.
A global discussion and conclusions (CHAPITRE V) close up this work.

Figure I.7. Summary of the different questions assessed throughout the experiments conducted in this work.



Do plant-mediated influences affect genomic as well as functional bacterial community structures ?

Which bacterial functions are characteristic of root and of soil bacterial communities ?

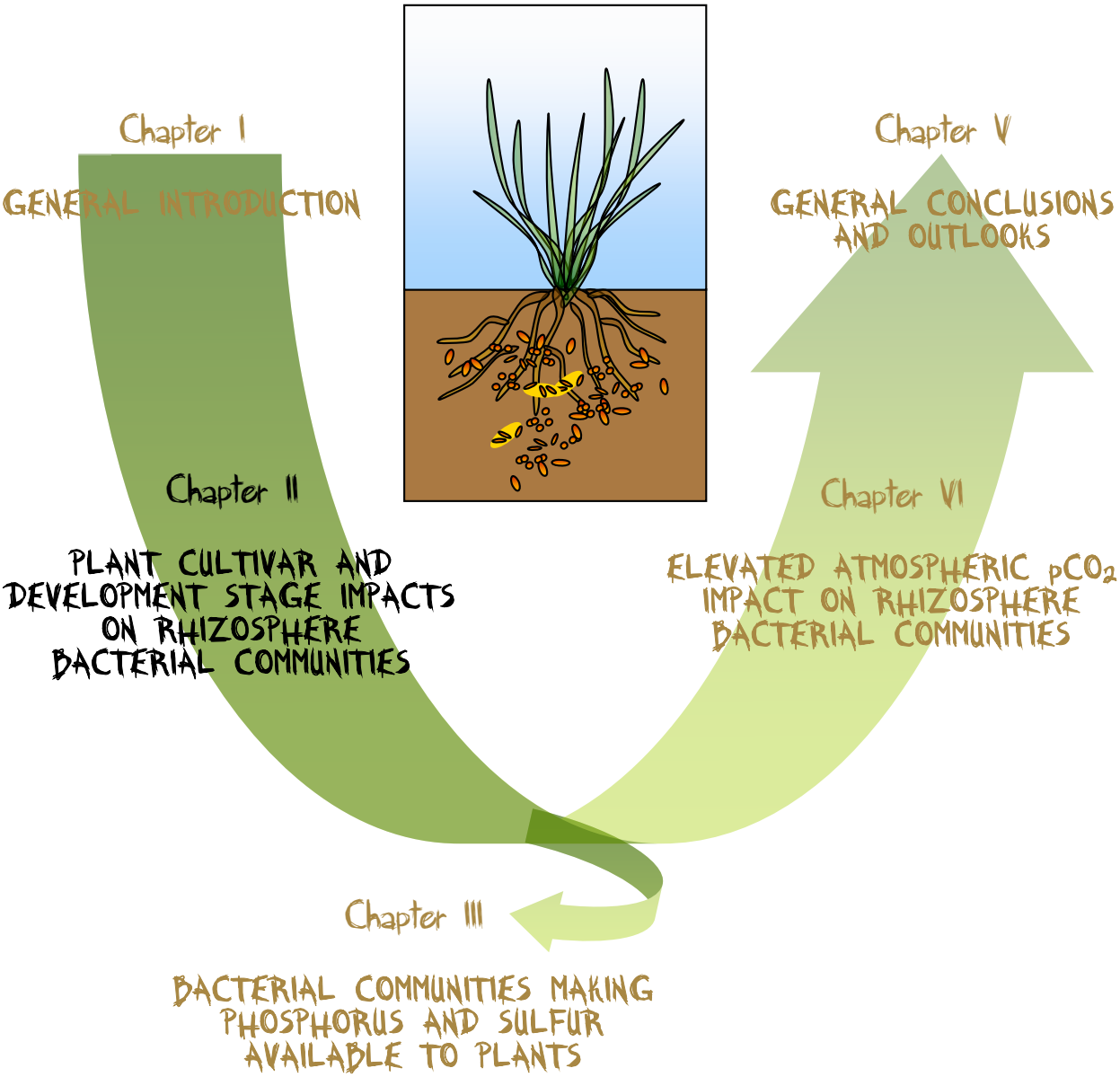
Which relationship exists between the different bacterial abilities ?

Plant mediated influences on bacterial communities :

How do plant cultivar, plant development stage and elevated atmospheric pCO_2 influence bacterial community structure ?

CHAPTER II :

PLANT CULTIVAR AND DEVELOPMENT STAGE IMPACTS ON RHIZOSPHERE BACTERIAL COMMUNITIES


Chapter I
GENERAL INTRODUCTION
Chapter II
PLANT CULTIVAR AND DEVELOPMENT STAGE IMPACTS ON RHIZOSPHERE BACTERIAL COMMUNITIES
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CHAPTER II : PLANT CULTIVAR AND DEVELOPMENT STAGE IMPACTS ON RHIZOSPHERE BACTERIAL COMMUNITIES

Overview

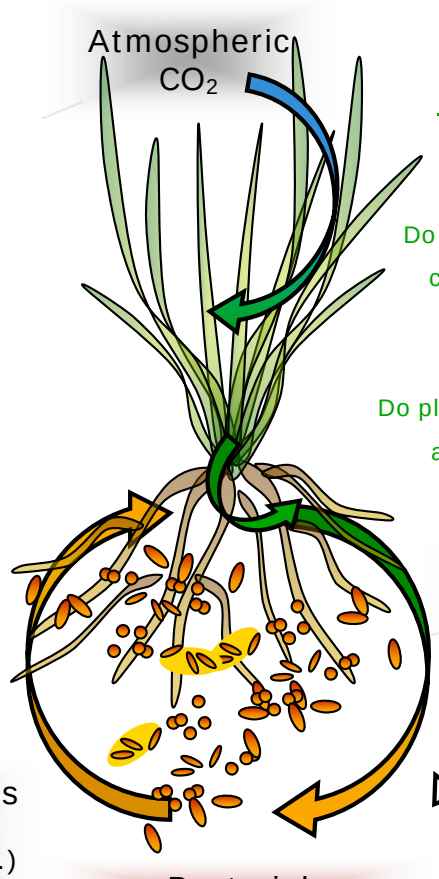
II.1. INTRODUCTION

II.1.1. CHAPTER PRESENTATION

The following chapter is divided in two experimental parts. In the first part (see chap. II.3), the genomic and functionnal structure of bacterial communities has been investigated in the rhizosphere of four cultivars of *L. perenne* in order to determine the impact of plant cultivar, development stage, and root proximity on the structure of rhizosphere bacterial communities. Total and metabollicaly active communities structure was assessed using genomic approaches (see chap. II.3.1), and total heterotrophe aerobic cultivable communities structure using in vitro functionnal characterisation approach (see chap. II.3.2).

In the second part (see chap. II.4), soil was characterised after plant growth to determine the nutrient status in the rhizosphere of the different cultivars (see chap. II.4.1). Root exudates of the cultivars were then characterised (see chap. II.4.2) in order to evaluate their degree of differenciation. Emphasis was put on organic acids and phenolic compounds secretion patterns.

Synthesis of the results obtained for these two approaches (see chap. II.5.1 / II.5.2) was performed to investigate the involvement of root exudates in the structuring of rhizosphere bacterial communities associated with the different plant cultivars by discussion and correlation of the cultivar-induced modifications observed on root exudates patterns and of those observed on bacterial communities structure (see chap. II.5.3).
The importance of the different bacterial abilities for root colonisation (see chap. II.5.4), and the impact of plant development on bacterial genomic community structure and functional abilities of bacterial populations (see chap. II.5.5) were also discussed.
A general conclusion close up this chapter (see chap. II.6).



Bacterial activities :

Which are the active key populations in the rhizosphere ?

Which are the most reactive populations in response to changes mediated by plant rhizodeposition and exudation ?

What is the frequency and taxonomic diversity of bacteria able to make P and S available for plants ?

Plant-mediated influences on rhizosphere environment :

Do plant cultivar influence nutrient content and conditions of their surrounding soil ?

Do plant cultivar have different organic acids and phenolic compounds exudation patterns ?

Are the differences observed in root environment consistant with those observed on bacterial community structure ?

Root selection :

Do plant-mediated influences affect genomic as well as functional bacterial community structures ?

Which bacterial functions are characteristic of root and of soil bacterial communities ?

Which relationship exists between the different bacterial abilities ?

Plant mediated influences on bacterial communities :

How do plant cultivar, plant development stage and elevated atmospheric pCO_2 influence bacterial community structure ?

II.1.2. ROOT EXUDATION

II.1.2.1. ROOT EXUDATION AND CARBON FLOW IN THE RHIZOSPHERE

Rhizodeposition (see chap. I.3.3) constitute a complex mixture of sugars, organic acids, amino acids, phytosiderophores, vitamins, aromatic compounds, purines, nucleosides, inorganic ions, gaseous molecules, enzymes and root border cells. Root exudates, the major part of rhizodeposition, mainly consist of carbon-containing compounds (Uren, 2000). In the case of meadow plants, up to 30-50% of total assimilated carbon can be translocated to the soil through living roots (Kuzyakov, 2001).

The continual release of carbon compounds from the root into the soil falls into two classes:

(i) exudates which are lost simply as a result of passive diffusion and over which the plant exerts little control (basal exudation)

(ii) exudates which are released for a specific purpose and over which the plant exerts a close degree of control.

In the second category, diffusion may still be the primary mechanism of exudate release, however, the opening of membrane pores (e.g. anion channels), and the use of vesicle transport may increase the effective diffusion rate by several orders of magnitude (Jones, 1998).

The membrane permeability determines if direct diffusion through the lipid bilayer is possible. This depends on the physiological state of the root cell and on the polarity of the compounds to be exuded. It is difficult operationally to divide exudation from living or senescing roots from C addition by dead or dying roots.

The definition employed here is that exudation only occurs from metabolically active respiring roots while loss of soluble C from non-metabolically active roots is termed lysis.

Root exudation involves the release of a wide variety of organic compounds, usually separated into low-molecular weight compounds (e.g. amino acids, organic acids, sugars, phenolics) and high-molecular weight compounds (e.g. mucilage and extracellular enzymes) (Marschner, 1995).

Theoretically, almost any soluble component present inside the root can be lost to the rhizosphere; however, current evidence suggests that exudation is dominated by low molecular weight solutes such as sugars, amino acids and organic acids that are present in the cytoplasm at high concentrations (Farrar *et al.,* 2003).

Functional roles of the root exudates components in the rhizosphere have been investigated (Jones *et al.*, 2004). Carbohydrates, organic acids, amino acids and purines provide nutrient and energy sources and promote the growth of microorganisms (Deubel *et al.,* 2000). Organic substances released from roots not only provide an energy and nutrient source for rhizosphere microorganisms, but also promote the chemotaxis of organisms towards roots (de Weert *et al.*, 2002; Bauer and Caetano-Anolles, 1990). Phytosiderophores, organic acids and phenolics are also chelatators of poorly soluble nutrients (Crowley, 2000). For a complete and recent review on root exudate-mediated interactions in the rhizosphere see Bais *et al.* (2006).

Carbon flow in the rhizosphere is often assumed to be a unidirectional flux whereby C moves out of roots into the surrounding soil. Hydroponic studies give now a significant body of evidence to suggest that a number of exudate components can be actively taken back into the root (Jones and Darrah, 1993; Muhling *et al.,* 1993). This re-absorption of C largely applies to those compounds lost in greatest quantities from roots (i.e. sugars and amino acids).
However, roots can also take up a variety of compounds which do not represent major components of root exudate and for which a functional role remains unclear (e.g. polyamines such as putrescine; Hart *et al.,* 1992; Kuiper *et al.,* 2001). It has been shown that the influx and efflux of C compounds at the soil–root interface occurs simultaneously, and that unlike the efflux component, the influx component is under direct plant control (Farrar *et al.,* 2003).

II.1.2.2. ORGANIC ACIDS

Apart from sugars and amino acids, organic acids typically represent the next largest exudate group (Jones *et al.*, 2004, Hodge *et al.,* 1998). Because of the negative charge associated with organic acids in the cytoplasm (e.g. succinate 2– and citrate 3–) their rate of exudation is enhanced by the electrochemical potential gradient across the membrane (Jones, 1998).

For unknown reason, a lack of uptake system for organic acids in roots was observed, but it is speculate that organic acids act in nutrient acquisition and consequently there is little benefit in recapture, particularly as the efflux rate is low in comparison to sugars and amino acids (Jones *et al.,* 2004).

Studies on the rhizosphere showed that organic acids secreted by plant roots could have an important influence on bacterial community structure (Singh and Mukerji, 2006; Baudoin *et al.,* 2001).

Weisskopf *et al.* (in prep) showed that bacterial diversity was negatively correlated with the amounts of organic acids exuded. This suggests that these compounds could act as elective and/or selective agents in the rhizosphere, stimulating the growth of particular bacterial populations and/or inhibiting other populations. Variation of the low molecular weight organic acids exuded by different cultivars of flax have been reported (Cieslinski *et al.,* 1997). The changes in organic acids exudation of different cultivars is likely to be sufficiant to induce a differentiation of their associated communities.

II.1.2.3. PHENOLIC COMPOUNDS

Plant produce a great variety of organic compounds that are not directly involved in primary metabolic processes of growth and development. These secundary metabolites appeared to function mainly in defense against pathogens and predators, and in chemoattraction.

Most of these secundary metabolites can be classified into three major groups : terpenoids, alkaloids and phenolic compounds. The last one were shown to influence growth and development of surrounding plants and soil microorganisms (allelopathy), and to play a role in a number of plant-microbes associations such as legume-*Rhizobium* symbiosis (Bertin *et al.,* 2003; Jones *et al.*, 2004).

Phenolic compounds are therefore a low-molecular weight compound category of relevance in root exudate. These compounds are synthetised primarily from products of the shikimic acid pathway and gather mainly flavonoïds (anthocyanins, isoflavones...) and phenolic acids (tannic acid, vanillin...).

Phenolics were already shown to be abundant component of *L. perenne* root exudates (Hodge *et al.,* 1998) and many other plants (Weiskopf 2005, Wu *et al.,* 2001). Moreover, difference was observed between phenolic acids exudation from different cultivars of *Triticum aestivum* L. (Wu *et al.,* 2001). This suggests that a part of cultivar-induced modifications of rhizosphere bacterial communities could be linked with phenolic compounds secretion pattern.

II.1.2.4. ROOT EXUDATES MODIFICATIONS

Components of exudation are regulated over longer timescales in response to physiological changes and gene expression patterns at the whole plant level. For example, in many cases a period of days is required to induce enhanced exudation in response to mineral deficiency (e.g. phytosiderophore release under Fe deficiency; Ma *et al.,* 2003).

The amount of root exudates varies considerably according to many abiotic and biotic factors, such as the plant species and development stage (Jones *et al.,* 2004) and cultivar (Wu *et al.,* 2001). It has been well documented in a number of species such as shorghum and wheat that root exudation decreases with age of plant and increases with soil stress such as compaction, drought, and low nutrient supply (Brady and Weil, 1999; Brimecombe *et al.,* 2001; Uren, 2000; Nguyen, 2003). Global climate changes, such as atmospheric pCO_2 elevation could also alter root exudation qualitatively and quantitatively (Nösberger *et al.,* 2006; Hodge *et al.,* 1998; Darrah, 1996), For a complete review on regulation of root exudates, see Jones et al (2004).

II.1.2.5. EXUDATES CHARACTERISATION : METHODS AND LIMITATIONS

Immediate consumption of soluble organic compounds by microorganisms (Hodge *et al.,* 1998) is a major difficulty associated with exudates caracterisation experiments conducted in soil. Therefore, the exact composition of root exudates in natural conditions and the influence of these compounds on soil fauna and microflora are at this day still largely unknown (Bais *et al.,* 2006).

Most experiments quantifying C exudation have used sterile, hydroponic cultures where exudates are typically collected over a period of days or weeks. Under these conditions, where the microbial sink has been removed, sugars and amino acids are simply lost and then reabsorbed (Jones *et al*., 1996). As a result, only a small accumulation is observed in the external medium representing the equilibrium between passive efflux and active influx (Kraffczyk *et al*., 1984). In vegetative cereal and grass crops, photosynthetically fixed C is transported very rapidly below ground and can be detected in the environment external to the root in less than 1 h from photosynthetic fixation (Minchin *et al.,* 1994; Dilkes *et al.,* 2004). From studies on excised root tips of maize it has been ascertained that roots can remain metabolically active for at least 48 h before their internal C reserves become exhausted by respiration (James *et al*., 1993; Xia and Roberts, 1994).

In a summary of 95 whole plant ^{14}C labelling studies performed in soil on a broad range of plant species, it has been estimated that approx. The equivalent of 5–10% of the total net fixed C is lost by root exudation, while experiments performed in hydroponics show that typically only 0.5–1.5% of fixed C is lost (Farrar *et al*., 2003). This may be due to a methodological bias causing underestimation of rhizosphere C flow in hydroponics and overestimation of rhizosphere C flow in soil (Meharg, 1994).

II.1.3. BACTERIAL COMMUNITIES IN THE RHIZOSPHERE

II.1.3.1. THE RHIZOSPHERE EFFECT

It is a well-known fact that plant species differ in their impact on the structure of rhizosphere microbial communities (Westover *et al.,* 1997; Smalla *et al.,* 2001; Marschner *et al.,* 2005; Costa et al., 2006; Patra et al., 2006), but until now, the extent to which this effect might be linked to the differential exudation behaviour of the various plant species has remained an open question.

A large amount of organic compounds is released by the roots in their surrounding soil, and can be used by microorganisms as carbon and energy source. In addition to the elective effect due to the enrichment in root-exudated organic compounds (see chap. II.1.2), plants exert a selective effect on bacterial communities by secretion of specific compounds favoring or inhibiting microbial growth (see chap. II.1.2), or by physicochemical modification of the rhizosphere environment (see chap. I.3.2).
Soil inhabiting bacterial community will react to root influences by adjustment of the activity and abundance of the different populations (species proportions), or by modification of their abilities (genetic plasticity). The effects of plants on the surrounding microflora might lead on one hand to a higher bacterial density, due to root carbon input in soil. On the other hand, it might lead to a lower diversity of bacteria in the rhizosphere compared to the bulk soil (Marilley *et al.,* 1998; Tarnawski *et al.*, 2003), due to competition and root selection.

As mainly measured with culture-dependant methods, the root impact on bacterial density also reflect a change in cultivability due to a shift in bacterial growth strategy (Gobat *et al.*, 2004): From the bare soil slow growing bacteria (K-strategists) to the fast growing bacteria (r-strategists). The K-strategist are usually more stable and permanent members of the communities (Varma *et al.,* 2004). In the rhizosphere, most of the active bacterial populations are r-strategists adapted to the increased carbon and energy flux, the proportion of culturable populations is then higher (Benizri *et al.,* 2007, Morgan and Whipps, 2000) than the 1-10 % currently obtained in the bare soil.

The investigation of plant-bacteria interactions taking into account a broad bacterial diversity remains highly complex (see chap. II.1.3.2), thus most studies are conducted with model bacterial populations. Due to their metabolic versatility and high growth rate, bacteria belonging

to the r-strategists genus *Pseudomonas* were shown to be particularly adapted to the rhizosphere environment and are currently considered as model rhizobacteria, in particular for plant growth promotion and root colonisation investigations (Lemanceau *et al.,* 1995; Rainey, 1999; Latour et al., 2003; Tarnawski, 2004; Lucy *et al.*, 2004). Some *Pseudomonas* activities are currently known to influence plant-bacteria interactions :
(i) nitrate dissimilation, an anaerobic respiratory process (Ghiglione *et al.,* 2000), (ii) production of auxin, an hormone involved in root elongation (Glick, 1995; Persello-Cartieaux *et al.,* 2003); (iii) production of hydrogen cyanide, a secondary metabolite which can inhibit the growth of rhizosphere inhabiting bacteria and fungi (Castric, 1977; Blumer and Haas, 2000), (iv) production of siderophores organic ligands that complex iron, providing a competitive advantage to producing organisms in the rhizosphere (Lugtenberg and Dekkers, 1999), and (v) production of antibiotics (Crowley, 2000).

Modifications in the structure of root-associated bacterial communities, at the genotypic level as well as at the functional level, affect bacterial productions, plant-bacteria interactions, and may generate a beneficial or deleterious feed-back effect on plant (see chap. II.1.3.3).

II.1.3.2. BACTERIAL DIVERSITY

Althought plants are known to have an evident impact on rhizosphere bacterial diversity, the methodological biases limits diversity investigations. As already mentionned (see chap. I.4.3), the image obtained from communities differs in relation with the methological approach used.
Kleenberger *et al.* (1983) suggested that pseudomonads, representing 50-70 % of culturable bacterial species, were the predominant bacteria in the rhizosphere of wheat and barley. A predominance of pseudomonads was also observed using genetic approach in the rhizosphere of *L. perenne* (Marilley *et al.,* 1999).

In contrast, Lilley *et al.* (1996) revealed that the 10 most common cultivable genera in rhizosphere of sugar beet were *Bacillus* (14 %), *Arthrobacter* (12 %), *Pseudomonas* (11 %), *Aureobacterium* (9 %), *Micrococcus* (6 %), *Xanthomonas* (5 %), *Alcaligenes* (4 %), *Flavobacterium* (3 %), *Agrobacterium* (3 %) and *Microbacterium* (3 %).

Other studies using genetic approaches of bacterial communities suggest that there may be as many as 4000 species per gram of soil, and that up to 64 % of the bacterial rhizosphere and soil species identified from 16S rDNA library were completely unknown (Torsvik and Goksoyr, 1990; Kuske *et al.,* 1997).

The parrallel investigation of bacterial communities through different points of view could therefore provide complementary informations and conduct to more complete knowledges on the diversity and behaviour of the entire communities.

Some minimum number of species is essential for ecosystem functioning under steady conditions but a large number of species is probably essential for maintaining stable processes in changing environments (Loreau *et al.,* 2001). As a complex changing environment, soil is also characterised by a redundancy of functions (Nannipieri *et al.,* 2003; Hamelin, 2003) and a high diversity of organisms.

Bacteria and fungi are highly versatile microbial communities and can carry out almost all known biological reactions. The diversity of the performing microorganisms for a given function depend mainly on the available ecological niches (number and characteristics of the soil microhabitats, interactions with other organisms), and their activity depends on the amounts of available metabolic substrates.
These functional richness and redundancy of functions is paradoxally linked to a lower genomic diversity in the root environment compared to bulk soil (Marilley *et al.,* 1998). As an interface area, rhizosphere, is the site of intense activities mainly due to the increased nutrient flux. However rhizosphere is also the site of complex interactions, including competition for nutrients and predation (Gobat et al., 2004), increasing the selection of the more competent populations and limiting genomic diversity (Marilley *et al.,* 1998; Tarnawski *et al.*, 2003).

In order to analyze the changes in bacterial genomic diversity, Shannon indices could be calculated from the genomic profiles of bacterial communities. Though initially designed for the description of the community structure of multicellular organisms (Simpson, 1949; Shannon and Weaver, 1963), diversity indices can also be used to describe microbial communities (for a review, see Hill *et al.,* 2003). The Shannon index is one of the most commonly used diversity index, yet it is not free of bias, e.g. by giving more weight to rare than to common species. Another biais, which is not linked to the index itself but to the profiling method, is the diversity and multiplicity of bacterial operons, which can give rise to an artificially high diversity. Despite these draw-backs, the Shannon index is suitable for the comparison of bacterial community profiles, and has often been used for such purposes (Simpson et al., 1999; Ampe and Miambi, 2000; Kocherginskaya *et al.,* 2001; Marschner *et al.,* 2001; McCaig *et al.,* 2001; Ogino et al., 2001).

II.1.3.3. BACTERIAL FUNCTIONS INVOLVED IN PLANT-BACTERIA INTERACTIONS

Considering that 80-90 % of the processes in soil are reactions mediated by microbes (Nannipieri *et al.,* 2003), microorganisms could be considered as key players in soil functioning (Gobat *et al.,* 2004). The impacts of microorganisms activities are multiple in the rhizosphere. Many of them are implicated in geochemical cycles (figure I.3) and therefore in nutrient availability. Among these activities, many allow the transformation and release of nutrients (e.g. Nitrogen fixation, Nitrification, inorganic phosphate solubilisation and organic phosphate mineralisation, organic sulfate mineralisation, siderophores production, protease production…), and some others lead to elements temporary sequestration (e.g. P precipitation, carbonatogenese…).

Bacteria are implicated in plant nutrition, directly (plant growth promoting rhizobacteria) or indirectly (mycorrhizal helper bacteria) (Andrade *et al.,* 2004; Hampp and Maier, 2004; Frey-Klett *et al.*, 2007). Indeed, mycorrhizal fungi, as well as bacteria, in addition to filling their own needs, can improve plant growth by the supply of mineral nutrients. Most plants rely on mycorrhizal fungi for their mineral nutrient acquisition, particularly for P uptake. The mycorrhizal symbiosis changes thus the nutritional and physicochemical conditions of the rhizosphere with a direct nutritional flux from soil to root, and has a large impact on the functional groups of microorganisms (Andrade, 2004; Roesti *et al.*, 2006).

Some rhizobacteria have a deleterious effect on plant growth (DRB) but plant damages are nevertheless mainly due to pathogenic fungi (Brimecombe *et al.,* 2000).

The beneficial root colonising rhizosphere bacteria, called plant growth promoting rhizobacteria (PGPR), are defined by three intrinsic characteristics :

(i) ability to undergo root colonisation

(ii) survival and multiplication in the rhizosphere, in competition with other rhizobacteria

(iii) ability to promote plant growth (Mahaffee and Kloepper, 1994; Brimecombe *et al.,* 2000)

The biochemical mechanisms of bacterial activities implicating a positive effect on plant growth have mostly been well studied (Brimecombe *et al.,* 2000), but the knowledges about the ecology and physiology of the performing microorganisms are still scrappy. The PGPR are known to carry out many important processes, such as those involved in the biological control of plant pathogens, nutrient cycling and seedling establishment (Oberhansli *et al.,* 1991; Lugtenberg *et al.,* 1991; Glick, 1995). Many bacterial taxa may include PGPR strains, however,

Pseudomonas and *Bacillus* are the most commonly described genera possessing PGPR ability (Barea *et al.,* 2004; Lemanceau, 1992).
When considering biofertilisation efficience, the use of a broad microbial community, constituted of many populations able to perform a given function but which have different ecological requirements, may have an enhanced effect on plant growth compared to single species inoculums by improving the survival probabilities of the functionality in soil.

In this study, emphasis will be put on bacterial abilities develpped in the following sections, which present the advantages to be wildely distributed among rhizosphere bacterial communities, to be recognised as PGP traits, and to be useful for further development of biofertilizers. Additionnaly, a broad bacterial diversity was investigated in order to improve the knowleges about the diversity and ecology of root-associated functional communities.

II.1.3.3.1. CELL-CELL COMMUNICATION : QUORUM SENSING

As the interface between plant, soil, fauna and microflora, rhizosphere environment is a complexe interaction system. The discovery that bacteria are able to communicate with each other, and with plants, changed the general perception of organisms as single, simple units inhabiting the soil.

As communication methods, bacteria use signalling molecules which are released into the environment. The quantity of these signal molecules in the environment is correlated to bacterial density. As well as releasing the signalling molecules, bacteria are also able to measure the number (concentration) of the molecules within a population. This phenomenon is currently called “Quorum sensing” (QS, see Waters and Bassler, 2005; Fuqua and Greenberg, 2002; Miller and Bassler, 2001). When concentration is high enought, signal molecules are detected by specific ligands, and the resulting complexe activate the transcription of target genes.

Various classes of signalling molecules are used, implicating that a population cannot necessarily talk to all other bacteria. In some cases a single bacterial species can have more than one QS system and therefore use more than one signal molecule. Today, several quorum sensing systems are intensively studied in various organisms such as marine bacteria and several pathogenic bacteria. Eukaryotic interferences with bacterial quorum sensing by production of NAHL-like compounds has been demonstrated with the seaweed *Delisea pulchra*

(Miller and Bassler, 2001), some quorum sensing molecules are also thought to be implicated in communication with plant (Williams *et al.,* 2007).
Quorum sensing enables bacteria to coordinate their behaviour, an important advantage in changing environments because of the need to respond quikly in order to survive. These responses include adaptation to availability of nutrients, defence against other microorganisms which may compete for the same nutrients and the avoidance of toxic compounds potentially dangerous for the bacteria. It is also very important for pathogenic bacteria during infection of a host.

Many bacterial activities implicated in plant-bacteria interactions, such as production of extracellular proteases (Swift *et al.,* 1997; Lynch *et al.,* 1999), siderophores (Lewenza *et al.,* 1999), antibiotics (McClean *et al.,* 1997; Bainton *et al.,* 1992), exopolysaccharides (Beck von Bodman and Farrand, 1995), swarming activities (Eberl *et al.,* 1996) and activities related to pathogene infection (Dunphy *et al.,* 1997), have been shown to be regulated by this density-dependant system.

Gram positive bacteria have been shown to communicate using a number of different QS signals. Many employ different post-translationally modified peptides. These systems are involved in the regulation of such diverse processes as virulence in *Staphylococcus aureus*, competence for DNA-uptake in *Bacillus subtilis* and *Streptococcus pneumoniae*, sporulation in *B. subtilis*, conjugal plasmid transfer in *Enterococcus faecalis*, and bacteriocin production in lactic acid bacteria (Kleerebezem *et al.,* 1997, Lazazzera and Grossman, 1998; Kuipers *et al.,* 1998). A second signal molecule employed for QS in Gram positives is the butyrolactone. This is used by several Streptomyces species to control production of antibiotics and antibiotic resistance (Bibb, 1996).

In Gram negative bacteria, QS communication is mostly mediated by N-acyl- homoserine lactones (NAHLs), low molecular weight signal molecules also implicated in chemoattraction, and differing mainly in the lenght and substitution of their carbon chain. Their synthesis is catasysed by an AHL synthase, enzyme which can direct the synthetis of several different AHL. The main known system synthase/receptor is the LuxI / LuxR found in *Vibrio fischerii* and in many other bacteria. NAHL synthesis was shown to be based on two precursors (Watson *et al.,* 2002; Hoang *et al.,* 2002), (i) Acyl-Acyl Carrier Protein (Acyl-ACP), coming from the biosynthesis pathway of fatty acids, (ii) S-adenosylmethionin (SAM), synthetised from methionin and ATP.

II.1.3.3.2. PLANT NUTRITION : THE NUTRIENT CYCLES

The return of nitrogen to atmospheric N_2 result of two types of microbial activities: denitrification and anammox. If such a return is a loss of available nitrogen for plant and soil microflora, at the global scale, it is the way to maintain the atmospheric nitrogen pool while avoiding the disastrous consequences of medium- and long-term accumulation of nitrate in the hydrous chain.

The dissimilatory reduction of nitrate to nitrite (both forms available for plant) can be followed by a reduction of nitrite to either ammonium during nitrate ammonification or to gaseous nitrogen compounds during the anaerobic respiratory denitrification process (figure I.3, Gobat *et al.,* 2004).

Denitrification is currently described as the main process of nitrate dissimilation (Gobat *et al.,* 2004), especially in soils (Gamble *et al.,* 1977). Ammonia oxydizing bacteria (anamox), in revanch, are poorly studied in this environment. Recent studies on marine sediments (Rysgaard *et al.*, 2004) and upwelling systems highlight the importance of Anamox bacteria in the returning of nitrogen to atmospheric pool (Francis *et al.*, 2007), suggesting that we also could have underestimated the importance of anamox bacteria in soils.

In the case of denitrification, the loss of nitrogen due to these processes generates a competition for nitrate between plants and bacteria in nitrogen limiting conditions (Fromin *et al.,* 2005). Nitrate and nitrite reductases producing strains are generally inhibited at root proximity, but their frequency is sometimes also shown to be higher in the rhizosphere than in bulk soil (Johansen and Binnerup, 2002; Roussel-Delif *et al,* 2005).

Another bacterial ability implicated in N cycling is the production of proteases, extracellular peptidases implicated in protein digestion into oligopeptides and amino acids (figure I.3, Gobat *et al.,* 2004). Proteases have also been shown to be implicated in plant resistance against pathogenic fungi (Szekeres *et al.,* 2004). Even if extracellular proteases production seems not to be necessary for root colonisation (O'sullivan *et al.,* 1991), the proportion of protease producing strains was reported to be higher in the rhizosphere than in bulk soil (Johansen and Binnerup, 2002). Roesti (2005) reported that the ability to secrete proteolytic enzymes is an important trait related to mycorrhizal helper bacteria. Extracellular protease activity seems also to be favoured by root exudation and organic matter amendment leading to an incread nitrogen mineralisation (Badalucco *et al.,* 1996; Marcote *et al.,* 2001).

Apart from the well studied importance of nitrogen in biological functions, some other nutrients are also significant for growth (figure I.3), and are still few investigated in the field of microbial ecology. Phosphorus and sulfur (see chap. III) are essential elements for biological

functions and are currently known to be broadly present in soils but only poorly available (Arnou, 1953, Schachtman *et al.,* 1998; Kertesz and Mirleau, 2004).

In agricultural soils more than 95% of the sulfur is present as sulfate esters or as carbon-bonded sulfur (sulfonates or amino acid sulfur), rather than inorganic sulfate (Jokic *et al.,* 2003; Prietzel *et al.,* 2003). Organic sulfur, not directly available for plant nutrition, is present partly included in microbial biomass and partly in the soil organic matter (Kertesz and Mirleau, 2004). Microbial death and protozoal predation release bacterial cellular contents (C-bounded sulfur) into the soil environment. Mineralisation of organic sulfates, catalysed by the mean of sulfatases, is microbially mediated (Vermeij *et al.,* 1999; Kahnert *et al.,* 2000), but it is not yet known whether specific members of the soil microbial community play a dominant role in catalysing this process. As constituant of essential biological compounds, such as amino acids and metallo-proteins, sulfur is an important element in biological functions.

Phosphorus is also an essential element because of its role in the near totality of biological processes. Organisms have developped several mecanisms to increase P availability at different levels. Inorganic P forms are known to be highly reactive and to be frequently found in soils as precipitated or complexed forms. Solubilisation of inorganic P is performed by secretion of organic acids or protons. Bacteria able to solubilise inorganic P were shown to be favoured at root proximity and to have plant growth promoting properties (Rodriguez and Fraga, 1999; Roesti, 2005). However, the main forms of P found in soil are organic compounds, in particular phytates (penta- and hexa-phosphate esters of inositol). These organic forms have further to be mineralised, by the mean of phosphatases, to allow availability for plants. Proportion of strains producing alkaline and acid phosphatases have also been shown to be incrased in the rhizosphere compared to bulk soil (Johansen and Binnerup, 2002). Phytates mineralisation is catalysed by the specific phytases. As well as inorganic phosphate solubilisation, production of phytases may also be an important advantage for plant and microorganisms nutrition.

Siderophores from bacterial and vegetal orignin are organic ligands that complex iron (figure I.3, Neilands, 1995). This complexation leads to the unavailability of siderophores-bounded iron to native microflora (Kloepper *et al.,* 1980), providing a competitive advantage to siderophore-producing organisms in iron-depleted environment. Some bacterial populations also produced siderophores allowing iron availability to plants (Lugtenberg and Dekkers, 1999). The PGP property of siderophore production is currently demonstrated and known to be well distributed among *Pseudomonas* spp. (Kloepper *et al.,* 1980). A part from their main implication in iron nutrition, siderophores are also suspected to be implicated in P nutrition by the release of inorganic and organic P forms from soil complexes in acidic soils (see chap. III).

II.1.3.3.3. PLANT DEVELOPMENT AND RESISTANCE AGAINST PATHOGENS

Many bacterial productions, such as exopolysaccharides, hormones, and antibiotics have an influence on plant development and resistance against pathogens.

Exopolysaccharides are polymers principally implicated in cell adhesion, protection, and in soil aggregate formation. Bollag and Huang (1998) showed that some bacteria produce polysaccharides interacting with clay particles and that these clay-polysaccharide complexes can persist, even after the death of the microbes. Production of exopolysaccharide is also generally important in biofilm formation, and likewise can effect the interaction of bacteria with roots (Bianciotto *et al.,* 2001a; Bianciotto *et al.,* 2001b).
As constituant of the root-surrounding mucilage, exopolysaccharides have an implication in plant physical resistance by protecting roots. For plant growth promoting as well as pathogen bacterial population, exopolysaccharides are also implicated in bacteria recognition by plant (Squartini, 2000 ; Varma *et al.,* 2004).

One of the most known hormones influencing plant development is indol-acetic acid. Auxin (Indole-3-acetic acid, IAA) is implicated in root ramification and elongation (Glick, 1995; Persello-Cartieaux *et al.,* 2003), and production of IAA by bacteria is a well known plant growth promoting traits. Tryptophan is the main precursor of IAA synthesis by microorganisms and enhances its production. Both beneficial and pathogenic microorganisms could be able to produce IAA. Plant growth-promoting bacteria produce IAA via the indolepyruvic acid pathway, in contrast to plant pathogens, wich seem to preferentially synthetise IAA via the indoleacetamide pathway (Patten and Glick, 2002). The ability for auxin production has been found among various phototrophic and heterotrophic bacteria, not only pathogenic or symbiotic species but also free-living microorganisms not associated with plants (Tsavkelova *et al.,* 2005).

Ethylene is a simple unsaturated carbon gas, identified as a common constituant of the atmosphere of both aerobic and anaerobic soils, and is well known for its strong effect on plant growth (Smith, 1976; Arshad, 1992; Glick, 1995). It is synthesized by most bacteria and fungi, but is only oxidized by a limited range of microorganisms. This hormone is implicated in: breaking of dormancy; regulation of swelling and elongation; hypertrophy; promotion of adventitious roots; modification of root growth; promotion of root hairs; epinasty; hook closure; inhibition of leaf expansion; control of flower induction; exudation; ripening; senescence, and is also involved in some host-pathogen interactions (Primrose, 1979; Bertin *et al.,* 2003).

The producton of secundary metabolites with antimicrobial properties has long been recognized as an important factor for disease supression (see review Haas and Defago, 2005). However, volatile compounds such as ammonia, ethylene and hydrogen cyanide (HCN) produced by a number of bacteria are also believed to play a role in biocontrol. HCN is a secondary metabolite which can inhibit the growth of rhizosphere inhabiting bacteria and fungi by repression of the cytochrome c oxydase (respiratory chain) and several other metalloenzymes (Castric, 1977; Blumer and Haas, 2000), and which sometime interfere with root growth (Bakker and Schippers, 1987). In the rhizosphere, about 32% of isolated strains were found to be cyanogenic (Kremer and Souissi, 2001), however bacterial cyanogenesis seems to be essentially restricted to the proteobacteria (Blumer and Haas, 2000), and it is already known that iron has a stimulatory effect on cyanogenesis (Blumer and Haas, 2000).

II.2. EXPERIMENTAL DESIGN

II.2.1. BACTERIAL COMMUNITIES STRUCTURE DETERMINATION AND SOIL CHARACTERISATION

II.2.1.1. PLANT MATERIAL AND SAMPLING

Model plant

As an enhancement of root influence on the surrounding soil is linked to plants that maintain from year to year at the same location, focus was here put on perennial meadow. *Lolium perenne* was choosen as model plant. Growing fast, it also presents both advantages to be frequently used for forage and to account for a large part of terrestrial natural ecosystems, and has been used as model plant in many other studies, including previous researches conducted in our laboratory (see chap. I.6). Since plant type and species are commonly known to influence bacterial community structure (Westover *et al.*, 1997; Smalla *et al.*, 2001; Marschner *et al.*, 2005; Patra *et al.*, 2006; Costa *et al.*, 2006), cultivar impact is currently less studied (Dalmastri *et al.*, 1999; Varma *et al.*, 2004). We selected different *L. perenne* cultivars to investigate the impact of minor plant variability on bacterial communities and to evaluate the response sensitivity of bacterial communities. Four cultivars of *L. perenne*, two tetraploïds (Anaconda and Bastion) and two diploïds (Cavia and Lipresso), with different ecolocical traits were selected for the experiments (table II.1).

Sterilization of seeds

Sterilisation of seeds was performed for the four cultivars using peracetic acid 0.5% $^{v}/_{v}$ (Paterson and Sim, 1999) and pre-germination (first root length < 0.5 cm) of sterilized seeds on nutrient agar allowed to check for their sterility.
The soil used in this experiment was a non-steril agricultural soil preadapted to Bastion cultivar. Sterilisation of the seeds was performed in order to limit the introduction, in our system, of preadapted bacterial populations coming from seeds of the differents cultivars.

Table II.1. Description of the cultivars (Mosimann *et al.*, 2004). Traits* are evaluated using a qualitative scale from 1 (best) to 9 (worse).

Cultivar	ANACONDA	BASTION	CAVIA	LIPRESSO
Ploïdy	tetraploïd	tetraploïd	diploïd	diploïd
Ear development period (10 flower / m^2)	6-10th May	11-15th May	6-10th May	6-10th May
Yield*	4.5	4	5.2	4.4
Seedling growth speed*	3.9	?	4.6	4
Concurrence force*	4.1	5	4.5	3.2
Persistance*	5.5	5	5.5	5
Resistance against snow mold*	5.6	5	6.3	5.4
Resistance against powdery mildew*	2.3	3	2.9	3.5
Resistance against rust*	2.9	?	3.4	4.9
Resistance against leaf pathogens*	4	5	4	?
Resistance to altitude*	6.2	5	5.7	5.4
Digestibility*	5	5	5	?

Soil

A fertile Eutric Cambisol (FAO classification, table II.2.), which harboured a monoculture of *L. perenne* cultivar Bastion since 11 years (Nösberger *et al.,* 2006), was collected (4-30 cm) in the field site of the Institute of Plant Science (ETHZ) in Eschikon (8°42'E, 47°27'N, 550 m a.s.l.), and used as bacterial reservoir and growth substrate for all the cultivars used in this experiment. 700g of non-sterile sieved (∅ 2 mm.) soil were used per pot.

Eschikon, Lindau, CH
Field site of the Institute of Plant Science (ETHZ)
Source : Google Earth

Table II.2. Soil description

Soil		Lüscher *et al.,* 1998	This study, 2004	
Structure (%)	Clay	28		-
	Silt	32		-
	Sand	36		-
pH [H_2O]		6.5 – 7.6	6.63	(± 0.04)
pH [KCl]		-	5.66	(± 0.01)
Organic matter ($mg.g^{-1}$)		2.8 – 5.1	73	(± 1.3)
TOC ($mg.g^{-1}$)		-	25.5	(± 0.99)
MINC ($mg.g^{-1}$)		-	2.8	(± 0.17)
NH_4^+ ($mg.g^{-1}$)			-0.02	(± 0.004)
NO_3^- ($mg.g^{-1}$)		-	0.07	(± 0.03)
Ntotal ($mg.g^{-1}$)		-	3.35	(± 0.76)
Pav ($mg.g^{-1}$)		-	0.10	(± 0.003)
Ptotal ($mg.g^{-1}$)		-	0.54	(± 0.08)

Plant growth conditions

Sterilised seeds of four cultivars of *L. perenne* were grown in pots under greenhouse in semi-controlled conditions with 12 hour of photoperiod and without any fertilisation. The number of plants per pot was 10 for pots corresponding to the first development stage, 6 for pots corresponding to the second stage and 3 for other stages.

Watering was performed using Blumat© system (Water dispenser with suction tubing with a nearby container with water) for pot sizes of above 15 cm (length of ceramic cone- about 8 cm). As the soil dries, the ceramic cone delivers water gradually into the surrounding earth, thereby creating a vacuum within the cone, which draws water from the nearby container of water.

Cultures
(A) Blumat system
(B) Example of pot
(C) Global view

Sampling of roots and soil

Samples were collected for the four cultivars (Anaconda, A; Bastion, B; Cavia, C; and Lipresso, L) at four development stages (hypocotil development, 1; 4 leaves, 2; Ear development, 3; ear maturity, 4), and in parallel for control soil incubated without plants.

For each development stage three replicates pot experiments were performed. Two rhizosphere fractions (washed roots, R; rhizospheric soil, RS) and one bulk soil control fraction (soil without plant, S) were recovered (figure II.1). For planted pots corresponding to the first development stage, plants of the three replicates were pooled for each cultivar because of the low quantity of biomass.

Plant age and shoot-root ratio were determined for each development stage (table II.3).

Sample recovering for bacterial communities structure determination

2 series of 92 samples, constituted of 0.5 g of root or soil, and 1 ml of rhizosphere soil solution (figure II.1) were collected, as described in Jossi et al (2006, see chap. IV), for determination of the genomic community structure by DGGE analyses of 16S rDNA (total communities) and cDNA of 16S rRNA (metabolically active communities).

Figure II.1. Sample recovering (performed for each replicate pot).

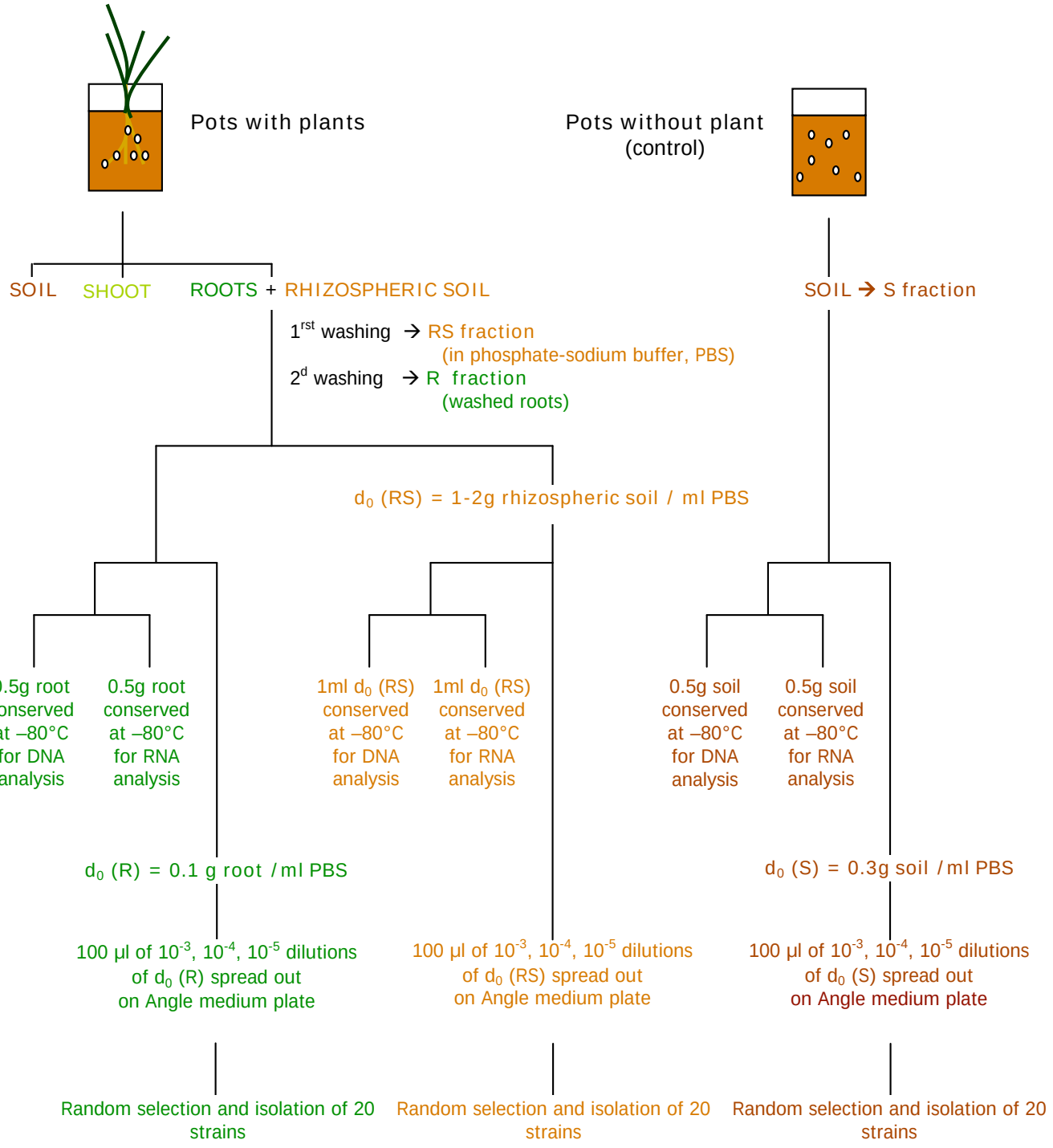


Table II.3. Shoot-root (S/R) ratio and plant age for each cultivar at the moment of the sampling.

Plant development stage	cultivar	age of plants (days ±SD)	S / R
Hypocotil development (~1 - 2 cm)	Anaconda	5*	0.1*
	Bastion	9*	0.02*
	Cavia	5*	0.03*
	Lipresso	4*	0.02*
Four leaves	Anaconda	16 (0)	1.4 (0.5)
	Bastion	25 (0)	1.3 (0.7)
	Cavia	16 (0)	1.1 (0.41)
	Lipresso	17 (0)	1.3 (0.4)
Ear development (before flowering)	Anaconda	117 (26)	2.5 (0.5)
	Bastion	107 (31)	4.2 (2.8)
	Cavia	83 (3)	1.4 (0.7)
	Lipresso	82 (3)	1.5 (0.9)
Ear maturity (after flowering)	Anaconda	221 (28)	1.4 (0.1)
	Bastion	237 (0)	2.8 (0.9)
	Cavia	175 (12)	3 (2.1)
	Lipresso	168 (0)	4.8 (2.8)

* For this stage the three replicated were mixed because of the low biomass production

Bacterial plate counts and strains isolation

Bacteria plate counts were performed. For each root, rhizosphere and bulk soil sample (92 samples) suspension-dilutions have been spread out on Angle medium (Angle *et al.,* 1991) plates using glass beads (3 replicate plates per dilution) and number of CFU per gram of root, rhizospheric soil and control soil have been determined after 72 hours of incubation at room temperature. This incubation period allows the stabilisation of CFU number, which significantly increased before this time.

For each root, rhizosphere and bulk soil sample, 20 strains were randomly selected (using dilution corresponding to plates which present 10 to 100 CFU) and isolated on Angle medium for determination of the functionnal structure of cultivable heterotrophe aerobic bacterial communities. Colony morphology of each strain has been described using the following criteria: pigmentation, elevation, perimeter and texture. Purity of each strains was controled by streaking on Angle medium plate (1g glucose/l), and microscopic control was performed when culture were suspected to be contaminated. All the isolates were maintained on Angle medium (at 4°C) and conserved in 40% glycerol solution (at –80°C) for further analyses.

Sample recovering for soil analyses

Soil samples were collected before incubation, after 7 month of cultivation with each plant cultivar, and after 7 month of incubation without plant (control soil). Soils were then air dried for further analyses.

II.2.1.2. MOLECULAR FINGERPRINTING OF BACTERIAL COMMUNITIES

Community profiles obtention

Community profiles were obtained using a (RT-)PCR-DGGE approach. Extraction of total genomic DNA and RNA, reverse transcriptions of genomic RNA, PCR amplification of the V3 region of 16S rDNA, as well as denaturing gradient gel electrophoresis (DGGE) and pictures normalisation were performed as described in Jossi *et al*. (2006, see chap. IV), excepted that PCR amplification of the V3 region of 16S rDNA has been directly performed on nucleic acids extracts without the total 16S rDNA gene amplification intermediary step. For further analyses, DGGE profiles were converted into a numerical matrix which contains the environmental variables describing each sample, and gives the relative intensity and position of each bands of the corresponding DGGE profile.

II.2.1.3. FUNCTIONAL FINGERPRINTING OF BACTERIAL COMMUNITIES

The isolates were described (colony morphology) and checked for some putative functions implicated in plant-soil-bacteria interactions (plant growth and resistance, nutrient availability, cell-cell communication). Sixteen bacterial traits (table II.4) corresponding to enzymatic (cellular and extracellular), and secretion activities were tested.

Morphology

(0) Colony morphology (pigmentation, form, elevation, border, surface and texture) was determined on Angle medium (Angle *et al.,* 1991) plates after 72h of incubation in the dark at room temperature.

Example of morphological description: isolate 47

Pigmentation: yellow
Form: regular
Elevation: slightly convex
Border: diffuse
Surface and texture: smooth-shinning

Enzymatic activity : Cellular

(1) Aminopeptidase acivity was measured in order to determine the Gram cell-wall type. L-alanine aminopeptidase is an enzyme localised in the bacterial cell wall that cleaves L-alanine from various peptides. It is found almost only in Gram-negative microorganisms. Studied Gram-positive and Gram-variable microorganisms show no, or very weak activity. Test strips (Fluka, Sigma-Aldrich Co.) with reaction zone containing 0.5 mmole L-alanine-4-nitroanilide and buffering agents allows to detect L-alanine aminopeptidase activity. Strains presenting no aminopeptidase activity were considered as Gram positive.

(2) Oxidase activity has been determined as described in Gerhardt *et al.* (1981) with aqueous solution of 1% N, N, N', N'- tétraméthyl- 1,4- phénylènediamine di-hydrochlorure (TMPD). This reagent is reduced in the presence of cytochrome c oxidase and produce a purple coloration.

(3) – (5) reduction of nitrate to nitrite, to gaseous compounds (N_2,N_2O) or to ammonium (NH_4^+). Nitrate nutrient broth was used for growth of the isolates, detection of nitrate and nitrite was done using Griess reagent, and inverted Durham tube was used to detect gaseous nitrogen (Roussel-Delif *et al.,* 2005; Tarnawski *et al.*, 2006). Denitrifiant and nitrammonifiant isolates were also taken into account as nitrate-reducing strains.

Enzymatic activity : Extracellular

(6) – (7) production of proteases on SMB agar plates with 15 $g.l^{-1}$ of skim-milk powder for detection of casein hydrolysis (Gerhardt *et al.,* 1981), and in tubes containing 4ml of mineral Schlegel medium with 30 $g.l^{-1}$ of gelatin for detection of gelatin hydrolysis (Gerhardt *et al.,* 1981). In the case of casein hydolysis, strains strains presenting a proteases activity harbour a clearing zone around their colonies. Gelatin hydrolysis was detected by the liquefaction of the medium.

(8) production of aryl-sulfatases in inorganic sulfur limiting conditions on modified Angle medium plates with 40 $mg.l^{-1}$ X-sulfate as only sulfate source, and in inorganic sulfur non-limiting conditions on modified Angle medium plates with 40 $mg.l^{-1}$ X-sulfate and 710 $mg.l^{-1}$ Na_2SO_4 (Kertesz *et al.,* 1993; Kertesz and Mirleau, 2004). The degradation of X-sulfate by the mean of aryl-sulfatases produce a blue coloration around colonies (for details see chap. III)

(9) production of phytases on modified Angle medium with Na-IHP 10 $g.l^{-1}$ (Richardson and Hadobas, 1997) as only phosphate source. Lecture was done as described by Bae *et al.* (1999), using a cobalt chlorur solution and coloration with a molibdo-vanadate solution (for details see chap. III)

Secretion activity

(10) solubilisation of inorganic phosphate on Phosphate Solubilising Bacteria medium (Kim *et al.,* 1997) with 5 $g.l^{-1}$ of $Ca_3(PO_4)_2$ (for details see CHAP. III). Ca-phosphate (Ca-P) was used because it is the most significant P form in calcareous soils.

(11) production of siderophores in iron-limiting conditions on CAS medium (Schwyn and Neilands, 1987) as describe by Tarnawski et al. (2006) (for details see CHAP. III).

(12) production of indole-3-acetic acid (IAA) on Angle liquid medium supplemented with L-tryptophan as describe in Tarnawski *et al.* (2006).

(13) production of hydrogen cyanide (HCN) on synthetic medium containing glutamate, glycine and methionine as precursors of hydrogen cyanide (Castric and Castric, 1983) as described in Tarnawski *et al.* (2006).

(14) production of exopolysaccharides (glucanes) on Angle medium (Angle *et al.,* 1991) supplemented with 1 $g.l^{-1}$ of glucose.

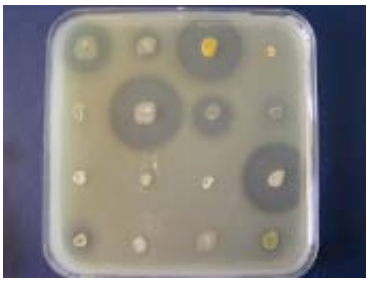
(15) – (16) production of N-acyl-homoserines lactones (NAHLs). Test was performed as describe in Delorme (2001). LB agar plates with 5$g.l^{-1}$ NaCl (Miller, 1972) was used with the biosensor *Chromobacterium violaceum* CV026 (McClean *et al.,* 1997) for detection of oxo- or C_3 non-substituted short carbon chain NAHLs (C_4 to C_8). AB agar plates (Chilton *et al.,* 1974) supplemented with 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal 40 $mg.ml^{-1}$) was used with the biosensor *Agrobacterium tumefasciens* NTLR4 (Shaw *et al.,* 1997) for detection of C_3 substituted or non-substituted long carbon chain NAHLs (C_6 to C_{12}).

Preculture of each strain was performed on Angle medium (Angle *et al.,* 1991), or on Angle medium modified without inorganic phosphorus and sulfur sources respectively for phytases and sulfatases production. Incubations were performed in the dark at room temperature for precultures and cultures. Reference strains were used as positive and negative control for each test (table II.4).

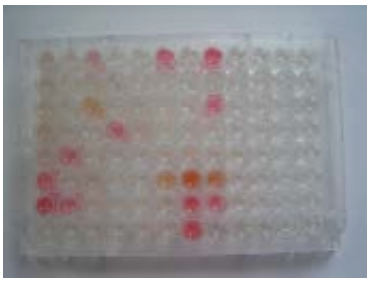
Lectures were done after a 72h period of incubation for Ca-phosphate solubilisation, IAA, HCN, siderophores, NAHLs, and proteases production, and after one week of incubation for nitate dissimilation, phytase and sulfatases production.

In vitro tests of isolate abilities

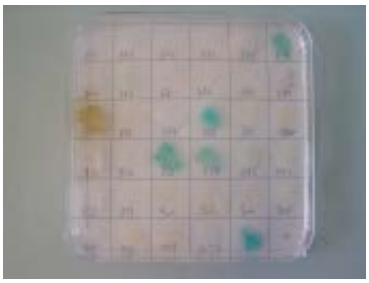
Proteases production (Casein hydrolysis)



Auxin production

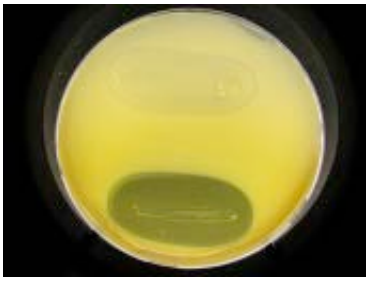


Aryl-sulfatases production

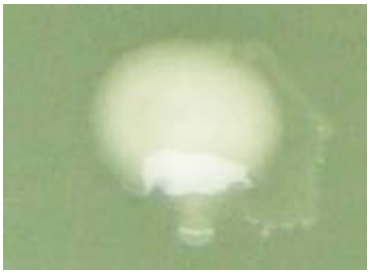


Hydrogen cyanide production

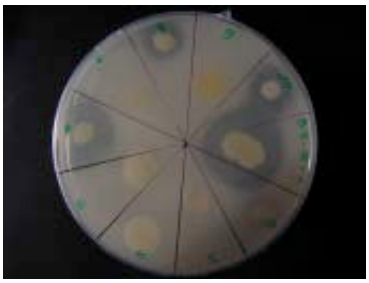
Phytases production



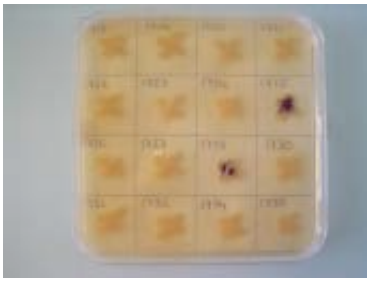
Exopolysaccharides production



Ca-phosphate solubilisation



Production of NAHL C_4-C_8 Biosensor : *C. violaceum*



Siderophores production

Production of NAHL C_6-C_{12} Biosensor : *A. tumefasciens*

Table II.4. Implications of the enzymatic (A) and secretion (B) activities tested, and reference strains used as positive and negative control.

NEU : Bacterial Strains Collection, University of Neuchâtel

CFBP : Collection Française de Bactéries Phytopathogènes

DSM : Deutsche Sammlung von Microorganismen

(A) Enzymatic activities Implications	Positive control strain	Negative control strain
(1) Aminopeptidase		
Gram type	*Bacillus megaterium* NEU 16	*Escherichia coli* NEU 1006
(2) Oxidase		
Cytochrome c detection	*Pseudomonas aeruginosa* NEU 1023	*Escherichia coli* NEU 1006
(3) Nitrate reduction		
N cycle	*Escherichia coli* NEU 1006	No inoculum
(4) Denitrification		
N cycle	*Pseudomonas aeruginosa* NEU 1023	No inoculum
(5) Nitrammonification		
N cycle	No inoculum	No inoculum
(6) Casein hydrolysis		
N cycle	*Bacillus megaterium* NEU 16	*Bacillus sphaericus* NEU 1003
(7) Gelatin hydrolysis		
N cycle	*Bacillus megaterium* NEU 16	*Bacillus sphaericus* NEU 1003
(8) Aryl-sulfatases		
S cycle	Rhizosphere soil isolate	*Enterobacter aerogenes* NEU 1036
(9) Phytases		
P cycle	*Bacillus amyloliquefaciens* DSM 7	*Bacillus subtilis* NEU 1

(B) Secretion activities Implications	Positive control strain	Negative control strain
(10) Solubilisation of Ca-phosphate		
P cycle	*Pseudomonas lini* CFBP 5738	Rhizosphere soil isolate
(11) Siderophores		
Fe cycle	*Pseudomonas fluorescens* CFBP 2127	*Escherichia coli* NEU 1006
(12) Auxin production		
Root elongation and ramification	*Pseudomonas fluorescens* CFBP 2123	*Pseudomonas fluorescens* NEU 1201
(13) HCN production		
Resistance against pathogens	*Pseudomonas fluorescens* NEU 1201	*Enterobacter cloacae* NEU 1027
(14) Exopolysaccharides production		
Cell adhesion and protection	*Rhizobium trifolii* NEU 1136	*Bacillus sphaericus* NEU 1003
(15) NAHLs C_4 to C_8 production Cell-cell communication		
Functional genes regulation	*Pseudomonas syringae* CFBP 2067	*Pseudomonas fluorescens* NEU 1201
(16) NAHLs C_6 to C_{12} production Cell-cell communication		
Functional genes regulation	*Pseudomonas syringae* CFBP 2067	*Pseudomonas fluorescens* NEU 1201

II.2.1.4. SOIL ANALYSES

Analyses were performed on soil before and after growth of each cultivar and on the control soil incubated without plant, in collaboration with the Soil and Vegetation Laboratory, and the Geochemical and Environmental Analyses laboratories, University of Neuchâtel (NE, Switzerland).

Available nitrogen (NO_3^-, NH_4^+) was extracted from 15g of soil with 100 ml of KCl 15%. The soil suspension was mixed one hour in a rotary shaker and centrifuged 10 min at 3000 rpm. The supernatant was taken and the pellet was resuspended with 50ml of KCl15% and mixed a half hour in a rotary shaker and centrifuged 10 min at 3000rpm. The two supernatants were filtrated on paper filter (17 $^1/_2$ Schleicher and Schuell AG, Riehen, Switzerland) and pooled in volumetric flask 250ml. Filtrate solutions were adjusted to 250 ml with deionised water. Distillation, and trapping of NH_4^+ and NO_3^- were done according to Cline and Kaplan (1975), and titration was performed using Tashiro indicator and Büchi system (Laboratoriums-Technik AG, Flawil, Switzerland).

Available phosphorus (Pav) was revealed according to Olsen *et al.,* (1954) by shaking 2.5 g of soil with 60 ml of sodium bicarbonate $NaHCO_3$ (0.5 N, pH 8.5) for 30 min. After filtration (512$^1/_2$, Schleicher and Schuell AG, Riehen, Switzerland), P was determined colorimetricaly at 880 nm using the molybdate procedure (Murphy and Riley, 1962).

Total nitrogen (Ntot) and phosphorus (Ptot) were measured from 2.5 g of soil using the mineralisation process described by Kjeldahl (1883). For Ptot determination, mineralisation was followed by the colorimetric detection of P by the ascorbic acid-molybdate blue method (Murphy and Riley 1962). For Ntot determination, distillation, trapping and titration of N were also performed as described above for available nitrogen.

Organic matter (OM) content was assessed on 10 g of soil by the loss on ignition (LOI) method at 450°C (Allen, 1974; Anderson and Ingram, 1993).

Soils were on the other hand air-dried and finely crushed with agate system, and 50 mg of each sample were analysed by Rock-Eval 6 (Re6) pyrolysis (Behar *et al.,* 2001) in order to determine organic (TOC) and mineral (MINC) carbon percentages and to characterise organic matter.

$pH_{[H2O]}$ (free or active acidity, activity of H_3O^+ ions of the soil solution) and $pH_{[KCl]}$ (total exchange acidity, activity of H_3O^+ ions of the soil solution and the soil complex) were also determined. Twenty grams of soil were placed in a beaker with 50ml of deionised water or 50ml of KCl 1M for $pH_{[H2O]}$ and $pH_{[KCl]}$ measurement respectively. The soil suspensions were mixed energetically using a glass rod, and pH measurements were done after one hour.

II.2.1.5. STATISTICAL ANALYSES

Molecular and functional fingerprinting of bacterial communities

Data analyses were performed in three steps, in the same way, for both genomic and functional bacterial communities structure.
The matrix giving the relative intensity (p_i , for each profile $\Sigma p_i = 1$) and position of bands from each DGGE profile, and the presence-absence matrix containing results from the sixteen functional traits tested for each isolate, were previously Hellinger transformed (Legendre and Gallagher 2001), since PCA and RDA, the ordination methods which will be used here, are not directly appropriate for the analysis of matrix with high number of zeros (Legendre and Legendre 1998).

To measure the variation between bacterial community profiles from the different cultivars, evolution stages and replicate pots, partial RDA was first calculated. For genomic approach (DGGE community profiles) RDA analysis was performed on four subsets of samples, i) DNA-based root profiles, ii) DNA-based rhizosphere soil profiles, iii) RNA-based root profiles and iv) RNA-based rhizosphere soil profiles. For functional approach (isolates abilities) RDA analysis was performed on two subsets of isolates, i) root profiles, ii) rhizosphere soil profiles. These partial RDA allowed the extraction of the variation in the community profiles explained by sets of explanatory variables and shared by these data sets (see Borcard *et al.,* 1992; Okland and Eilertsen, 1994 for details, and Jossi *et al.,* 2006 for an example with DGGE data). Significiance of the results was assessed using Monte Carlo test (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$) .

Cultivar and evolution stage influence on root, rhizosphere soil, and bulk soil community structures were then further assessed by applying PCA analyses, on the complete dataset, for each genomic and functional approach. Ordination analysis of the community profiles allows knowing which communities present similar or different profiles among the different cultivars and evolution stages. Two plot representation were used, i) plot of the centroïds corresponding to root and rhizosphere communities associated with each cultivar, and to bulk soil communities, independently from the evolution stage, ii) plot of the centroïds corresponding to root, rhizosphere and bulk soil communities at each evolution stage, independently from the plant cultivar. Plot representations were based on axis 1 and 2. For functional approach, vectors indicating the correlations between their corresponding functional traits were also represented on the PCA plots (vector size was reduced 30 times in order to fit to the plot). All analyses were performed with the statistical software R 2.3.1 (R Development Core Team, 2006).

In a third step, the cultivar-induced modifications of bacterial genomic and functional community structures were closer investigated using different methods.

Richness, diversity and evenness were calculated and compared for DGGE profile of each cultivar and fraction. Richness was obtained by calculation of the number of bands (S) presents in each profile. Diversity was assessed using Shannon index ($H' = -\Sigma p_i * LNp_i$), and evenness using richness and Shannon index ($R = H' / H'max = H' / \log_2 S$). Standard deviation was calculated between values of samples from each cultivar and fraction, and Student test was used to assess the significiance of the obtained results.

Frequency of functional groups was investigated based on the in vitro functional characterisation of bacterial isolates from root and rhizosphere soil of each cultivar, and from control soil. Comparison of proportion of positive strains for each ability between root, rhizosphere soil and bulk soil, between *L. perenne* cultivars, and between the evolution stages was performed using a generalised linear model (glm) analysis with a logistic regression model. Results were compared, and significiance was tested using the Tukey Kramer HSD post hoc test. The tests were performed using S-plus 6 Statistical Software (Insightful Corporation, Seattle, Washington).

Soil data

Soil data obtained from $pH_{[H2O]}$, $pH_{[KCl]}$, OM, TOC, MINC, Ptot, Pav, Ntot, NO_3^-, and NH_4^+ analyses of initial soil, control soil incubated without plant, and soil collected after growth of each plant cultivar were checked for normality using Shapiro-Wilk and Kolmogorov-Smirnov tests. Effects of plant cultivar on the characteristics of soil were compared using variance analyses (ANOVA) followed by Tukey Kramer HSD post hoc test. All analyses were performed with the statistical software R 2.3.1 (R Development Core Team, 2006).

II.2.2. ROOT EXUDATES CHARACTERISATION

II.2.2.1. PLANT MATERIALS AND SAMPLING

Model plant

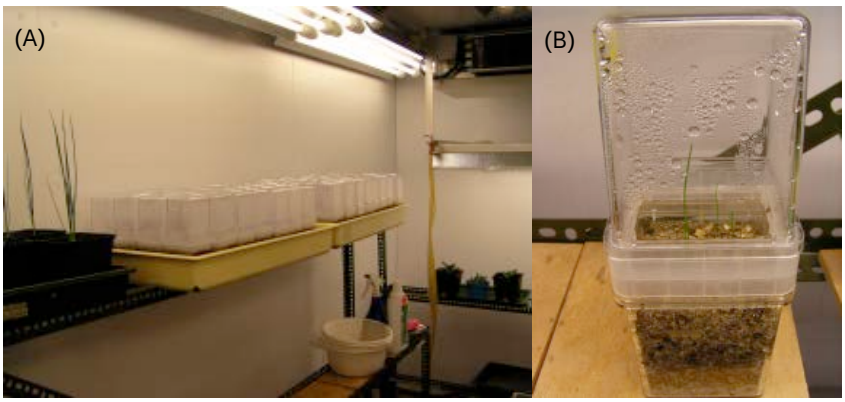
Three cultivars of *L. perenne*, one tetraploïd (A) and two diploïds (C, L), were choosen as model plants (table II.1), the fourth cultivar B is missing because the germination percentage of this seed batch was very low and we did not have enough germinated seeds to perform this experiment).

Sterilization of seeds and plant growth conditions

In order to avoid bacterial consumption, culture and collection of exudates were performed in sterile conditions. Seeds were sterilized by washing with 50 vol% ethanol for 1 min, 70 vol% for 2 min and 5 % NaOCl for 10 min (modified after Hensel *et al.,* 1990, Kreutzer *et al.*, 2006), and pregerminated individually in microplate containing 200µl of nutrient broth.

Five germinated seeds were planted in each sterile microcosms (two Magenta GA-7 Vessel 77mm x 77 mm x 97 mm H with coupler, Sigma-Aldrich, Co.), which contained 100g of vermiculite $(H,Na,Ca_{1/2})_x(Mg_xAl_{2-x})Si_4O_{10}(OH)_2$ and was supplemented with 100 ml of water, 1 ml of basic fertilisation solution A (K_2SO_4 15g, $CuSO_4$ 420 mg, $ZnSO_4$ 1.08 g, $MnSO_4$ 2.1 g, $CoCl_2$ 67 mg, $MgSO_4$ 9 g, Na_2MoO_4 36 mg, nanopure water ad 500ml), 100 µl of basic fertilisation solution B ($CaCl_2$ 15 g, nanopure water ad 100 ml), 200 µl of phosphate fertilisation solution (KH_2PO_4 12 g, KPO_4 * $7H_2O$ 0.228 g, nanopure water ad 100ml), and 200 µl of nitrogen fertilisation solution (NH_4NO_3 8.22 g, nanopure water ad 100 ml). Incubation was performed at 20°C, with 16 hour of photoperiod.

Cultures
(A) Global view
(B) Exemple of microcosm



Sampling design

Two series of 5 replicate microcosms were planted for three of the four cultivars (Anaconda, A; Cavia, C; Lipresso, L). Each replicates contained five plants. Plants of each microcosm were collected after 30 days of growth. Root exudates were recovered on one hand from cut off roots (one cutting performed at root base) and on the other hand from intact plants. They were collected by roots incubation in sterile desionised water (4 ml per ~0.3 g fresh weight of roots) as described in Weisskopf (2005). Exudates were filtered at 0.2µm, freezed at -80°C and concentrated by lyophilisation. Lyophilisates were conserved at -80°C.

II.2.2.2. ORGANIC ACIDS ANALYSES

Organic acids present in root exudates were determined by HPLC analysis. Samples were resuspended after lyophilization in nanopure water in proportion to the root fresh weight (0.4 $\mu l.mg^{-1}$ fresh weight of root), and were filtered through 0.2 µm syringe filters (Semadeni SA, Ostermundigen, Switzerland).

50 µl of the samples were then injected into a cation-exchange column (BENSON polymeric^{TM}, spherical sulfonated styrene-divinylbenzen resin in the H^+ form, with a porosity of 10 µm, a length of 300 mm and an internal diameter of 7.8 mm), used to separate the different organic acids at room temperature with an isocratic flow of H_2SO_4 20 mM at 0.7 $ml.min^{-1}$. Separation was achieved in 45 minutes and absorbance was monitored at 210 nm. All analyses were performed on extracts obtained from cut off root and intact plants.

Calibration curves with standard organic acids were performed for quantification of the most abundant organic acids present in the analyzed samples. Different volumes of a standard solution containing acetic, butyric, citric, formic, fumaric, gallic, gluconic, glutaric, lactic, maleic, malic, malonic, oxalic, propionic, pyruvic, succinic, and isocitric acid were injected in the column (50 µl, 25µl, 10 µl, 5 µl and 2 µl of standard solution containing respectively 4.5 µg, 2.3 µg, 0.9 µg, 0.5 µg and 0.2 µg of each of the eleven organic acids)

II.2.2.3. PHENOLIC COMPOUNDS ANALYSES

Phenolic compounds analysis of root exudates was performed as described in Weisskopf (2005). Phenolics were extracted from exudates lyophilisates using methanol 80% (1 ml per lyophilisate obtained from ~0.3 g of roots). Samples were vigorously shaked (vortex) and filtered at

0.45µm. After solvant evaporation, extracts were resuspended in the first HPLC solvent (A) in proportion to the root fresh weight (0.75 µl. mg root fresh weight^{-1}). 100 µl of the samples were then injected into a reverse phase C18 column (NUCLEOSIL 250x4.6mm, 7.0 µm) for analysis. The elution solvents consisted of water, acetonitrile and acetic acid in the following proportions (A) 93:5:2, (B) 23:75:2. Solvent gradient started with 10% of B and reached 100% in 40 minutes, with a flow rate of 0.4 ml.min^{-1}. Absorbance was monitored at 263 nm. All analyses were performed on extracts obtained from cut off root and intact plants.

II.2.2.4. STATISTICAL ANALYSES

Analyse of the organic acids and phenolic compounds secretion profiles corresponding to exudates from each cultivar was performed in three steps. Dataset matrix of peak areas and retention times were obtained from HPLC-UV chromatograms (absorbance was monitored at 210 nm and 263 nm respectively for organic acids and phenolics). The peaks with an area (mV*min) lower than 0.2, and peaks appearing in only one or two of the five replicates, were not taken into account.

Redundancy analyses (RDA) and principal component analyses (PCA) were first performed on organic acids and phenolic compounds dataset to have a global view of the results. To measure the variation of the organic acids and phenolics secretion profiles which was related to plant cultivar, partial RDA (see chap. II.2.1) was calculated for each compound type on two subsets of samples, i) cut off root exudates profiles, ii) intact plants exudates profiles.

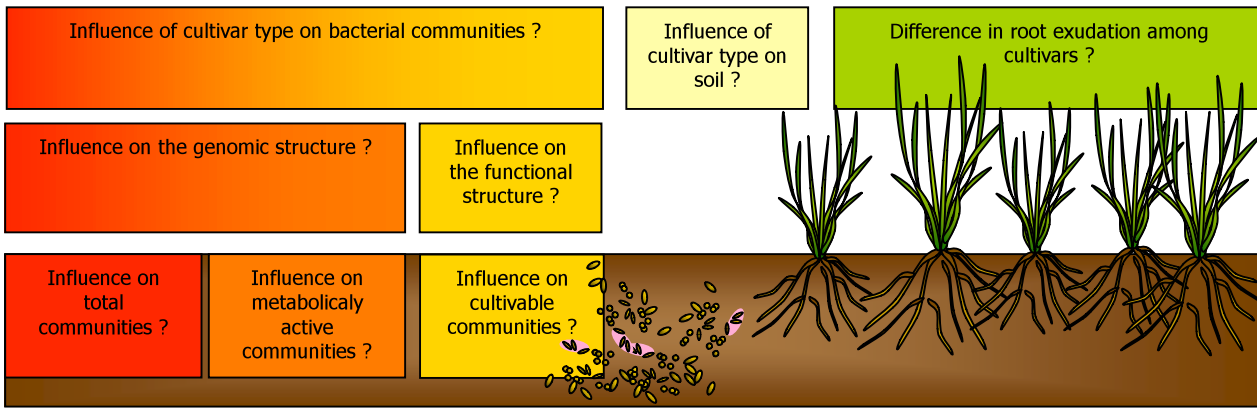
PCA analysis (see chap. II.2.1) was used to plot the centroïds of samples from each cultivar and root treatment regarding to their secretion patterns. Plot representations were based on axis 1 and 2. All analyses were performed with the statistical software R 2.3.1 (R Development Core Team, 2006).

The typical organic acids and phenolics profile of each cultivar has then been represented by calculation of the average area and standard deviation of peaks obtained from the five replicates, in order to evidence the organic acids and phenolics which are responsible of the differences observed between the cultivars.

II.2.3. SYNTHETIC OVERVIEW OF THE EXPERIMENT

A synthetic overview of the objectives and the samplings design is presented in figure II.2.

Figure II.2. Synthetic overview of the experiment



Bacterial communities structure determination and soil characterisation

4 cultivars + **1 control** (soil without plant)
→ A (L.p. cv. anaconda), B (L.p. cv. bastion), C (L.p. cv. cavia), L (L.p. cv. lipresso)
→ T (control)

4 plant development stages
→ 1 (hypocotil development), 2 (four leaves), 3 (ear development), 4 (ear maturity)

3 replicates

2 fractions / cultivar
→ roots (R), rhizosphere soil (RS)
1 control fraction
→ soil without plant (S)

Plant culture : greenhouse, non-sterile conditions
Pot contains 700g of soil (Eutric cambisol, CH-Eschikon) sieved at 2mm
3 (stage 3, 4), 6 (stage 2) and 10 (stages 1) plants / pot

Photoperiod : 10h (natural light + prolongation with artificial light if necessary)
Temperature : ~20°C
Watering (manual) : every 3 days

92 DNA samples

92 RNA samples

1950 bacterial strains

18 soil samples

Genotypic profiles of total bacterial communities

(based on 16S rDNA)

Genotypic profiles of active bacterial communities

(based on 16S rRNA)

Phenotypic profiles of cultivable communities

(16 functions tested in vitro)

Soil pH, organic matter and nutrient contents

Root exudates characterisation

3 cultivars
→ A, C, L

1 plant development stage
→ 30 days (intermediary of stage 2 and 3)

2 series of 5 replicates

2 exudates collection methods
→ cut off roots (C)
→ intact plants (NC)

Plant culture : phytotron, sterile conditions
Microcosme contains 100g of vermiculite
4 plants / microcosme

Photoperiod : 16h
Temperature : ~20°C
Watering (initial, single) : 100ml

2 series of 30 root exudates samples

Root exudates patterns (organic acids, phenolic compounds)

II.3. PLANT CULTIVAR AND DEVELOPMENT STAGE IMPACTS ON THE STRUCTURE OF BACTERIAL COMMUNITIES

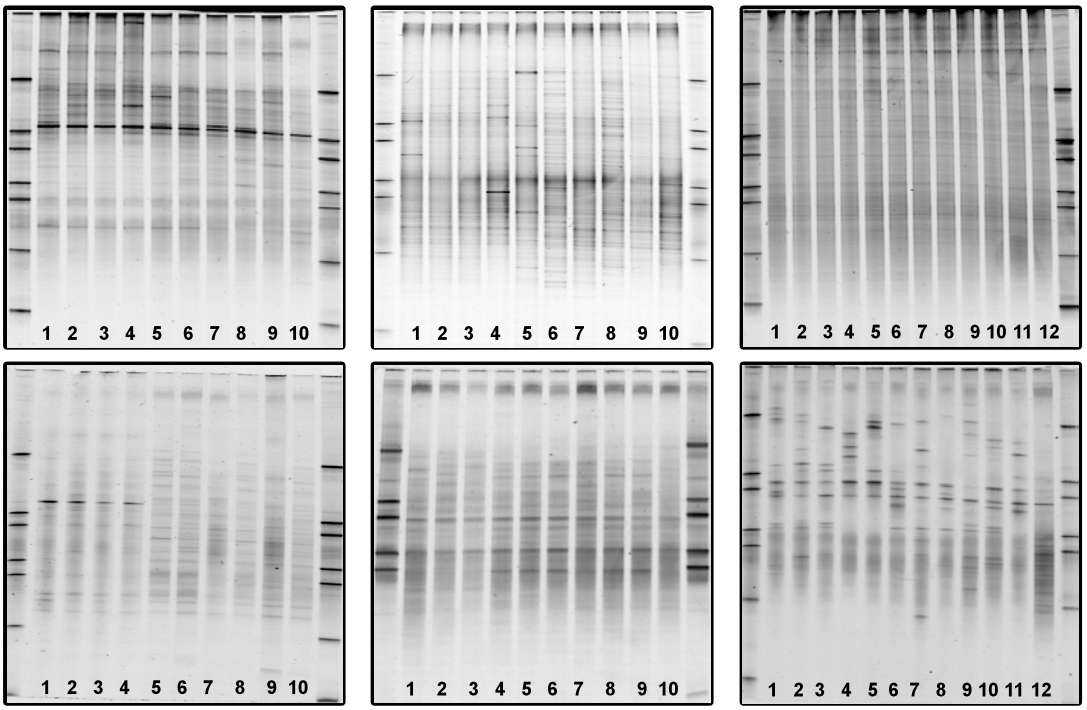
II.3.1. RESPONSES OF TOTAL AND ACTIVE BACTERIAL COMMUNITIES

II.3.1.1. OVERVIEW OF THE RESULTS

The 92 (DNA-based approach) and the 92 (RNA-based approach) DGGE community profiles (figure II.3.1) obtained from root and rhizosphere soil of the four cultivars, and from control bulk soil, each at four evolution time, were normalised and numersised. The obtained matrix contained for each sample the relative intensity and position of each band of the profile.

Figure II.3. Examples of DGGE profiles from root, rhizorphere and bulk soil communities

The gels presented here correspond to root (left) and rhizosphere soil (middle) communities associated to Anaconda cultivar, and to bulk soil (right) community profiles. Gels in the upper part are based on 16S rDNA and those in the lower part are based on 16S rRNA. First and last lane of each gel corresponds to the reference pattern. For root and rhizosphere soil communities, samples were ordered as follow: (1) Development stage-1 (3 replicates pooled), (2)-(4) Development stage-2, (5)-(7) Development stage-3, (8)-(10) Development stage-4. For bulk soil communities, samples were ordered as follow: (1)-(3) Development stage-1, (4)-(6) Development stage-2, (7)-(9) Development stage-3, (10)-(12) Development stage-4.



Community profiles were first globally analysed using variation partitioning analysis (RDA) in order to determine the importance of plant cultivar and development stage in the structure of associated bacterial communities (see chap. II.3.1.2).

Cultivar-induced modifications of the total and active bacterial communities structure were then investigated, using principal component analysis (PCA), to allow the distinction between root, rhizosphere and bulk soil communities, and between communities associated with the different cultivars (see chap. II.3.1.3).
Richness, Shannon diversity index, and evenness index were then compared for the different fractions and cultivars to closely assess the impact on bacterial community structure (see chap. II.3.1.4).

Temporal evolution of root, rhizosphere and bulk soil communities was also globally assessed using PCA analyses in order to estimate the plant and the soil evolution influence on total and active bacterial community structure (see chap. II.3.1.5). Richness, Shannon diversity index, and evenness index were also compared for the different stages to closely assess the evolution of bacterial community structure during plant growth (see chap. II.3.1.6).

II.3.1.2. IMPORTANCE OF PLANT CULTIVAR AND DEVELOPMENT STAGE INFLUENCES

Variation partitioning (figure II.4) was performed on DNA- and RNA-based data obtained from the DGGE profiles of the root and rhizosphere soil samples.

Significant effect ($p \leq 0.001$) of plant cultivar was detected on bacterial root (8 % and 18 %) and rhizosphere soil (12 % and 24%) community profiles using both DNA and RNA-based approaches. Influence of cultivar on community profiles was higher for metabollicaly active communities (RNA-based approach) than for total communities (DNA-based approach) for both root and rhizosphere soil fractions. As shown in Jossi et al. (2006) in the case of an atmospheric pCO_2 elevation (see chap. IV), environmental perturbations of the rhizosphere affect more the structure of active than of present bacterial communties.

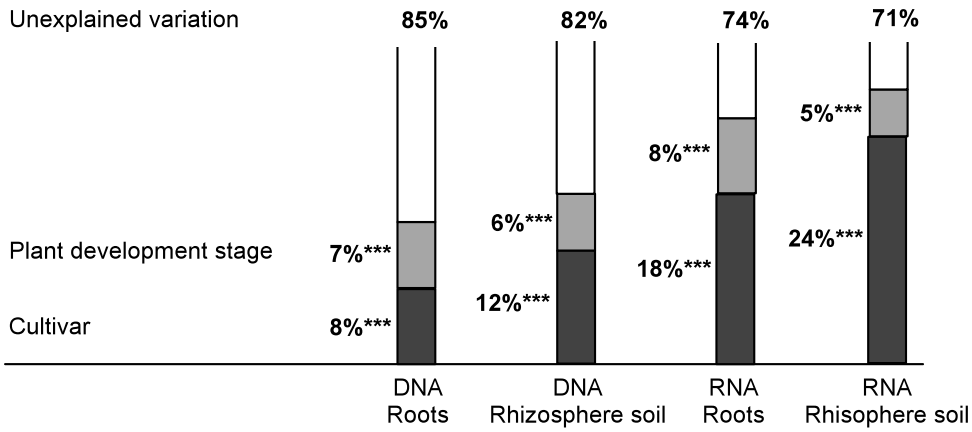
Interestingly, the cultivar effect was here more important on the structure of the rhizosphere soil than the root communities for both RNA- and DNA-based approach, contrasting with the known gradual root influence due to the diffusion of root productions from the root vicinity to the bulk soil.

Significant effect of plant evolution stage was also detected on bacterial root (7 % and 8%) and rhizosphere soil (6 % and 5 %) community profiles using respectively DNA and RNA-based approaches.

No shared variation of DGGE profiles was observed between plant cultivar and evolution stage influences, indicating that cultivar and evolution stage effects on bacterial communties are independent. In every case the effect of cultivar on community profiles was more important than the evolution stages one.

Figure II.4. Variation partitioning for DGGE-based community profiles: Impact of plant cultivar and development stage

Variation partitioning on data obtained from DNA- and RNA-based dgge profiles from root and rhizosphere soil samples. Percentages correspond to the part of the profiles variation explained by plant cultivar or development stage (Monte Carlo test for significance, * p≤0.05, ** p≤0.01, *** p≤0.001), and the part of the variation not explained by these two variables. Variation explained by the replicates was always null and insignificant.



II.3.1.3. CULTIVAR-INDUCED MODIFICATIONS: GLOBAL VIEW

To assess the plant cultivar-induced variation of the structure of total and active bacterial communities, the DGGE profiles were submitted to principal component analysis (PCA). The plot representation (figure II.5) shows, for DNA- and RNA-based community approaches, the centroïds corresponding to all the replicates and evolution stages of root and rhizosphere communities for each cultivar and for control soil communities.

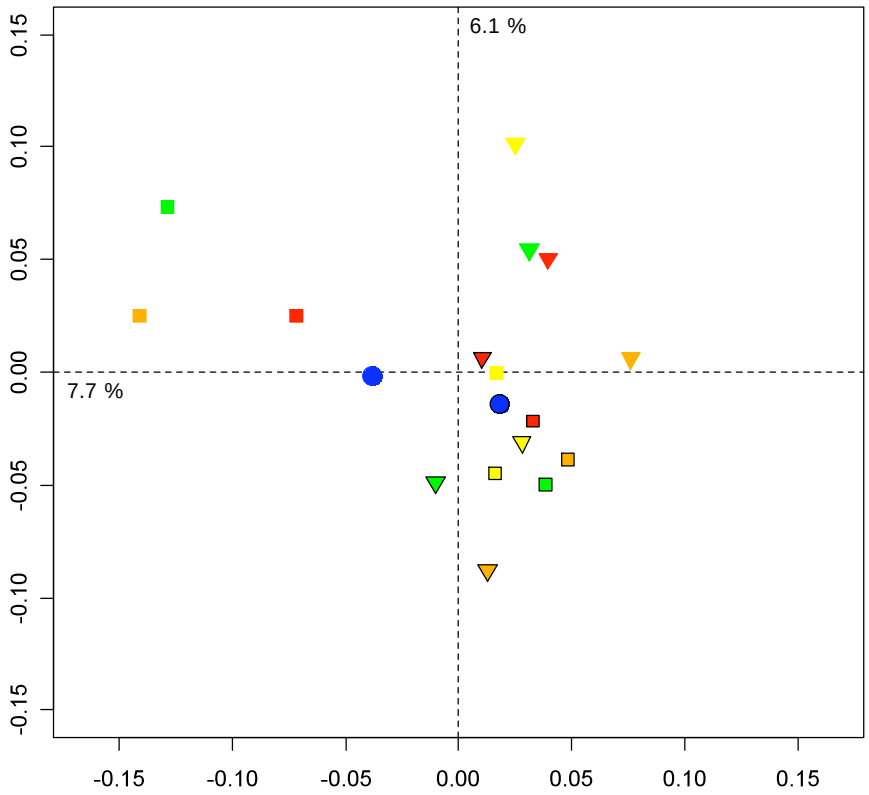
A clear separation was observed between present (DNA-based profiles, colour-filled symbols with black margin) and active (RNA-based profiles, colour-filled symbols) communities. Sames tendencies were observed on both total and actives communities but variations were much higher among active communities.

Clear separation was observed between root (triangles) communty profiles and rhizosphere (squares) and bulk soil (circles) profiles. Cultivar harbouring the most similar root and rhizosphere communities was Cavia (yellow symbols). Indeed, comparison between root and rhizosphere soil communities of the four cultivars showed that Cavia rhizosphere soil communities were more similar to root communities than to other cultivar rhizosphere soil communities. The higher differentiation between root and rhizosphere soil communities was observed for Bastion cultivar (Orange symbols).

A high similarity was observed between root active communities associated to Anaconda (Red symbols) and Lipresso cultivars (Green symbols).

Figure II.5. Principal component analysis for DGGE-based community profiles: Impact of plant cultivar

Plot of the principal component analysis (PCA) with centroïds corresponding to DGGE profiles from total (colour-filled symbols with black margin) and active (colour-filled symbols) bacterial communities of root (triangles) and rhizosphere soil (squares) from the differents cultivars (Anaconda: red, Bastion: Orange, Cavia: yellow, Lipresso: green), and of control bulk soil (blue), independently from the evolution stage.



II.3.1.4. CULTIVAR-INDUCED MODIFICATIONS: RICHNESS, DIVERSITY AND EVENNESS

Richness (number of bands), Shannon-Weaner diversity index ($H' = -\Sigma p_i * LNp_i$) and evenness index ($R = H' / H'max = H' / \log_2 S$) were determined for each DGGE profile (annexe II.1), and average values were then calculated for root and rhizosphere soil communities of each cultivar and for control bulk soil, independently from the evolution stage.

Richness values ranged from 16 to 36, Shannon index from 2.64 to 3.48, and Evenness index from 0.66 to 0.69 (Evenness = 1 correspond to an equal number of representent for each population of the community).

Richness (figure II.6.1) and Shannon-Weaner diversity index (figure II.6.2) of the DGGE profiles were higher ($p \leq 0.001$) for DNA (total communities) than RNA (metabolicaly active communities) based profiles, indicating that only a part of the total communities is metabolically active. In the particular case of Bastion root-associated communitiy profiles, richness values and Shannon-Weaner diversity index seems to be similar between total and active communities.
For Bastion root-associated total communities, richness ($p \leq 0.001$) and diversity ($p \leq 0.01$) were significantly lower than for other cultivars root-associated communities. The differences observed for Bastion cultivar could be linked with the nature of the soil used in this experiment, and be due to the particular adaptation of the soil inhabitant bacterial populations to the rhizosphere of Bastion because of the previous 11 years of cultivation with this plant cultivar. We suppose that Anaconda, Cavia and Lipresso cultivars select other populations than those originally dominant in the soil preadapted to Bastion, leading to the detection of a higher bacterial diversity.

No clear tendencies were observed among the Evenness values of the different cultivars. (figure II.6.3).

The higher cultivar-induced variation was previously observed on active bacterial communties (figure II.4 / II.5), but when regarding the richness and diversity of communities, no significant effect of plant cultivar was observed on active community profiles (figure II.6.1 / II.6.2). Only a small but significant ($p<0.01$) variation was visible on the richness and diversity of total communities associated with Bastion cultivar.

Figure II.6. Richness, diversity and evenness of total and active bacterial communities for root and rhizosphere soil of each plant cultivar and for control bulk soil

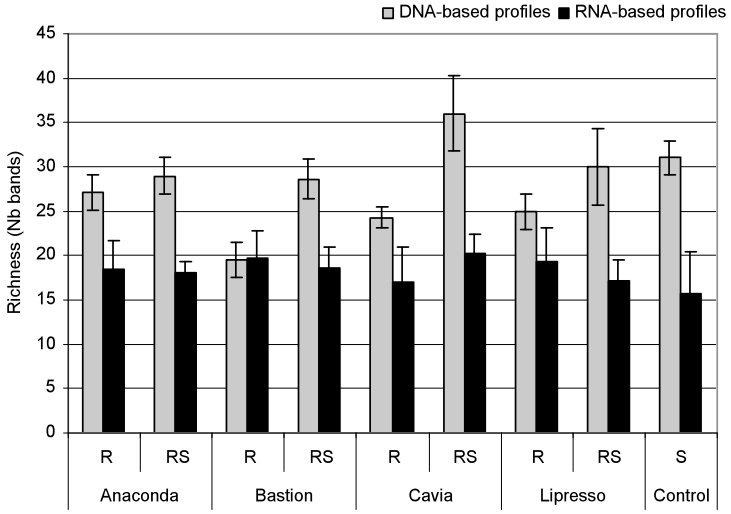


Figure II.6.1. Richness (number of bands) on DGGE community profiles obtained from root (R) and rhizosphere soil (RS) of each cultivar, and from the control bulk soil (S), independently from the evolution stage.

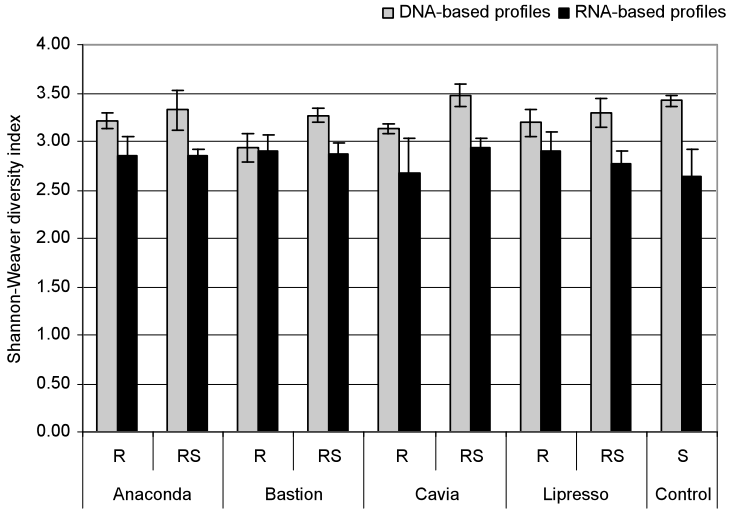


Figure II.6.2. Shannon diversity index of DGGE profiles obtained from root (R) and rhizosphere soil (RS) of each cultivar, and from the control bulk soil (S), independently from the evolution stage.

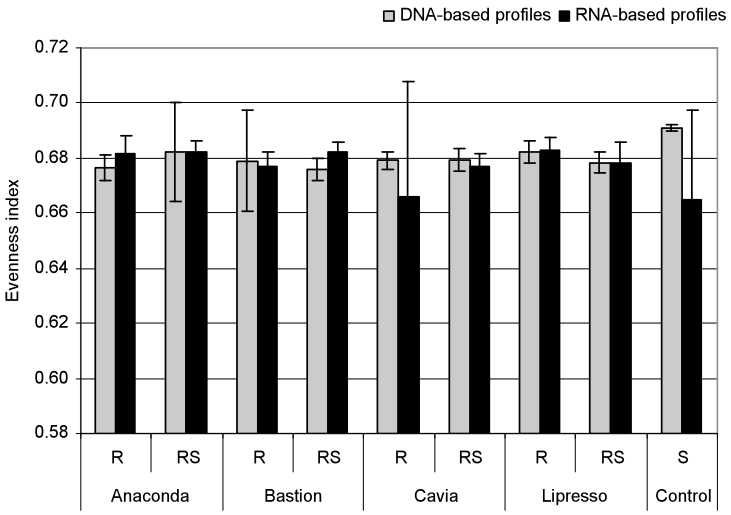


Figure II.6.3. Evenness index of DGGE profiles obtained from root (R) and rhizosphere soil (RS) of each cultivar, and from the control bulk soil (S), independently from the evolution stage.

II.3.1.5. TEMPORAL VARIATIONS: GLOBAL VIEW

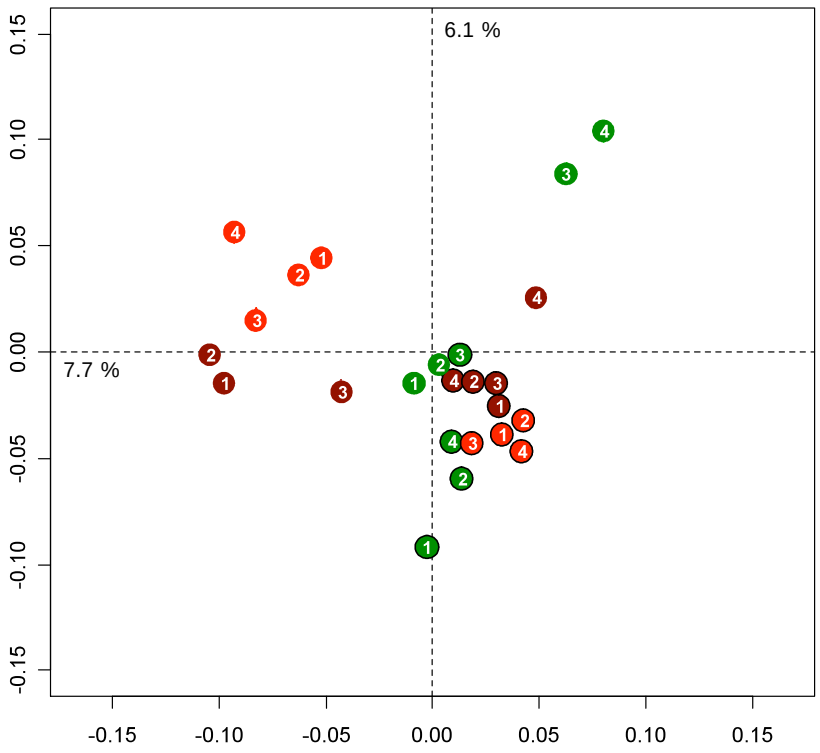
To assess the temporal variation of the bacterial communities, another plot representation (figure II.7) of the PCA analysis described above (see chap. II.3.1.2) has been performed based on the same dataset. It shows the centroïds corresponding to root, rhizosphere and to bulk soil communities, for each evolution stage, independently from the plant cultivar.

As already observed for plant cultivar effect, results presented here showed a clear separation between present (DNA-based profiles, colour-filled symbols with black margin) and active (RNA-based profiles, colour filled symbols) communities and a higher variation between profiles of active than total communities. Clear separation was also observed between root community profiles, and rhizosphere and bulk soil profiles.

Profiles corresponding to early stages (1 and 2) differ from to those corresponding to late stages (3 and 4). This difference could be attributed to soil temporal evolution (independently from plant influence) because same tendency is observed on control bulk soil communities. Separation of profiles corresponding to the different stages was more pronounced on root than on rhizosphere and bulk soils bacterial communities.

Figure II.7. Principal component analysis for DGGE-based community profiles: Temporal evolution

Plot of the principal component analysis (PCA) with centroïds corresponding to DGGE profiles from total (colour-filled symbols with black margin) and active (colour-filled symbols) bacterial communities of root (green), rhizosphere soil (red) and bulk soil (brown) at each evolution stages (hypocotil development: 1, four leaves: 2, ear development: 3, ear maturity: 4), independently from the plant cultivar.



II.3.1.6. TEMPORAL VARIATIONS: RICHNESS, DIVERSITY AND EVENNESS

Richness (number of bands), Shannon-Weaner diversity index ($H' = -\Sigma p_i * LNp_i$) and evenness index ($R = H' / H'max = H' / \log_2 S$) were determined for each DGGE profile (annexe II.1), and average values were then calculated for root, rhizosphere and bulk soil communities at each evolution stage, independently from the plant cultivar. Richness values ranged from 15 to 33, Shannon index from 2.34 to 3.48, and Evenness index from 0.66 to 0.69 (Evenness = 1 correspond to an equal number of representent for each population of the community).

Richness (figure II.8.1) and Shannon-Weaner diversity index (figure II.8.2) of the DGGE profiles were higher ($p \leq 0.001$) for DNA (total communities) than RNA (metabolically active communities) based profiles, indicating that only a part of the total communities is metabolically active. The observed differences were more important for the richness than the Shannon index. No important differences of evenness values were observed between total and active communities (figure II.8.3).

No differences between profiles of the different evolution stages were detected on Richness, Shannon index and Evenness

Independently from development stage, total richness tended (not significant) to be higher for soil communities compared to root and rhizosphere soil. The inverse tendency was observed when regarding only active communities.

Figure II.8. Richness, diversity and evenness of total and active bacterial communities for root, rhizosphere and bulk soil at each evolution stage

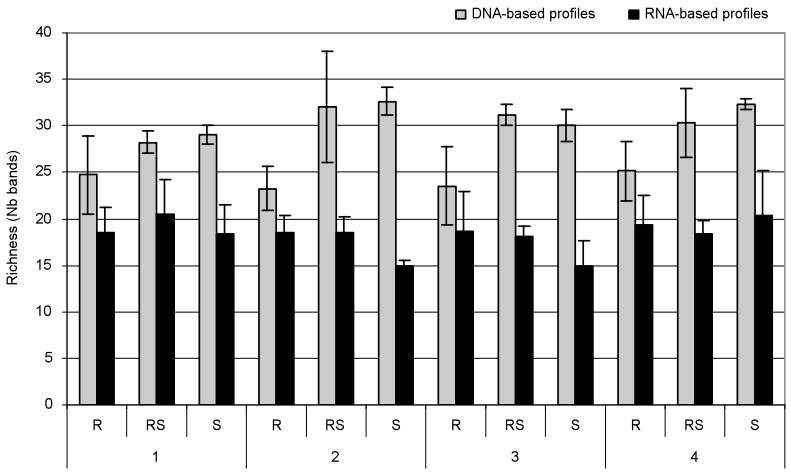


Figure II.8.1. Richness (number of bands) of DGGE community profiles obtained from root (R), rhizosphere soil (RS) and bulk soil (S) for each evolution stage, independently from the plant cultivar.

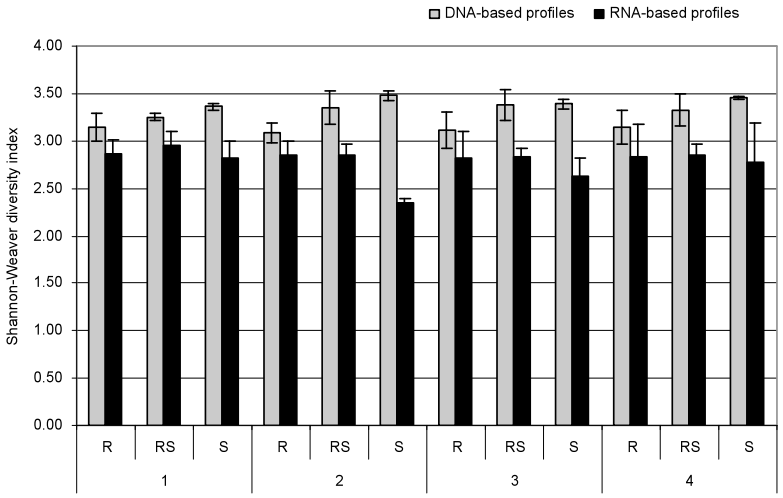


Figure II.8.2. Shannon-Weaner diversity index of DGGE profiles obtained from root (R), rhizosphere soil (RS) and bulk soil (S) for each evolution stage, independently from the plant cultivar.

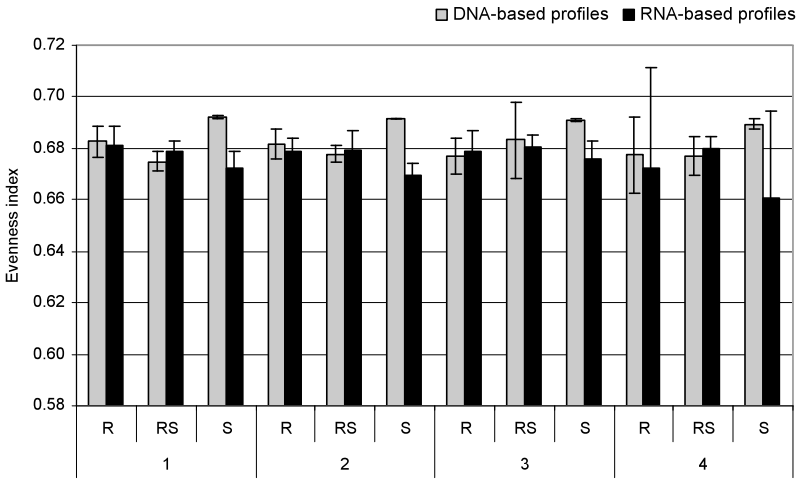


Figure II.8.3. Evenness index of DGGE profiles obtained from root (R), rhizosphere soil (RS) and bulk soil (S) for each evolution stage, independently from the plant cultivar.

II.3.2. RESPONSES OF CULTIVABLE HETEROTROPHIC BACTERIAL COMMUNITIES

II.3.2.1. OVERVIEW OF THE RESULTS

The 1950 collected isolates were morphologicaly described (annexe II.2) and tested for 16 bacterial abilities (table II.4, p. 67): Aminopeptidase activity (gram), oxydase activity (oxyd), glucan production (eps), siderophore production (sid), Ca-phosphate solubilisation (psb), phytase production (phyG, see chap. III), nitrate reduction in nitrite (nr), denitrification (d), casein hydolysis (case), gelatin hydrolysis (gelat), sulfatase production (sulf, see chap. III), production of N-acyl homoserine lactones (nahl C_4-C_8 and nahl C_6-C_{12}), auxin production (iaa) and hydrogen cyanide production (hcn). A presence/absence matrix containing the functional profile and the environmental variables describing each strain was constituted, and proportions of positive strains were calculated for each bacterial trait (annexe II.3).

In order to investigate the functional traits characteristic of root, rhizosphere and bulk soil communities, proportion of positive isolates were calculated for each bacterial trait independently from the evolution stage and plant cultivar (see chap. II.3.2.2).

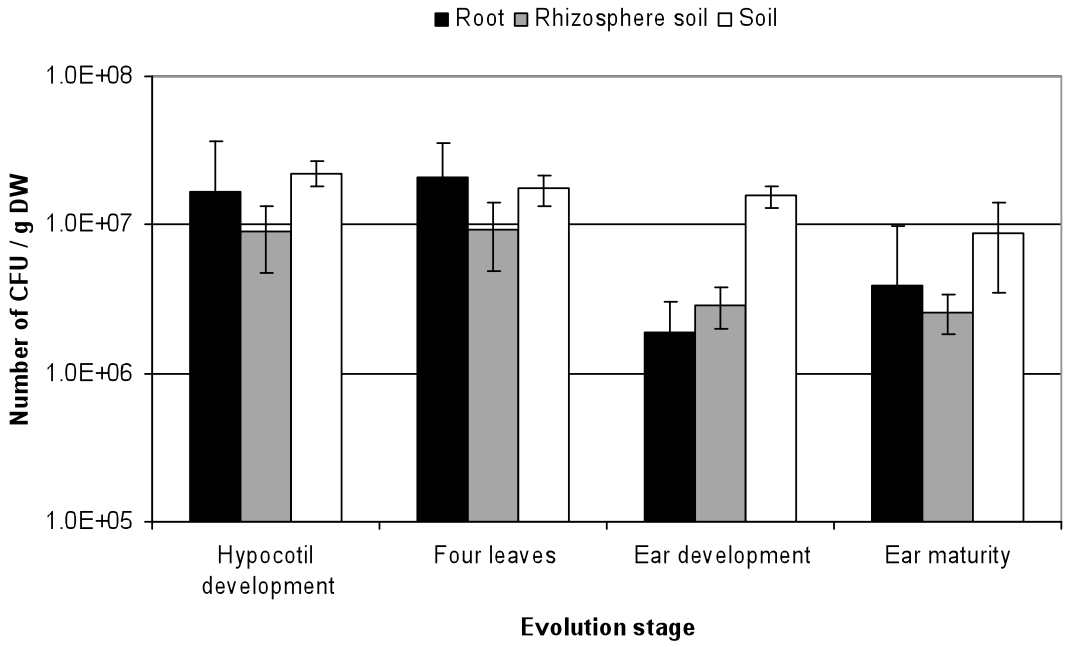
The importance of plant cultivar and development stage impacts on the structure of associated bacterial communities was estimated by performing variation partitioning of data corresponding to the phenotypic profiles of the isolates (see chap. II.3.2.3).

To allow the distinction between root, rhizosphere and bulk soil communities, and between communities associated with the different cultivars, and to highlight the interactions between the different bacterial abilities, frequency of bacterial abilities were then compared separately between the four cultivars allowing the identification of discriminant functions (see chap. II.3.2.5). Theses cultivar-induced modifications of the structure of cultivable heterotrophic bacterial communities were investigated, using PCA ordination analyses (see chap. II.3.2.4).
To estimate the plant and the soil evolution impact on cultivable heterotrophic bacterial community structure and functional abilities (see chap. II.3.2.6), frequencies of bacterial abilities were compared separately, as for detection of cultivar-induced modifications, between the four evolution stages to allow the identification of the discriminant functions (see chap. II.3.2.7). These temporal differentiations of root and rhizosphere soil communities, and of bulk soil communities was assessed using PCA analyses.

II.3.2.2. BACTERIAL DENSITY AND FREQUENCY OF FUNCTIONAL ABILITIES: ROOTS INFLUENCE

Number of bacterial CFU per gram dry weight of root, rhizosphere soil and bulk soil was determined on Angle medium plates (figure II.9). Bacterial density was slightly decreased in root and rhizosphere soil at flowering (ear development) and maturity stages, compared to hypocotil development and four leaves stages. In a less extend, a decrease was also observed during bulk soil evolution. No important difference was observed between bacterial densities associated to the different cultivars.

Figure II.9. Evolution of the number of bacterial CFU per gram (dry weight) of root and rhizosphere soil from all the cultivars, and of control bulk soil incubated without plant.

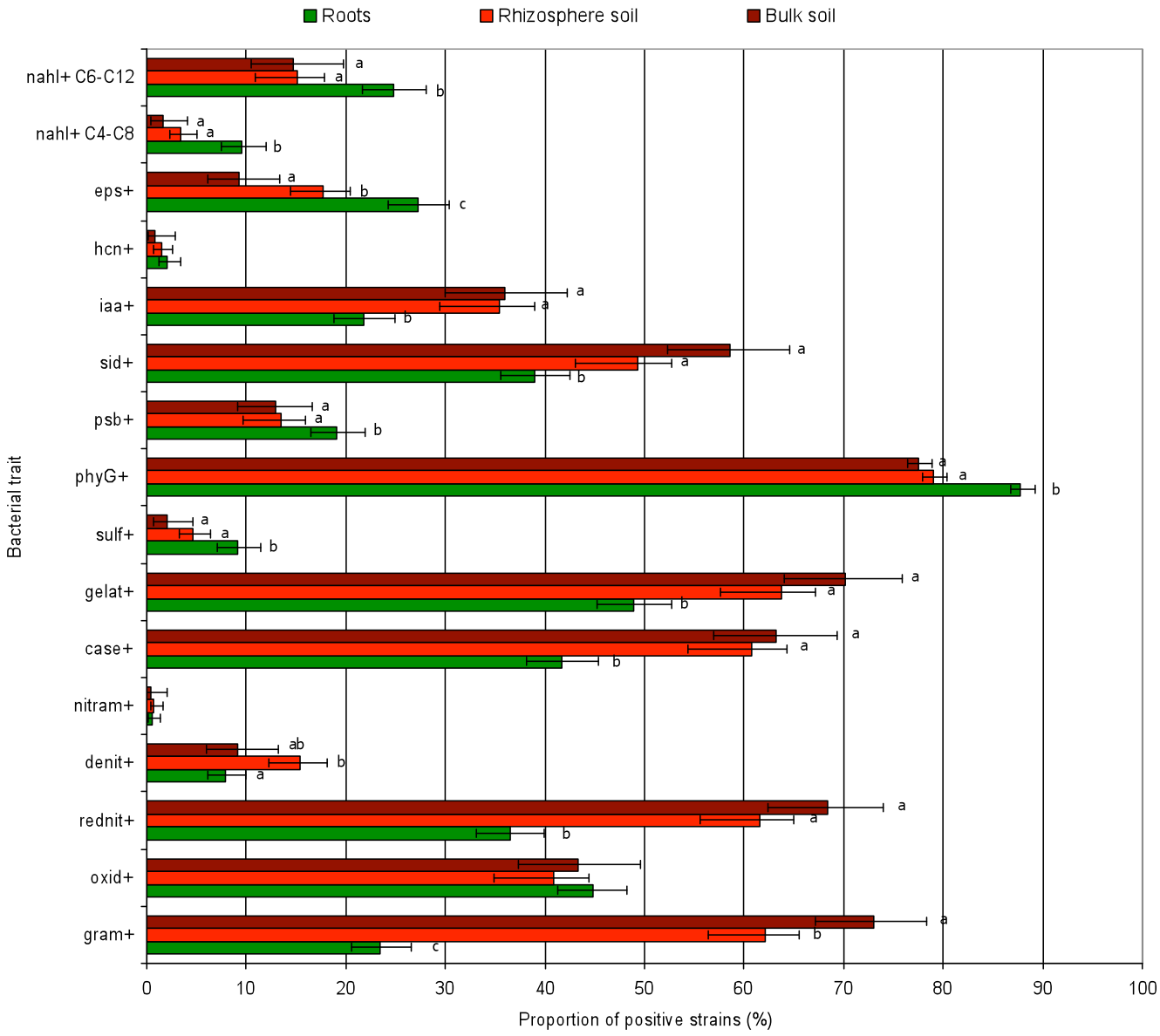


Proportion of positive strains was calculated for each fraction independently from the plant cultivar and development stage (figure II.10). Generalised linear model analysis and Tukey Kramer HSD post hoc test were used on the proportions data for each ability test in order to investigate differences between root and rhizosphere soil communities, and to determine the significance of these results (table II.5).

Differences between the proportions of root, rhizosphere soil and bulk soil isolates were observed for 13 of the abilities investigated. However, no significant differences were observed between root, rhizosphere and bulk soil communities for HCN production, nitrammonification and oxydase activity.

Figure II.10. Proportion of positive isolates in root, rhizosphere and bulk soil communities

Proportion (%) of positive strains isolated from root, rhizosphere soil and bulk soil. Percentages were calculated independently from the cultivar and the evolution stage for the seexteen bacterial traits investigated. Letters indicating significant differences are not mentioned for traits presenting no significant differences between the three fractions.



Root-associated bacterial traits (table II.5, figure II.10):

Proportions of positive isolates were higher for root than for rhizosphere and bulk soil communities for both C_6-C_{12} and C_4-C_8 Nahl production, for Ca-phosphate solubilisation, sulfatases (see chap. III) and phytases production (see chap. III). A decrease in the proportion of isolates able to produce exopolysaccharides (glucan) was observed from root to bulk soil.

Soil-associated bacterial traits (table II.5, figure II.10) :

Proportions were higher for rhizosphere and bulk soil than for root communities when regarding IAA production, siderophores production, gelatin and casein hydrolysis, and nitrate reduction in nitrite. A decrease in the proportion of Gram positive strains was observed from bulk soil to root vicinity. Proportion of denitrifiing isolates was higher in the rhizosphere soil than in root and bulk soil (not significant) communities.

Table II.5. Changes of the frequencies of each bacterial trait, in relation with root distance.
Differences in the proportion of positives strains isolated from the root (R), rhizosphere (RS) and bulk soil (S) communities independently from the plant cultivar and evolution stage.

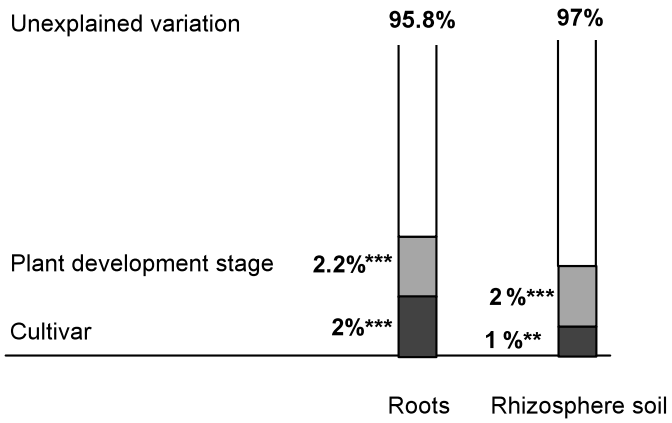
Secretion activities		R	RS	S	Enzymatic activities		R	RS	S
Nahl+ C6-C12	R	X	⇧***	⇧**	PhyG+	R	X	⇧**	⇧**
	RS		X	-		RS		X	-
	S			X		S			X
Nahl+ C4-C8	R	X	⇧***	⇧***	Sulf+	R	X	⇧*	⇧*
	RS		X	-		RS		X	-
	S			X		S			X
Eps+	R	X	⇧**	⇧***	Gelat+	R	X	⇩*	⇩**
	RS		X	⇧**		RS		X	-
	S			X		S			X
Iaa+	R	X	⇩***	⇩**	Case+	R	X	⇩*	⇩**
	RS		X	-		RS		X	-
	S			X		S			X
Sid+	R	X	⇩*	⇩***	Denit+	R	X	⇩*	-
	RS		X	-		RS		X	-
	S			X		S			X
Psb+	R	X	⇧*	⇧*	Rednit+	R	X	⇩**	⇩**
	RS		X	-		RS		X	-
	S			X		S			X
					Gram+	R	X	⇩***	⇩***
						RS		X	⇩*
						S			X

II.3.2.3. IMPORTANCE OF PLANT CULTIVAR AND DEVELOPMENT STAGE INFLUENCES

Variation partitioning (figure II.11) was performed on functional profiles obtained from strains isolated from root and rhizosphere soil in order to determine the importance of cultivar and development stage influence on bacterial community structure.

Figure II.11. Variation partitioning for functional community profiles: Impact of plant cultivar and development stage

Variation partitioning of the functional profiles of strains isolated from root and rhizosphere soil fractions. Percentages correspond to the part of the profiles variation explained by plant cultivar or development stage (Monte Carlo test for significance, * p≤0.05, ** p≤0.01, *** p≤0.001), and the part of the variation not explained by these two variables.



Slight but significant effect of plant cultivar was detected on the functionnal abilities of strains isolated from root ($p \leq 0.001$) and rhizosphere soil ($p \leq 0.01$). The variation of strains profiles, which is explained by plant cultivar, was higher for root communities (2%) than for rhizosphere soil communities (1%), contrasting with the results obtained on total and active communities structure. The genomic approach was non-oriented, but the functional approach used here focused on bacterial traits implicated in nutrition and plant-bacteria interactions, this might explain the contrasting results obtained using these two approaches.

Significant effect of plant development stage (2.2 % and 2 %) was detected on functional profile of strains isolated respectively from root and rhizosphere soil of the four cultivars.
No shared variation was observed between cultivar impact and those of development stages, indicating that, as observed on total and active communities, cultivar and development stage influence on heterotrophe bacterial communties are independent.

II.3.2.4. CULTIVAR-INDUCED MODIFICATIONS: GLOBAL VIEW

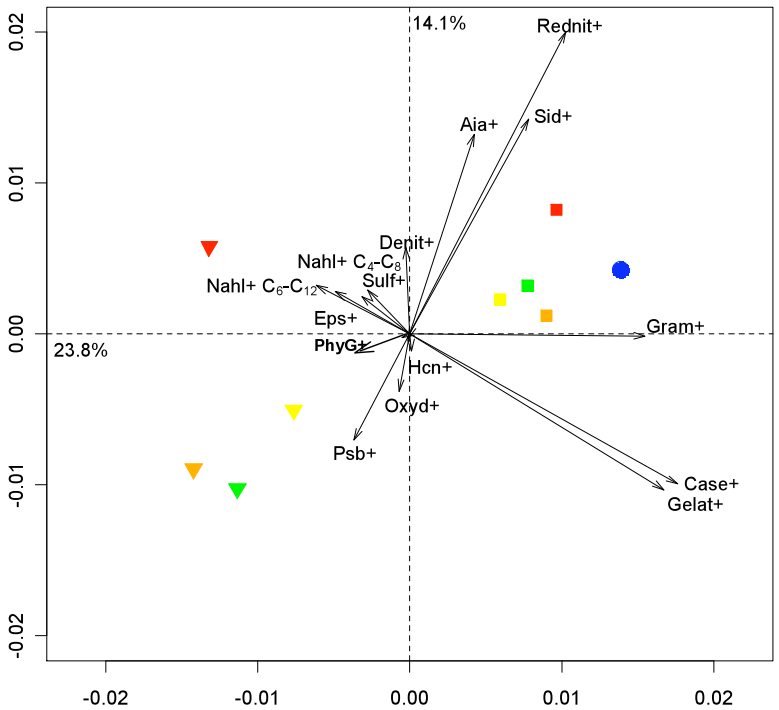
To assess the plant cultivar-induced variation on the functional structure of root and rhizosphere soil communities, strain profiles were submitted to principal component analysis (PCA). The plot (figure II.12) presented moreover highlights the relationships between the different bacterial traits.

A strong positive correlation has been demonstrated between some of the studied abilities: (i) NAHL, sulfatase and glucan production, (ii) casein and gelatin hydolysis, and (iii) siderophore production, nitrate reduction and auxin production abilities.

This analysis also showed a clear separation between functional structure of root communities and rhizosphere and bulk soil communities. Additionnaly, as observed on genomic structure of total and active communities, Cavia cultivar was shown to harbour the more similar root and rhizosphere soil cultivable communities. A higher variation between the different cultivar associated communities was observed in root compared to soil communities, and this variation was mainly observed on functional abilities of strains associated to Anaconda cultivar.

Figure II.12. Principal component analysis for functional community profiles: Impact of plant cultivar

PCA plot of strains functional profiles, with centroïds corresponding to strains isolated from root (triangles) and rhizosphere soil (squares) fraction for each cultivar (Anaconda: red, Bastion: orange, Cavia: yellow, Lipresso: green) and for control soil (blue circle), independently from the evolution stage of the samples. The discriminent bacterial traits are represented as vectors (for a better representation, the size of all the vectors has been divided by 30)



II.3.2.5. CULTIVAR-INDUCED MODIFICATIONS: FREQUENCY OF FUNCTIONAL ABILITIES

Percentages of positives strains isolated from roots and rhizosphere soil communities associated to the four cultivars were calculated for each bacterial trait separately (figure II.13). Generalised linear model analysis and Tukey Kramer HSD post hoc test were used on the proportions data for each ability test in order to investigate differences between root and rhizosphere soil communities of the four cultivars, and to determine the significance of these results.

In both root and rhizosphere soil fractions, no significant differences between cultivars were observed for proportion of oxydase positive strains, glucane, phytases, sulfatases, NAHLs C_4-C_8, cyanidric acid producing strains, Ca-phosphate solubilising, and nitrammonifiant strains.

Significant differences in the proportion of positive strains isolated from the four cultivars (figure II.13) were observed on 8 of the 16 bacterial traits investigated in this study (table II.6).

Among the 9 studied enzymatic activities, 5 were found to be affected by cultivar:

Proportion of strains able to produce proteases for casein hydrolysis was lower for Bastion than Cavia cultivar in the root fraction, and in rhizosphere soil, proportion of denitrifiing strains was lower for Bastion than for Lipresso cultivar.

In the rhizosphere soil, proportion of Gram positive strains was higher for Anaconda and Bastion than for Cavia cultivar. Proportion of nitrate-reducing strains was higher for Anaconda than for Bastion cultivar in the root fraction, and was higher in the rhizosphere soil for Anaconda than Cavia cultivar. Proportion of strains able to produce proteases for gelatin hydrolysis was lower in the root fraction for Anaconda than Cavia and Lipresso cultivars, and was lower in the rhizosphere soil for Anaconda than of other cultivars.

Among the 7 studied secretion activities, 3 were found to be affected by cultivar:

In the root fraction, proportion of auxin producing strains was lower for Bastion than for other cultivars. Proportion of root isolates producing C_6-C_{12} NAHL's was higher for Anaconda and Bastion than for Cavia cultivar, and was also higher for Bastion than Lipresso cultivar.

In the root fraction, proportion of strains able to produce siderophores was higher for Anaconda than for Bastion and Cavia cultivars.

Globally, Anaconda was the cultivar which harboured the most characteristic associated communities.

Among the 8 cultivars dependant traits (see chap. II.3.2.5) only 2 (Rednit+, Gelat+) were affected by cultivar type in both root and rhizosphere soil communities, and impact of plant cultivar was visible only on root communities for 4 of these 8 bacterial traits (Case+, Sid+, Iaa+, Nahl+ C6-C12). Interestingly some bacterial traits (Denit+, Gram+) are only affected by plant cultivar in the rhizosphere soil communities.

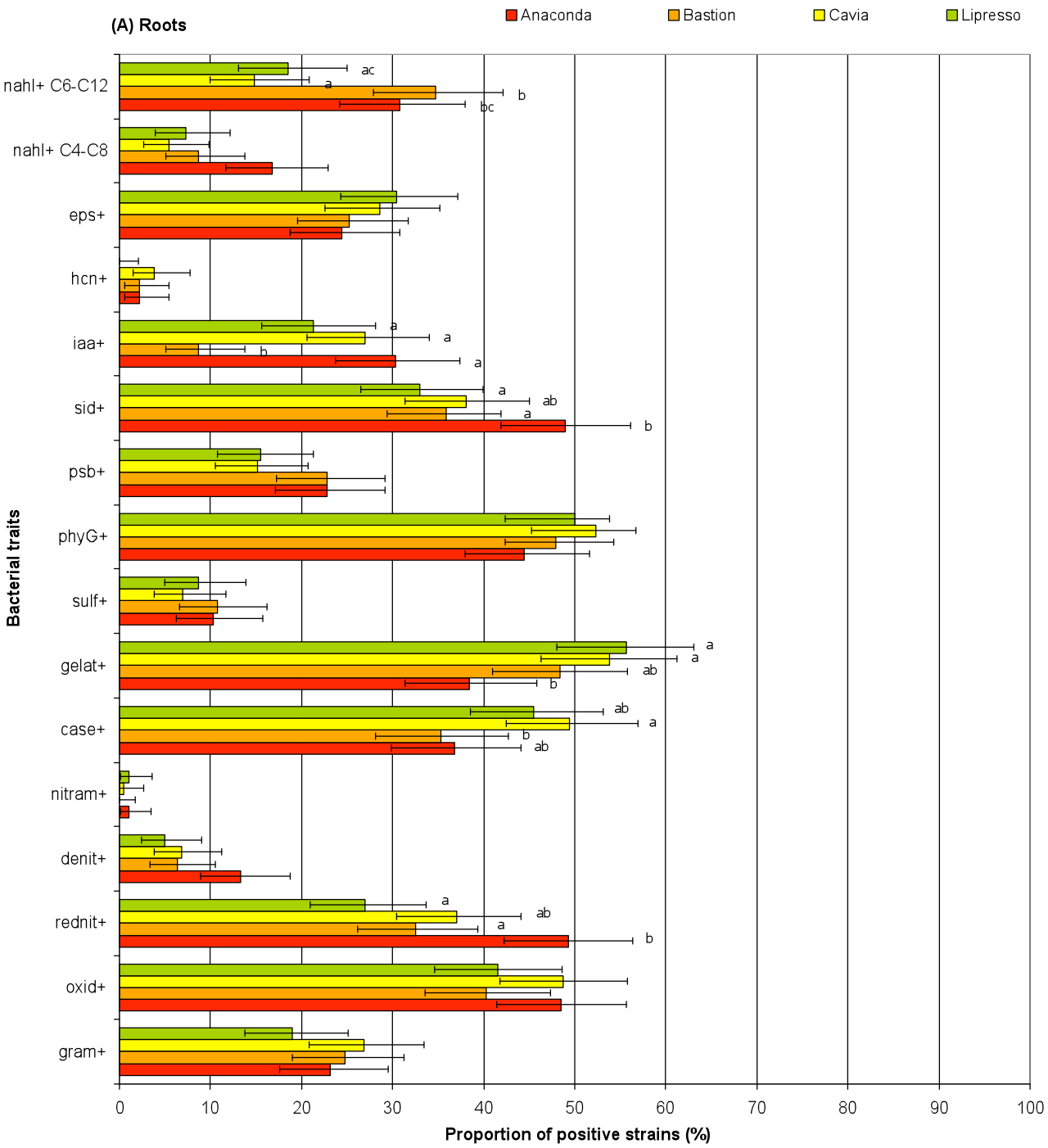
Table II.6. Changes of the frequencies of each bacterial trait, in relation with plant cultivar.

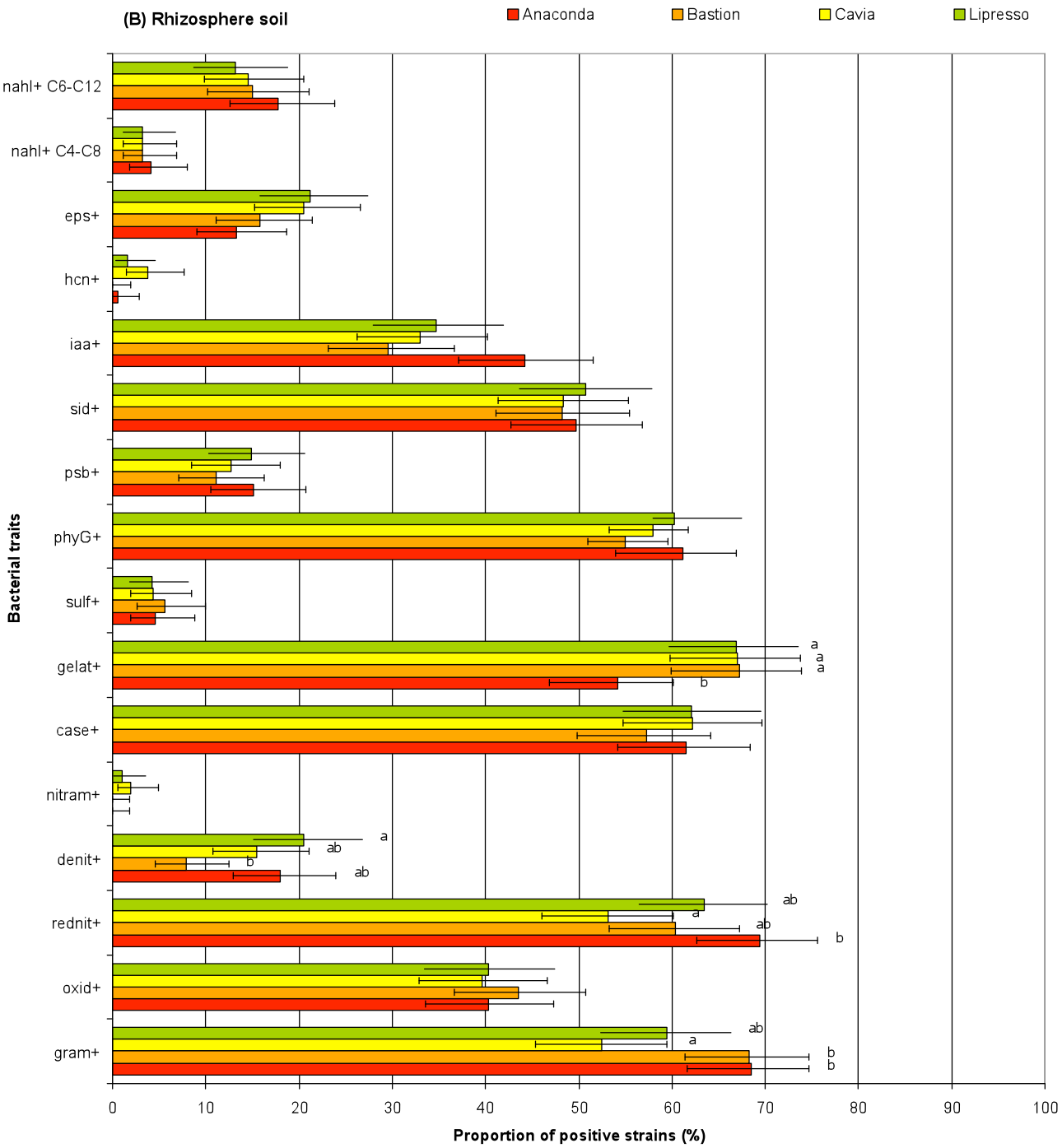
Differences in the proportion of positives strains isolated from the root and rhizosphere soil communities of the four cultivars (Anaconda: A, Bastion: B, Cavia: C, Lipresso: L), independently from the evolution stage. Arrows indicate a significant (* p≤0.05, ** p≤0.01, *** p≤0.001) increase (⇧) or deacrease (⇩) between proportions of positive strains of the compared cultivars.

Activities		Root communities					Rhizosphere soil communities			
		A	B	C	L		A	B	C	L
Nahl+ C6-C12	A	X	-	⇧*	-	A	X	-	-	-
	B		X	⇧**	⇧*	B		X	-	-
	C			X	-	C			X	-
	L				X	L				X
Iaa+	A	X	⇧***	-	-	A	X	-	-	-
	B		X	⇩**	⇩*	B		X	-	-
	C			X	-	C			X	-
	L				X	L				X
Sid+	A	X	⇧*	-	⇧*	A	X	-	-	-
	B		X	-	-	B		X	-	-
	C			X	-	C			X	-
	L				X	L				X
Gelat+	A	X	-	⇩*	⇩**	A	X	⇩*	⇩*	⇩*
	B		X	-	-	B		X	-	-
	C			X	-	C			X	-
	L				X	L				X
Case+	A	X	-	-	-	A	X	-	-	-
	B		X	⇩*	-	B		X	-	-
	C			X	-	C			X	-
	L				X	L				X
Denit+	A	X	-	-	-	A	X	-	-	-
	B		X	-	-	B		X	-	⇩**
	C			X	-	C			X	-
	L				X	L				X
Rednit+	A	X	⇧**	-	⇧***	A	X	-	⇧**	-
	B		X	-	-	B		X	-	-
	C			X	-	C			X	-
	L				X	L				X
Gram+	A	X	-	-	-	A	X	-	⇧**	-
	B		X	-	-	B		X	⇧**	-
	C			X	-	C			X	-
	L				X	L				X

Figure II.13. Proportion of positive isolates in the root and rhizosphere soil communities: Impact of plant cultivar

Proportion of positive strains among (A) root and (B) rhizosphere soil communities associated with the four cultivars (Anaconda: red, Bastion: orange, Cavia: yellow, Lipresso: green), independently from the plant development stage, for the seexteen bacterial traits investigated. Letters indicating significant differences are not mentioned for traits presenting no significant differences between the four cultivars.




(B) Rhizosphere soil
Anaconda
Bastion
Cavia
Lipresso
nahl+ C6-C12
nahl+ C4-C8
eps+
hcn+
iaa+
sid+
psb+
phyG+
sulf+
gelat+
case+
nitram+
denit+
rednit+
oxid+
gram+
Bacterial traits
a
a
a
b
a
ab
b
ab
ab
a
ab
b
ab
a
b
b
0
10
20
30
40
50
60
70
80
90
100
Proportion of positive strains (%)

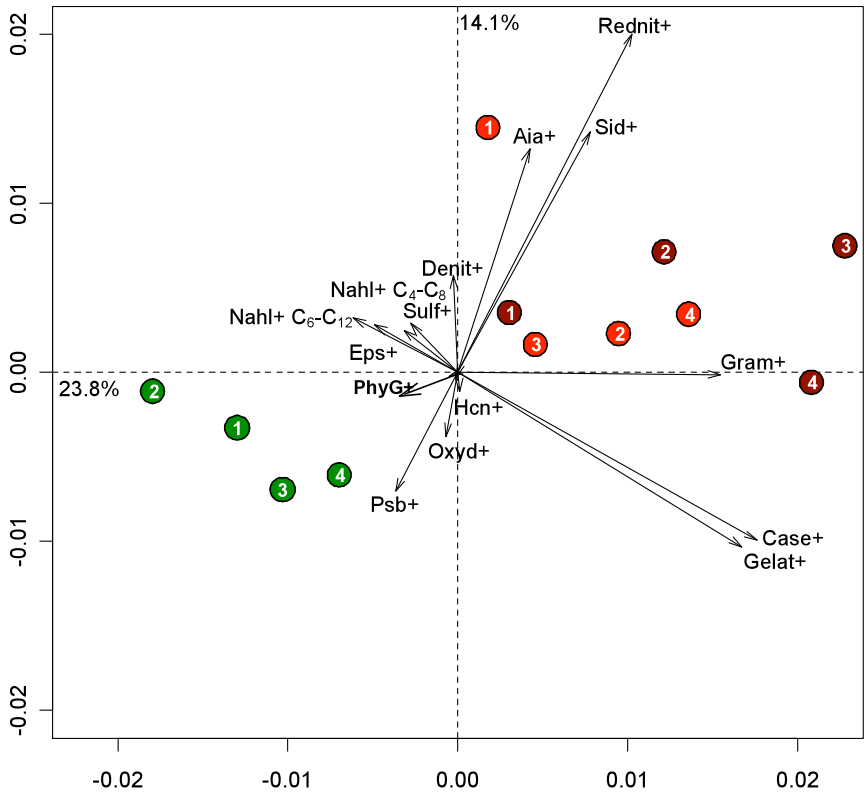
II.3.2.6. TEMPORAL VARIATIONS: GLOBAL VIEW

To assess the temporal variation of the culturable heterotroph bacterial communities structure, another plot representation of the PCA analysis described above (see chap. II.3.2.4) has been performed on the same dataset. This plot representation of the centroïds corresponding to the functional profile of root, rhizosphere and bulk soil communities at each evolution stage (figure II.14) showed a clear separation between root and soil communities.

Surprisingly, instead of the oriented nature of the approach on bacterial function implicated in plant-bacteria interactions, no global differences were observed between the evolution stages.

Figure II.14. Principal component analysis for functional community profiles: Temporal evolution

PCA plot of strains functional profile with centroïds corresponding to strains isolated from root (green circles), rhizosphere soil (red circles) and bulk soil (brown circles) fractions at each evolution stage (For plants, Hypocotil development: 1, four leaves: 2, ear development: 3, ear maturity: 4), and independently from the plant cultivar. The discriminent bacterial traits are represented as vectors (for a better representation, the size of all the vectors has been divided by 30).



II.3.2.7. TEMPORAL VARIATIONS: FREQUENCY OF FUNCTIONAL ABILITIES

Percentages of positives strains isolated from roots, rhizosphere and bulk soil communities corresponding to the four evolution stages (independently from the cultivar) were calculated for each bacterial traits separately (figure II.15). Generalised linear model analysis and Tukey Kramer HSD post hoc test were used on the proportion data to investigate differences between strains phenotype of communities corresponding to the evolution stages, and to test the significance of these results (table II.7).

In root, rhizosphere soil and bulk soil fractions, no significant differences between plant development stages were observed for proportion of strains able to hydolyse gelatin or casein, to produce sulfatases, HCN, or auxin, and for proportion of nitrammonifiant strains. Proportion of positive strains was affected by evolution stage for 10 of the 16 bacterial traits tested.

Among the 9 studied enzymatic activities, 4 were found to be affected by evolution stage:
As already documented by Miller *et al.* (1990), Gram positive strains become increasingly more abundant in the root and rhizosphere communities of maturing plants.
In the root and rhizosphere soil communities, the proportion of oxydase positive strains increased during plant development, the highest proportion was observed at flowering, and this proportion decreased at maturity stage.
In the root communities, a higher proportion of nitrate reducing strains was observed at the hypocotil development and the maturity stages and decreased in the intermediary stages.
A higher proportion of denitrifiant strains was observed in rhizosphere soil communities at flowering, and decreased at maturity stage. However, no significant differences were observed in the proportion of denitrifiers between evolution stages in the root and bulk soil communities.

Among the 7 studied secretion activities, 6 were found to be affected by evolution stage:
Proportion of C_4-C_8 and C_6-C_{12} Nahls producing strains in root and rhizosphere soil communities were higher at the beginning of plant development than at the later evolution stages. Interestingly, same tendency was observed for bulk soil communities but only for C_6-C_{12} Nahls.
A significant decrease in the proportion of exopolysaccharides producing strains was observed after flowering in the root and rhizosphere soil communities. This decrease was also observed in the bulk soil communities but in this case it appears immediately after the first evolution stage.
In the root-associated communities, the highest proportion of auxin producing strains was observed at the second evolution stage and decreased at flowering and maturity stages.

In the root communities, the highest proportion of siderophores producing strains was observed at flowering and decreased at maturity stage, a higher proportion of Ca-phosphate solubilising strains was also observed at the first evolution stage.
In all fractions, differences were observed between the different development stages for proportion of phytases producing strains (growth on angle medium P-free with Na-IHP as sole P source), but no general tendency was highlighted.

Temporal variation was visible on root, rhizosphere and bulk soil communities for 3 of the 10 affected traits (Eps+, Nahl+ C_6-C_{12} and Gram+), on root and rhizosphere soil communities for 2 of these 10 traits (Nahl+ C_4-C_8 and Oxyd+), and was visible only on root communities for 4 of these bacterial traits (Rednit+, Sid+, Psb+ and Iaa+). As observed for plant cultivar influence, Denit+ was affected by plant development stage only in the rhizosphere soil.

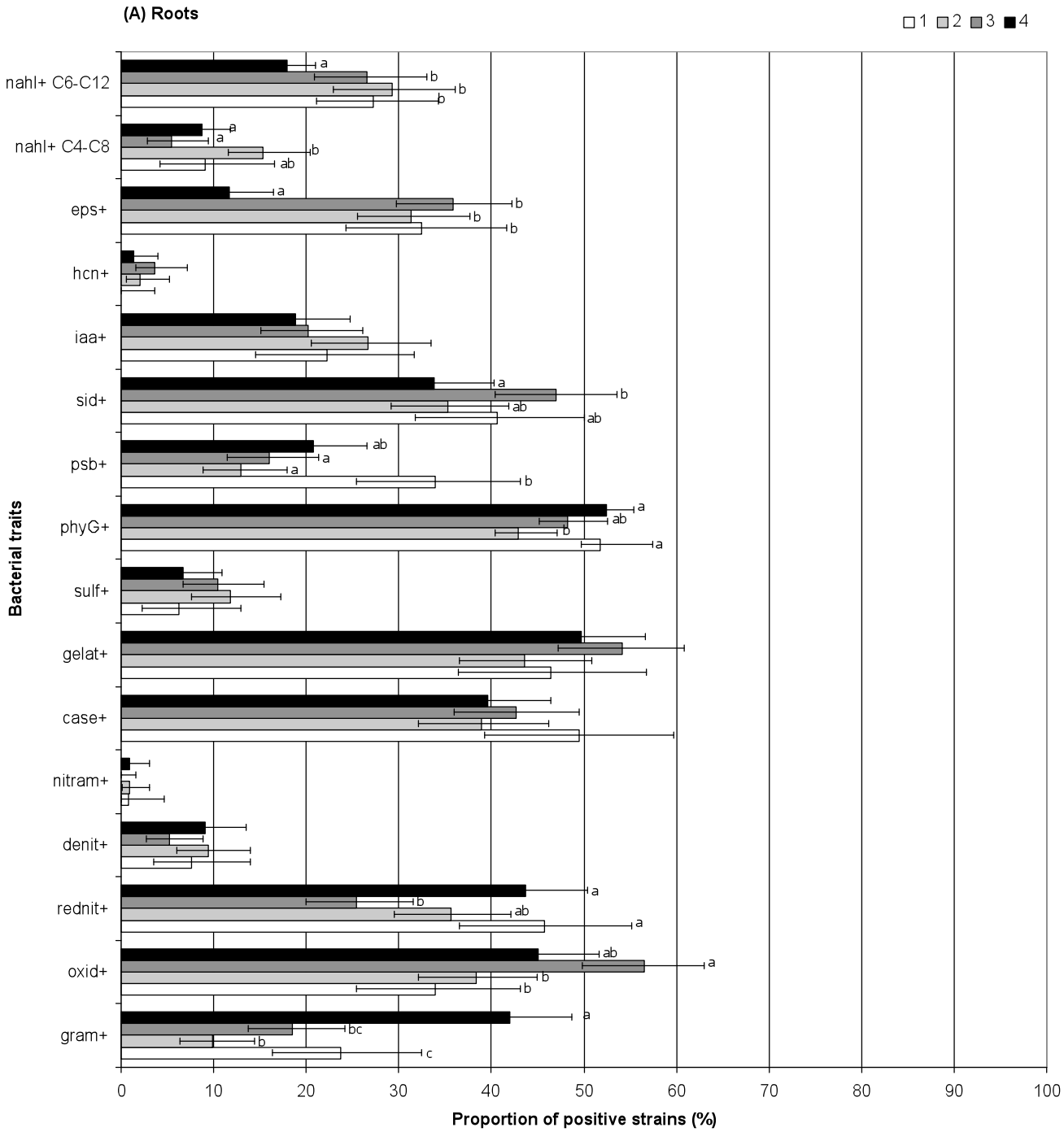
Table II.7. Changes of the frequencies of each bacterial trait, in relation with plant development and soil evolution.

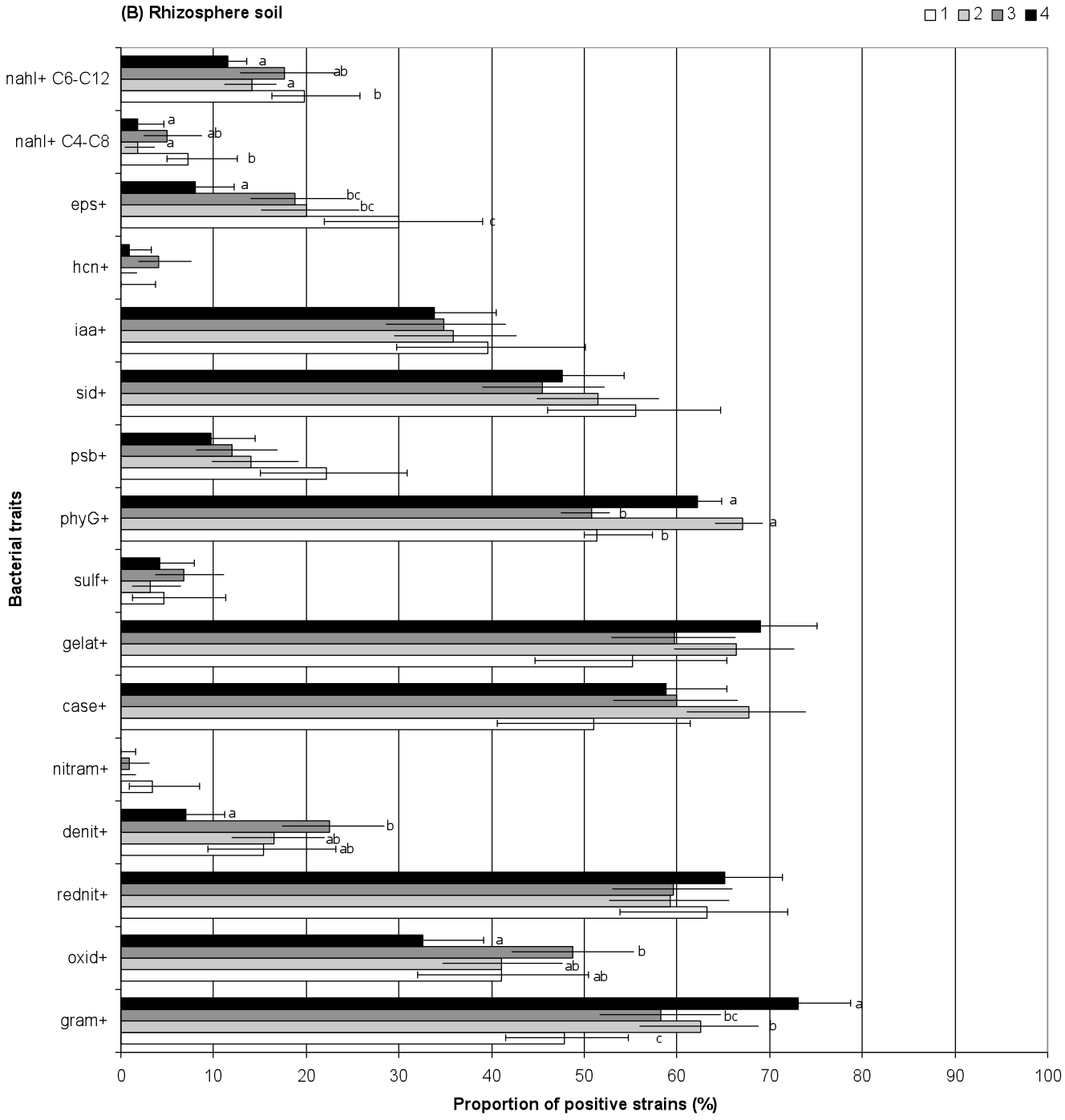
Differences in the proportion of positives strains isolated from the root and rhizosphere soil communities of the four evolution stages (hypocotil development: 1, four leaves: 2, ear development: 3, marurity: 4), independently from the plant cultivar. Arrows indicate a significant (* p≤0.05, ** p≤0.01, *** p≤0.001) increase (⇧) or deacrease (⇩) between proportions of positive strains of the compared cultivars.

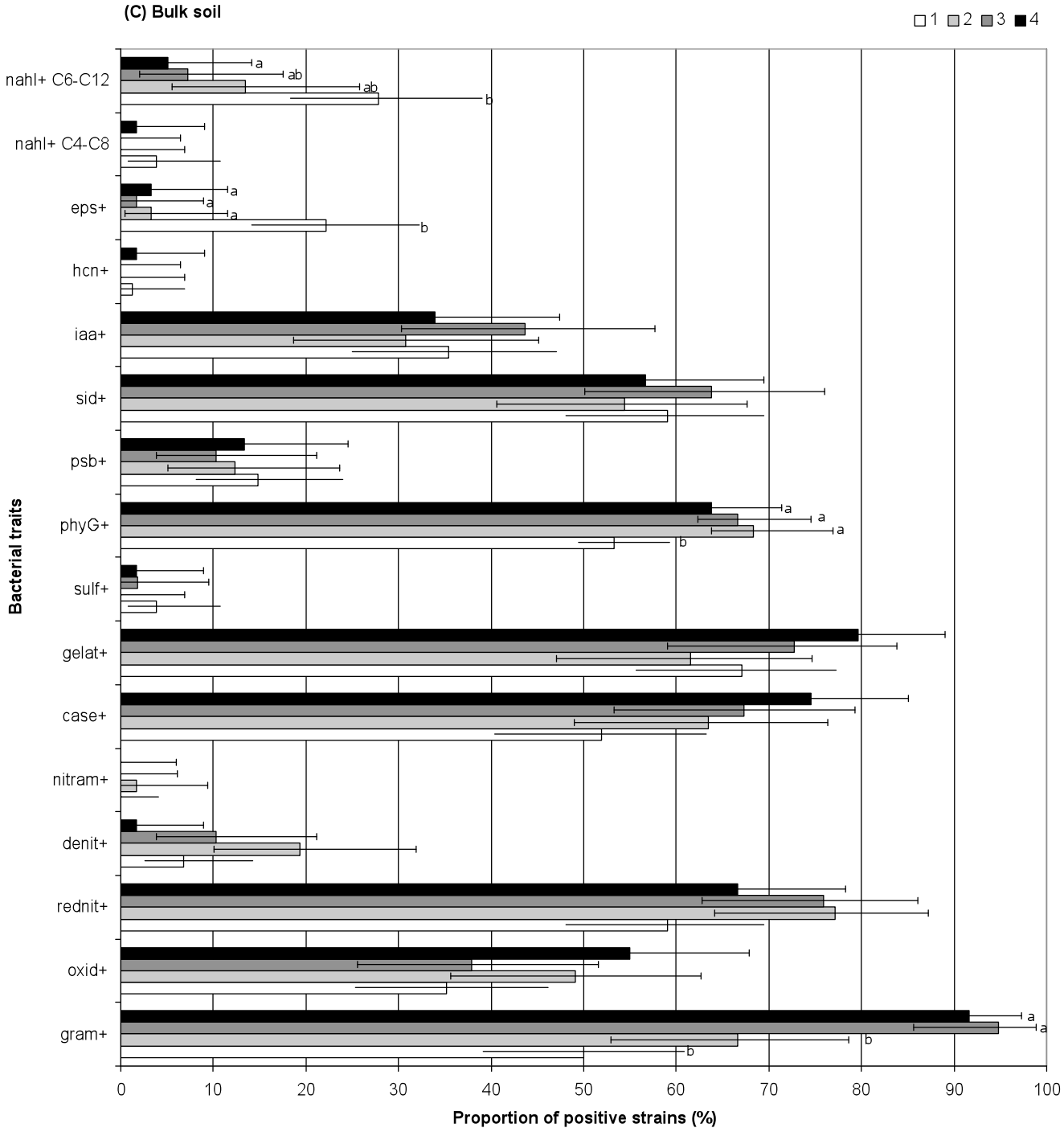
	Root communities					Rhizosphere soil communities					Bulk soil communities				
		1	2	3	4		1	2	3	4		1	2	3	4
Nahl+ C6-C12	1	X	-	-	⇧*	1	X	⇧*	-	⇧*	1	X	-	-	⇧*
	2		X	-	⇧⇧***	2		X	-	-	2		X	-	-
	3			X	⇧*	3			X	-	3			X	-
	4				X	4				X	4				X
Nahl+ C4-C8	1	X	-	-	-	1	X	⇧*	-	⇧**	1	X	-	-	-
	2		X	⇧***	⇧*	2		X	-	-	2		X	-	-
	3			X	-	3			X	-	3			X	-
	4				X	4				X	4				X
Eps+	1	X	-	-	⇧***	1	X	-	-	⇧***	1	X	⇧**	⇧***	⇧**
	2		X	-	⇧***	2		X	-	⇧**	2		X	-	-
	3			X	⇧***	3			X	⇧*	3			X	-
	4				X	4				X	4				X
Sid+	1	X	-	-	-	1	X	-	-	-	1	X	-	-	-
	2		X	-	-	2		X	-	-	2		X	-	-
	3			X	⇧*	3			X	-	3			X	-
	4				X	4				X	4				X
Psb+	1	X	⇧***	⇧**	-	1	X	-	-	-	1	X	-	-	-
	2		X	-	-	2		X	-	-	2		X	-	-
	3			X	-	3			X	-	3			X	-
	4				X	4				X	4				X
PhyG+	1	X	⇧**	-	-	1	X	⇩***	-	⇩**	1	X	⇩**	⇩**	⇩**
	2		X	-	⇩*	2		X	⇧**	-	2		X	-	-
	3			X	-	3			X	⇩**	3			X	-
	4				X	4				X	4				X
Denit+	1	X	-	-	-	1	X	-	-	-	1	X	-	-	-
	2		X	-	-	2		X	-	-	2		X	-	-
	3			X	-	3			X	⇧**	3			X	-
	4				X	4				X	4				X
Rednit+	1	X	-	⇧**	-	1	X	-	-	-	1	X	-	-	-
	2		X	-	-	2		X	-	-	2		X	-	-
	3			X	⇩***	3			X	-	3			X	-
	4				X	4				X	4				X
Oxyd+	1	X	-	⇩***	-	1	X	-	-	-	1	X	-	-	-
	2		X	⇩***	-	2		X	-	-	2		X	-	-
	3			X	-	3			X	⇧*	3			X	-
	4				X	4				X	4				X
Gram+	1	X	⇧*	-	⇩**	1	X	⇩*	-	⇩***	1	X	-	⇩***	⇩***
	2		X	-	⇩***	2		X	-	⇩*	2		X	⇩**	⇩**
	3			X	⇩***	3			X	⇩**	3			X	-
	4				X	4				X	4				X

Figure II.15. Proportion of positive isolates: Temporal evolution

Proportion of positive strains among (A) root, (B) rhizosphere soil, and (C) bulk soil communities corresponding to the four development stages (hypocotil development: 1, four leaves: 2, ear development: 3, ear maturity: 4), independently from the plant cultivar, for the seexteen bacterial traits investigated. Letters indicating significant differences are not mentioned for traits presenting no significant differences between the four stages.




(B) Rhizosphere soil
1 2 3 4
nahl+ C6-C12
nahl+ C4-C8
eps+
hcn+
iaa+
sid+
psb+
phyG+
sulf+
gelat+
case+
nitram+
denit+
rednit+
oxid+
gram+
Bacterial traits
0 10 20 30 40 50 60 70 80 90 100
Proportion of positive strains (%)


(C) Bulk soil
1 2 3 4
nahl+ C6-C12
nahl+ C4-C8
eps+
hcn+
iaa+
sid+
psb+
phyG+
sulf+
gelat+
case+
nitram+
denit+
rednit+
oxid+
gram+
Bacterial traits
0 10 20 30 40 50 60 70 80 90 100
Proportion of positive strains (%)

II.3.3. SYNTHESIS AND DISCUSSION: BACTERIAL COMMUNITIES

II.3.3.1. PLANT CULTIVAR IMPACT ON RHIZOSPHERE BACTERIAL COMMUNITIES STRUCTURE

In the first experimental part (see chap. II.3), the genomic and functionnal structure of bacterial communities has been investigated in the rhizosphere of four cultivars of *L. perenne* in order to determine the impact of plant cultivar on these community structures. Total and metabollicaly active communities were assessed using genomic approaches (see chap. II.3.1), and total heterotrophe aerobic cultivable communities using in vitro functionnal characterisation approach (see chap. II.3.2).
An influence of plant cultivar and development stage has been observed on community structure using the three approaches. In every case both cultivar and development stage influences were shown to be independant, indicating no differentiation of the plant cultivars at the level of their development. Using genomic approaches, impact of plant cultivar was more important than impact of development stage, however both effects were quite equivalent using functional approach.

Plant cultivar was shown to affect genotypic structure of metabollicaly active communities and, in a less extent of total community. The higher impact of plant-mediated changes observed here on the genotypic structure of active compared to total communities was not surprising. Indeed, the metabollic activity of most of the populations in the rhizosphere may be based on root exudates comsumption. Whereas RNA-based community profiles highlight active bacterial populations at the time of the sampling, DNA-based profiles display the most abundant populations independently of their current activity. The active part of the community may thus be more affected by changes in root exudation than total community, whatever the source of the modification (i.e. plant genotype or development stage, global climate change). Moreover, other studies on various systems, including perennial meadows (Jossi *et al.,* 2006), have reported similar discrepancies: Koizumi *et al.* (2003) observed higher differences in the structure of RNA-based community profiles between the upper and the lower layers of a lake sediment, and Mahmood and Prosser (2006) obtained quicker, finer-scaled and more reproducible shifts following treatment in ammonia-oxidizing RNA-based community profiles than in DNA-based community profiles.

These observations confirm the necessity of the inclusion of RNA-based fingerprinting techniques to the more commonly used DNA-based techniques, in order to gain a more complete understanding of the structure and function of bacterial communities.

No important differences between the four cultivars of *L. perenne* were observed on diversity and evenness of the dominant populations wathever the community considered, indicating, as already reported in the rhizosphere of *Thlaspi caerulescens* (Gremion, 2003), that plant did not exert a major influence on the overall bacterial diversity. It could thus be hypothetised that an equal number and proportion of the species should be necessary for the maintaining of rhizosphere functioning for the four cultivars. Taking into account the saturation state, which could occur when using diversity index calculations, and the methodological limitations linked with metagenomic approaches, we are not able to affirm that plant-mediated influences have no impact on rhizosphere population richness. But our results nevertheless shows, as already reported in the rhizosphere of *Thlaspi caerulescens* (Gremion, 2003), that plant did not exert a major influence on the overall bacterial diversity, and mainly affects the activity and abundance of specific populations.

Identification of the characteristic populations of communities associated to the different cultivars would be a further step for a closer investigation of the specific populations which fluctuate between the different cultivar associated communities.

Slight but numerous differences were noticed between functional structure of cultivable communities associated to the four cultivars (2n : Cavia, Lipresso; 4n: Anaconda, Bastion). This indicates that changes are moderate but affects many different functional groups. Indeed, 8 of the 16 bacterial trait investigated were affected by cultivar type. Differences were observed between all the cultivars, but Anaconda cultivar nevertheless presented the more extreme cultivable communities. Frequency of siderophore producing populations was notably higher in root fraction of Anaconda than in those of other cultivars. This could indicate a reduced competition for iron due to lower iron requirement of the plant tissues for this cultivar compared to others (Crowley, 2000). Moreover, its root and rhizosphere soil communities were characterised by a higher frequency of nitrate reducing populations, and a lower proportion of gelatin hydrolysing populations than those of other cultivars. Taking into account that competition between plant and bacteria for nitrate could inhibit nitrate reducing populations (Fromin et al., 2005), and that gelatin hydrolysis improves N mineralisation (Badalucco *et al.,* 1996; Marcote *et al.,* 2001), both results indicates a higher nitrate availability and a lower competition for this nutrient in the rhizosphere of Anaconda compared to other cultivars. Considering the high nitrogen requirement of *L. perenne* and the problems encountered with chemical fertilisation, this particularity of Anaconda cultivar could be an important advantage.

A higher impact of plant cultivar was observed on rhizosphere soil than on root total and active communities, but the inverse tendency was observed on cultivable community functional structure. This difference may be due to the root-oriented choice of the tested bacterial abilities. Indeed, the main part of the choosen traits were previously shown to be implicated in root competence and plant-bacteria interactions, and were supposed to be more influenced at root contact than in the rhizosphere soil. Among the 8 cultivar dependant traits only 2 (Rednit+, Gelat+) were affected by cultivar type in both root and rhizosphere soil communities. Impact of plant cultivar was visible only on root communities for 4 of these 8 bacterial traits (Case+, Sid+, Iaa+, Nahl+ C_6-C_{12}). Interestingly some bacterial traits (Denit+, Gram+) are only affected by plant cultivar in the rhizosphere soil communities.

Wathever the approach used for the investigations, the impact of cultivar on bacterial community structure seems to be independant from the plant ploïdy.

II.3.3.2. PLANT DEVELOPMENT STAGE IMPACT ON BACTERIAL COMMUNITY STRUCTURE

Global analyses of community profiles from the different evolution stages (see chap. II.3.1) showed no general tendency among total, active or cultivable communities, however the genomic approach based on 16S rRNA showed a clear separation between root active communities corresponding to the two first stages and those corresponding to the two last stages.

As for the different plant cultivars (see chap. II.3.3.1), no important differences between the plant development stages were observed on bacterial diversity and evenness of the dominant populations wathever the community considered, confirming that plant did not exert a major influence on the overall bacterial diversity, and that an equal number and proportion of the species should be necessary for the maintaining of rhizosphere functioning wathever the plant cultivar and development stage. It also suggest that plant-mediated influences affects more the activity and abundance of specific populations than the global diversity.

Identification of the characteristic members of communities associated to the different development stages would be a further step for a closer investigation of the specific populations which fluctuate during plant growth.

When regarding functional approach of cultivable heterotrophic communities a clear temporal variation was visible for 10 of the 16 bacterial traits studied (see chap. II.3.2).

Temporal variation was visible on root, rhizosphere and bulk soil communities for 3 of these 10 traits (Eps+, Nahl+ C_6-C_{12} and Gram+), on root and rhizosphere soil communities for 2 of these 10 traits (Nahl+ C_4-C_8 and Oxyd+), and was visible only on root communities for 4 of these bacterial traits (Rednit+, Sid+, Psb+ and Iaa+). As observed for plant cultivar influence, Denit+ was affected by plant development stage only in the rhizosphere soil.

At the beginning of plant development, root and rhizosphere soil bacterial communities were characterised by a higher proportion of strains able to produce Nahls. On the other hand, bacterial communities corresponding to the last plant development stage (ear maturity) presented the lowest proportion of glucan producing strains and the highest proportion of Gram positive strains in root and rhizosphere soil fractions.

The frequency of bacterial abilities which were shown to be favoured by root presence (see chap. II.5.4) generally tended to be higher among communities corresponding to early plant development stages (before flowering), whereas frequencies of abilities related to soil were higher among communities of mature plants. This differentiation seems to indicate that the root influence is stronger at the beginning of plant growth than after flowering. Root-associated population abilities tend to gather with bulk soil abilities after this key stage. This may be due to the massive plant carbon allocation to the aboveground biomass, which appears at flowering stage, and reduces plant organic input in soil.

Wathever the approach used for the investigations, the impact of plant development stage on bacterial community structure seems to be independant from the plant ploïdy.

II.4. INFLUENCE OF CULTIVAR TYPE ON RHIZODEPOSITION

II.4.1. CHANGES IN SOIL COMPOSITION

Total phosphorus (Ptot), available phosphorus (Pav), total nitrogen (Ntot), available nitrogen (NO_3, NH_4^+), organic matter content (OM), organic (TOC) and mineral (MINC) carbon percentages, and $pH_{[H2O]}$ and $pH_{[KCl]}$, which have been measured for initial soil, control soil incubated without plant and soil incubated with the different cultivars, are presented in table II.8. Data were checked for normality and analysed using Anova and Tukey Kramer HSD post hoc test for significiance.

After 7 month of incubation, pH of planted, as well as non-planted soil was significantly higher ($p < 0.05$) than pH of initial soil.

Nitrate content of the incubated soils, whatever they were planted or not, was lower ($p < 0.05$) than initial soil values. Soil planted with Anaconda cultivar presented a higher nitrate content ($p< 0.05$) than those planted with other cultivars and control soil without plant.
Plot representation of NO_3^- and NH_4^+ contents is presented in figure II.16, and highlight differentiation of Anaconda cultivar.

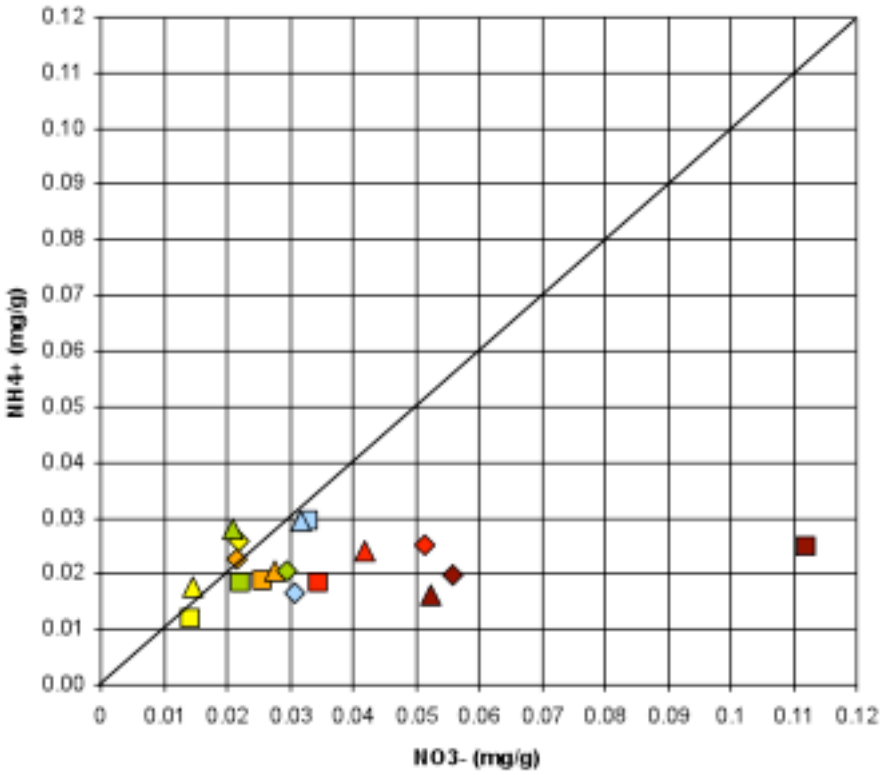
Other soil component measured here were constant during the experiment whatever the soil treatment considered.

Table II.8. Soil elements content and pH

pH, organic matter and nutrient content of initial soil, control soil incubated without plant, and soils after 7 month of cultivation of the different cultivars of *L. perenne* (Anaconda, Bastion, Cavia, Lipresso). Significant differences ($p<0.05$) are mentioned by small letters.

Soil	Initial soil	Control soil	Anaconda	Bastion	Cavia	Lipresso
pH [H_2O]	6.63 (0.04)^a	7.36 (±0.12)^b	7.45 (±0.1)^b	7.44 (±0.04)^b	7.33 (±0.08)^b	7.36 (±0.07)^b
pH [KCl]	5.66 (0.01)^a	6.37 (±0.18)^b	6.46 (±0.1)^b	6.43 (±0.07)^b	6.31 (±0.13)^b	6.37 (±0.08)^b
MO (%)	7.28 (±0.13)	7.36 (±0.35)	7.45 (±0.04)	7.52 (±0.16)	7.74 (±0.52)	7.57 (±0.53)
TOC (%)	2.55 (0.10)	2.45 (±0.14)	2.64 (±0.056)	2.66 (±0.171)	2.67 (±0.057)	2.57 (±0.043)
MINC (%)	0.28 (0.02)	0.31 (±0.01)	0.30 (±0.006)	0.31 (±0.026)	0.32 (±0.012)	0.34 (±0.035)
NH_4^+ (mg/g)	0.02 (0.00)	0.03 (±0.01)	0.02 (±0.004)	0.02 (±0.002)	0.02 (±0.007)	0.02 (±0.005)
NO_3^- (mg/g)	0.07 (0.03)^a	0.03 (±0.001)^b	0.04 (±0.008)^c	0.03 (±0.003)^b	0.02 (±0.004)^b	0.02 (±0.005)^b
Ntotal (mg/g)	3.35 (0.76)	3.38 (±0.43)	2.71 (±0.16)	2.83 (±0.34)	2.87 (±0.16)	2.91 (±0.27)
Pav (mg/g)	0.10 (0.00)	0.13 (±0.02)	0.09 (±0.004)	0.12 (±0.045)	0.12 (±0.006)	0.11 (±0.019)
Ptotal (mg/g)	0.54 (0.08)	0.63 (±0.06)	0.51 (±0.04)	0.54 (±0.07)	0.54 (±0.03)	0.52 (±0.03)

Figure II.16. Soil nitrate (NO_3^-) and ammonium (NH_4^+) contents. The three replicate samples (square, triangle, rhombus) are represented for initial soil (brown), soil cultivated with Anaconda (red), Bastion (orange), Cavia (yellow) and Lipresso (green) cultivars, and control soil incubated without plant (blue).



II.4.2. CHANGES IN ROOT EXUDATION

II.4.2.1. OVERVIEW OF THE RESULTS

The two series of 30 exudates samples corresponding to the five replicates obtained from intact plants and cut off roots of the three cultivars (the fourth cultivar was missing because of a shortage of seeds) were submitted to HPLC analyses for organic acids (see chap. II.4.2.2) and phenolics (see chap. II.4.2.3) patterns establishment. We also try to characterise the amino acids exudation pattern of each cultivar. We achieved the separation of 20 standard amino acids, but whatever the collection method used, the quantities exuded by plant root were so low that we could not detect it with UV detection system.

HPLC profiles of organic acids and phenolics were numerised, and the obtained matrix containing for each sample the retention time and area of each peak were analysed. Standards of the main organic acids found in the rhizosphere were also submitted to HPLC analyses in order to establish a correspondance with the results obtained for root exudates.

As described for bacterial communities investigations, analyses of organic acids and phenolics profiles were performed in three steps: (i) to determine the importance of plant cultivar and collection method in the composition of root exudates, organic acids and phenolics exudation profiles were submitted to variation partitioning. (ii) to allow the distinction between exudates collected from intact plants or cut off roots of the different cultivars, the organic acids and phenolics secretion patterns were in a second step investigated, using PCA ordination analyses. (iii) to highlight the compounds which differs between the cultivars and collection methods, histogramms of the secreted compounds were then established based on the HPLC-chromatogram of the five replicates of each sample, and standard deviation was calculated. For organic acids, the identification of the key compounds was performed based on data obtained from standards acids analyses.

II.4.2.2. ORGANIC ACIDS SECRETION PATTERNS

HPLC analyses were performed on root exudates samples in order to determine the organic acids secretion pattern for each cultivar. Eleven acids were used as standards for identification of the secreted compounds (table II.9).

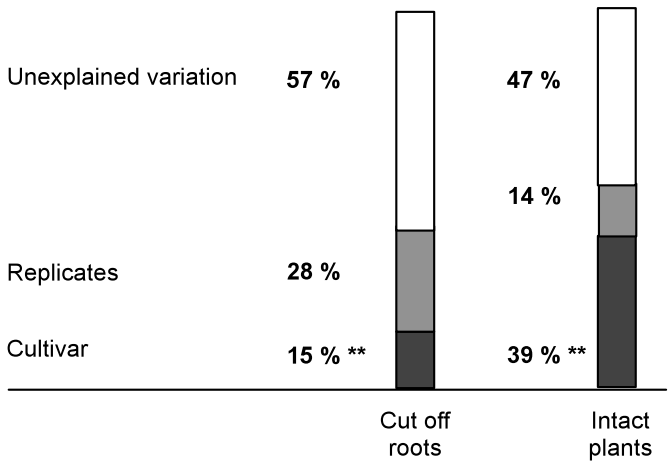
Table II.9. Organic acid standards used for HPLC analyses

N°	Organic acid	Retention time (min)	N°	Organic acid	Retention time (min)
(1)	oxalic acid	6.2	(8)	formic acid	13.0
(2)	citric acid	7.4	(9)	acetic acid	14.1
(3)	gluconic acid	7.9	(10)	glutaric acid	23.3
(4)-(5)	maleic and malic acids	8.9 (15.9)	(11)	gallic acid	34.1
(6)-(7)	lactic and succinic acids	11.5			

Partial RDA of the obtained organic acids profiles from the different cultivars (figure II.17) showed a significant difference (p=0.008) between the cultivars on samples obtained from intact plants (39% of profiles variability explained by the cultivar type) and from cut off roots (15% of profiles variability explained by the cultivar type).

Figure II.17. Variation partitioning for organic acids secretion pattern : Impact of plant cultivar

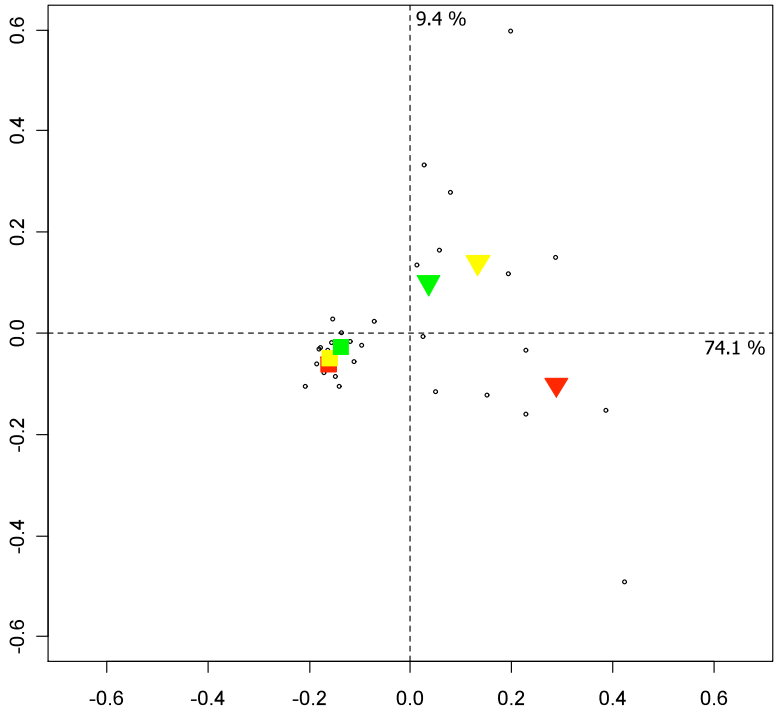
Variation partitioning on data obtained from HPLC-UV chromatograms (210 nm) corresponding to root exudates from cut off root and intact plants. Percentages correspond to the part of organic acids profiles variation explained by plant cultivar or replication (Monte Carlo test for significance, * p≤0.05, ** p≤0.01, *** p≤0.001), and the part of the variation which is not explained by these two variables.



PCA analysis showed also a higher variability between organic acids secreted from intact plants than from cut off roots of the different cultivars (figure II.18). PCA revealed that among samples collected from intact plants, Anaconda cultivar present a distinctive organic acids secretion profile.

Figure II.18. Principal component analysis for organic acids secretion patterns : Impact of plant cultivar

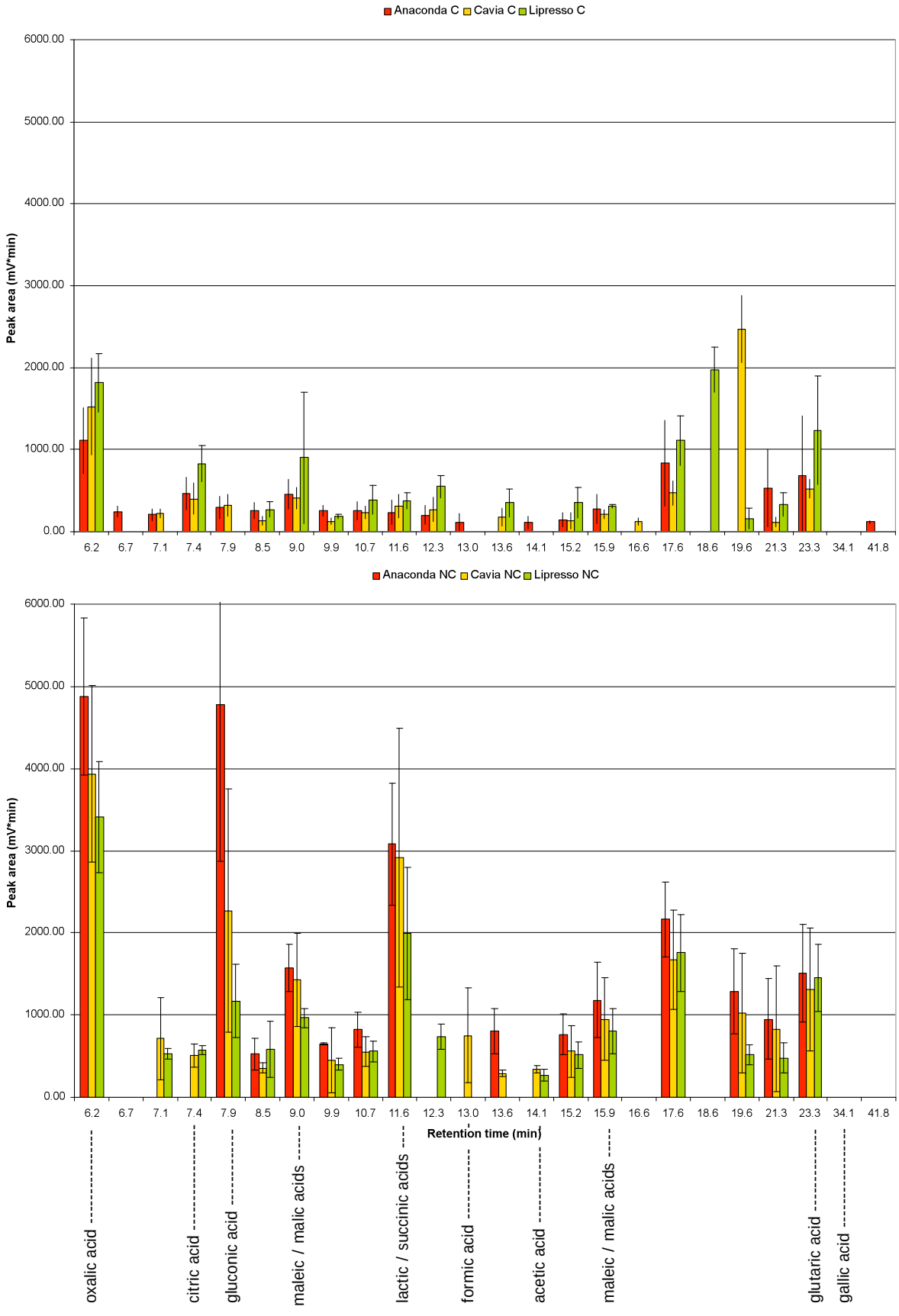
Plot of the principal component analysis (PCA) of organic acids HPLC profiles obtained from cut off roots (squares) and intact plants (triangles) of each cultivar (Anaconda : red, Cavia: yellow, Lipresso: green).



Organic acids profiles were different when collected from cut off root or intact plants (figure II.17 / II.18). The number of compounds was higher for cut off roots than for intact plants (figure II.19), however, for the same root biomass, the quantities of the organic acids exuded were globally higher when collected from intact plants than from cut off roots. HPLC-UV chromatogramm obtained from intact plants of Anaconda cultivar present some missing compounds (RT1 = 7.1 / RT2 = 7.4 / RT3 = 14.1) wich were present in the secretion profiles from the other cultivars (figure II.19). Two of these three compounds were identified as citric and acetic acids (table II.9). Some other compounds are discriminant between the different cultivars for both cut off roots and intact plants exudates, but they seem to be less important because no clear differences were observed on PCA plot for these cultivars.

Figure II.19. Organic acids secretion patterns for each cultivar

Histogramm based on HPLC-UV Chromatogramm (210 nm) of the five replicates obtained from cut off roots (C) and from intact plants (NC). Quantities (peak area) of each compound correspond to thoses exuded by 130 mg of fresh roots.

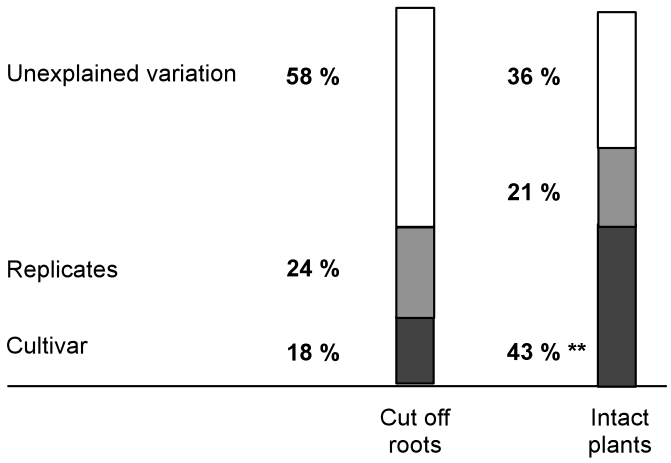


II.4.2.3. PHENOLIC COMPOUNDS SECRETION PATTERNS

Partial RDA of phenolic compounds profiles secreted from the different cultivars (figure II.20) showed a significant difference (p=0.004) between the cultivars only on samples obtained from intact plants (43% of profiles variability explained by the cultivar type). No significant differences were observed between profiles obtained from cut off roots of the different cultivars.

Figure II.20. Variation partitioning for phenolic compounds secretion pattern: Impact of plant cultivar

Variation partitioning on data obtained from HPLC-UV chromatograms (263 nm) corresponding to root exudates from cut off root and intact plants. Percentages correspond to the part of phenolic compounds profiles variation explained by plant cultivar or replication (Monte Carlo test for significance, * p≤0.05, ** p≤0.01, *** p≤0.001), and the part of the variation which is not explained by these two variables.

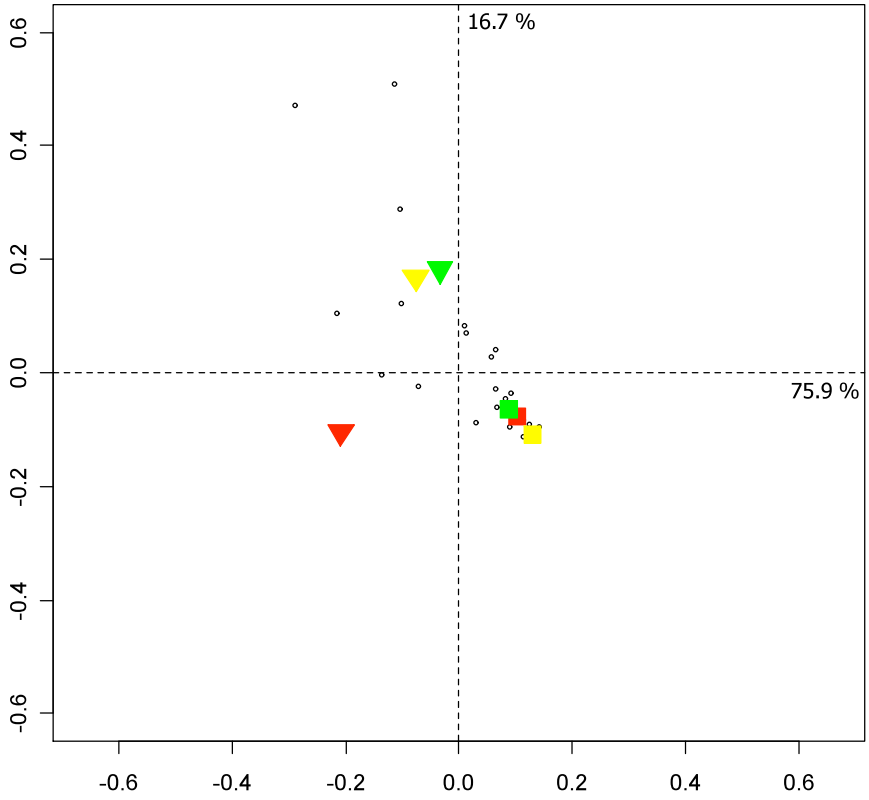


As observed for secretion organic acids,PCA analysis showed a higher variability between phenolic compounds secreted from intact plants than from cut off roots of the different cultivars (figure II.21). PCA revealed also that among samples collected from intact plants, Anaconda cultivar presents distinctive phenolics secretion profile.

Phenolic compounds profiles were very different when collected from cut off root or intact plants (figure II.21 / II.22), the number of compounds was higher for cut off roots than for intact plants. Most of the compounds which were detected only in exudates collected from cut off root, have a high retention time (RT > 28 min), and are present in small quantities. Globally, as observed for organic acids, the quantities of the phenolic compounds secreted were higher when collected from intact plants than from cut off roots.

Figure II.21. Principal component analysis for phenolic compounds secretion patterns: Impact of plant cultivar

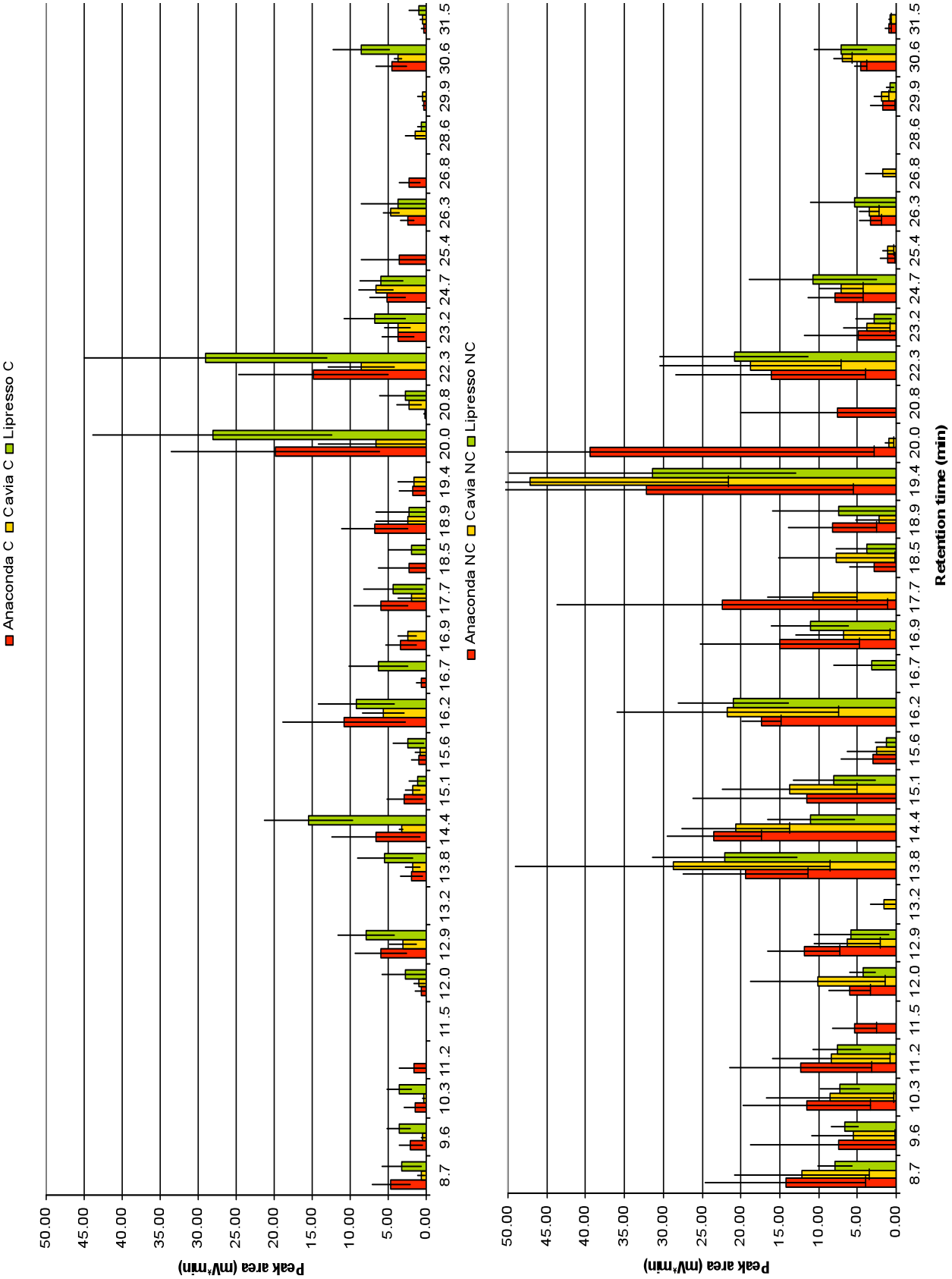
Plot of the principal component analysis (PCA) of phenolic compounds HPLC profiles obtained from cut off roots (squares) and intact plants (triangles) of each cultivar (Anaconda: red, Cavia: yellow, Lipresso: green).



HPLC-UV chromatogramm obtained from intact plants of Anaconda cultivar present two phenolic compounds (RT1 = 11.49, Peak area = 5.35 / RT2 = 20.08, Peak area = 7.64) wich were relatively abundant and were not found in profiles from the other cultivars (figure II.22). As already observed for organic acids, some other compounds are discriminant between the different cultivars for both cut off roots and intact plants exudates, but they seems to be less important because no clear differences were observed on PCA plot for these cultivars.

Figure II.22. Phenolic compounds secretion patterns of each cultivar.

Histogramm based on HPLC-UV Chromatogramm (263 nm) of the five replicates obtained from cut off roots (C) and from intact plants (NC). Quantities (peak area) of each compound correspond to thoses exuded by 130 mg of fresh roots.



II.4.3. SYNTHESIS AND DISCUSSION: RHIZODEPOSITION

In the second experimental part of this chapter (see chap. II.4), soil modifications after plant growth were first investigated (see chap. II.4.1) in order to evaluate the nutrient status in the rhizosphere of the different cultivars. Root exudates of the cultivars were then characterised (see chap. II.4.2) in order to evaluate their differenciation and its potential influence on bacterial communities. Emphasis was put on organic acids and phenolic compounds secretion patterns, which constitute a large part of root exudates, seems to be dismissed from plant re-absorption (Jones *et al*., 2004, Hodge *et al.,* 1998), and have already been shown to influence bacterial community structure (Singh and Mukerji, 2006; Baudoin *et al.,* 2001; Weisskopf *et al.,* in prep).

Whatever the cultivar, no significant differences in pH between planted and unplanted soils were detected. pH differences measured in soils, at the beginning and the end of the experiment, are explained by the use of calcareous tap water for irrigation.
Soil analyses, which were performed before and after growth of each plant cultivar, revealed a higher nitrate content in soil cultivated with Anaconda than with other cultivars. The difference was slight and this founding as to be verified, but this result indicates that nitrate availability could be higher in the rhizosphere of Anaconda compared to other cultivars.

Comparison of organic acids and phenolic compounds in root exudates evidence quantitative and qualitative differences between the plant cultivars, in particular for exudates obtained from intact plants of Anaconda. Cultivar-induced modifications of organic acids and phenolics exudation patterns, were clearly visible for intact plants but was quite absent when regarding profiles of exudates collected from cut off roots. This indicates that most of the organic acids and phenolic exudation differences between the cultivars depends on plant signalling. For both organic acids and phenolics, the number of exuded compounds was generally lower, and their amount was higher, for intact plants than for cut off roots. The higher number of collected compounds for cutt off roots compared to intact plant might be due to the release of intracellular constituants from the cutting. In a general way, quantitative and qualitative changes between the two exudate collection methods could be linked to the perturbation of plant signalling due to the cutting of roots, suggesting that the main part of exuded compounds are released by mecanisms dependent of the plant integrity. However, these changes between intact plant and cut off roots exudates could also be due to modifications of the root re-absorption processes. It therefore seems to be necessary to keep the plant integrity to have a more realistic characterisation of root exudates.

HPLC-UV chromatogramm obtained from intact plants of Anaconda cultivar present two phenolic compounds wich were not found in profiles from the other cultivars. Phenolic compounds were shown to influence growth and development of surrounding plants and soil microorganisms, and to play a role in a number of plant-microbes associations such as legume-Rhizobium symbiosis (Bertin *et al.,* 2003; Jones *et al.,* 2004).). Moreover difference has already been observed between phenolic acids exudation from different cultivars of *Triticum aestivum* L. (Wu *et al.,* 2001).

For organic acids, HPLC-UV chromatogramm obtained from intact plants of Anaconda cultivar present three missing compounds wich were present in the secretion profiles from all the other cultivars. Two of these missing compounds were identified as citric acid (triacid) and acetic acid (monoacid). Citrate has already been documented as important in phosphate mobilisation from inorganic sources (Gerke *et al.*, 1994; Jones and Darrah, 1994; Hinsinger, 1998; Jones, 1998; Weisskopf, 2005) by complexation of the cations in soil. It was also reported to increase Fe availability from mineral surfaces (Ström *et al.,* 1994), and from Fe-tri-hydroxyde (Jones and Darrah, 1996). Like citrate, but in a less extent, acetate is implicated in P availability through acidolysis (Fox *et al.,* 1990).
Organic acids have been reported to have an influence on root selection of bacterial populations (Jones *et al.,* 2004; Weisskopf *et al.,* in press). The differentiation of exudation pattern highlighted in the case of Anaconda cultivar should therefore be visible on associated bacterial communities. Cultivation of our bacterial isolates, as well as incubation of soil samples, in presence and in absence of the identified compounds, will certainly provide additonnal information's about the impact, on bacterial communities, of the differences observed on root exudates.

Both soil and exudates approaches showed that the rhizosphere of Anaconda cultivar may be different from those of the other cultivars. But when exudates were collected from cut off roots, no important differences in the exudation of the different cultivars were retrieved. But when collected from intact plants, exudates of the cultivars studied here were clearly shown to differ, indicating that the maintaining of plant control is essential to detect exudation variations. Further investigation of other component of the root exudates, such as amino acids and sugars, will be usefull to complete these results.

II.5. SYNTHESIS AND DISCUSSION

In this chapter we investigate the structure of bacterial communities in the rhizosphere of four *L. perenne* cultivars, in relation with plant development stage and fraction (see chap. II.3.3). We also investigate cultivar impact on root exudation and soil nutrient content (see chap. II.4.3). The aim was to answer the following questions:

(i) Do plant cultivar have an influence on bacterial community structure ?
(ii) Do plant cultivar influence nutrient content and conditions of their surrounding soil ?
(iii) Do plant cultivar have different organic acids and phenolic compounds exudation patterns ?
(iv) Are the differences observed in root environment consistant with those observed on bacterial community structure ?
(v) Which bacterial functions are characteristic of root and soil bacterial communities ?
(vi) Which relationship exists between these functions ?
(vii) How plant development influence bacterial community structure ?

Three approaches have been used to investigate the influence of plant cultivar and development stage on rhizosphere bacterial communities structure (i) genomic approach based on 16S rDNA community profiles was used for investigation of the total bacterial communities (see chap. II.3.1), (ii) genomic approach based on 16S rRNA community profiles was used for investigation of the metabolically active bacterial communities (see chap. II.3.1), and (iii) functionnal approach based profiles of communitiy potential abilities was used for investigation of culturable heterotrophic bacterial communities (see chap. II.3.2).
In contrast with the non-orientation of the two genomic approaches, the functional approach was oriented on bacterial traits implicated in nutrient cycles and plant-bacteria interactions.

Investigations have been performed for root and rhizosphere communities associated to four cultivars of *L. perenne* at four plant development stages, and in parallel for control soil (without plant) at four evolution stages.

Nutrient, organic matter content and pH of initial soil, of soil after growth of each cultivar and soil incubated without plant were determined (see chap. II.4.1). Two groups of components of root exudates were also characterised for the different cultivars: (i) organic acids (see chap. II.4.2), and (ii) phenolics (see chap. II.4.2).

II.5.1. BACTERIAL COMMUNITY STRUCTURE AND RHIZOSPHERE ENVIRONMENT

Genomic approaches revealed that plant did not exert a major influence on the overall bacterial diversity. It could thus be hypothetised that an equal number and proportion of the species should be necessary for the maintaining of rhizosphere functioning for the four cultivars. Indicating that plant cultivar affects more the activity and abundance of specific populations than the global diversity. The identification of the characteristic populations of communities associated to the different cultivars would be a further step for a closer investigation of the specific populations which fluctuate between the different cultivar associated communities.

The impact of plant on the structure of rhizosphere bacterial communities has been further assessed by correlation of the cultivar-induced modifications detected in the functional structure of culturable bacterial communities (see chap. II.3.2), and of the differences detected in soil (see chap. II.4.1) and root exudates patterns (see chap. II.4.2).

Nitrogen content was higher in the rhizosphere of Anaconda cultivar compared to others, suggesting that the frequencies of nitrate-reducing and of protease-producing populations retrieved in the rhizosphere of the different cultivars would also be higher. This hypothesis has been confirmed by in vitro abilities of bacterial isolates. Several hypotheses could be formulated to explain this difference in nitrate availability. It could be linked: (i) to a higher plant N input in soil (i.e. free amino acids), (ii) to a higher level of N fixation, (iii) to mineralisation, (iv) to a lower N absorption by the plant, and (v) to differences among the protozoa community structure, which also have an implication here considering that bacteria grazing by protozoa have an impact on N mineralisation. Indeed, bacterial predators have already been shown to alter the balance in nitrogen competition between plant and bacteria and to increase plant nitrogen uptake (Clarholm, 1985).

Additionnaly, considering that citrate is missing in the exudates of Anaconda cultivar, the higher frequency of isolates able to produce siderophores which was found in root communities of Anaconda could suggest that the enhancement of siderophore producing bacterial populations may compensate the lower Fe solubilisation which is related to the absence of citrate. On the same way, the absence of citrate and acetate in the exudates of Anaconda cultivar may be compensated by the activity of phosphate solubilisers and organic phosphate hydrolysers. But althought no significant differences in available P content of soils after growth of the four cultivars was detected, no enhancement of the frequency of strains able to solubilise Ca-phosphate or to produce phytases has been reported in the root and rhizosphere soil communities associated to Anaconda compared to other cultivars. This suggests that other processes, such as “classical” phosphatase activity, could be here involved in P supply.

II.5.2. ROOT COMPETENCE: WHICH BACTERIAL TRAITS ARE DETERMINANT?

Rhizosphere effect on functional structure of communities was investigated by comparison of the structure of root, rhizosphere and bulk soil cultivable communities associated with the different plant cultivars and development stages (see chap. II.3), in order to determine which bacterial traits are important for root colonisation and rhizosphere competence. As it has been performed by Delorme (2001), bacterial abilities which were correlated with root proximity (frequency of the performing populations higher for root than for rhizosphere, and bulk soil, communities) were here considered as root related traits which are favoured by root presence and may be determinant for root competence.

Wathever the plant cultivar and the development stage considered, functional, as well as genomic structure of root communities were clearly different from those of rhizosphere and bulk soil communities. As observed by Weisskopf et al. (in prep), only a few differences were observed between rhizosphere and bulk soils. Bastion and Lipresso cultivars presented the higher differentiation between the functional structures of their respective root and rhizosphere soil communities. Moreover, a higher differentiation of the genomic structure of root and rhizosphere soil communities was also obseved for Bastion cultivar. Whatever the approach used, Cavia cultivar harboured the most similar root and rhizosphere soil communities, indicating a less extended plant influence.

Independently from the plant cultivar, N-acyl homoserine lactone, glucan, phytase and sulfatase production, and Ca-phosphate solubilisation were, on one hand, shown to be favoured at root proximity.

These results are consistant with similar studies on Ca-phosphate solubilisation, organic phosphate mineralisation (Rodriguez and Fraga, 1999), and sulfatase production (Kertesz and Mirleau, 2004). In the particular case of phytase and sulfatse producing strains, the results obtained here, which are based on potential abilities of isolates, are supported by in situ activities measurement. Indeed, Tarafdar and Junk (1987) and Knauff *et al.* (2003) documented that phosphatases and sulfatases activities respectively were enhanced at root proximity.

Additionnally, NAHL's are the main molecules implicated in quorum sensing of Gram negative bacteria and are involved in the regulation of many bacterial functions related to plant-bacteria interactions (Fuqua and Greenberg 2002; Miller and Bassler, 2001). They are in particular involved in the regulation of the production of exopolysaccharide, which are implicated in cell adhesion and protection (Squartini, 2000 ; Varma *et al.,* 2004). Elasri et al. (2001), which also

reported a higher frequency of NAHL producers in the rhizosphere than in the bulk soil, showed that frequency of NAHL producers was higher when a close relationship exists between the plant and its bacterial communities. It might be the case in our experiment, considering that the model plant used here is grown in a pre-adapted soil. Additionnaly, as Gram negative bacteria constitute the main part of root communities, the relationship observed between root environment and these abilities thus was not surpising.

On the other hand, soil communities were characterised by a higher frequency of gram positive strains and of nitrate-reducing strains, siderophore, proteases and auxin producers. As already admitted, Gram positive strains (strategists k) seems here to be favoured in the nutrient limiting conditions of the soil environment (Gobat *et al.,* 2004).

As reported by Delorme (2001), the frequency of denitrifiing populations was found to be higher in rhizosphere soil than in the bulk soil, but was also higher than in root communities.

As rhizosphere is generally a carbon rich environment, nutrient limiting condition are frequent due to root uptake and stimulation of the growth of microorganisms by root input. Regarding the loss of available N due to denitrification activity, and the competition between bacteria and plants in nitrate-limiting conditions (Fromin *et al.,* 2005), the decrease in the abundance of nitrate-reducing and denitrifiing strains observed here in the root fraction is consistant.

Protease activity could improve N supply, and in addition plant resistance. Indeed, it was reported to increase N mineralisation (Badalucco *et al.,* 1996; Marcote *et al.,* 2001), and to be involved in plant resistance against pathogenic fungi (Szekeres *et al.,* 2004). O'sullivan *et al.* (1991) showed that extracellular proteases production was not necessary for root colonisation, and in the present work, frequency of isolates presenting this ability was found to decrease from bulk soil to root. The proportion of protease-producing populations has nevertheless already been reported to be higher in the rhizosphere than in the bulk soil (Johanson and Binnerup, 2002). Moreover, Roesti (2005) reported that the ability to secrete proteolytic enzymes is an important trait related to mycorrhizal helper bacteria (MHB) capabilities.

Auxin is a well-known plant growth-promoting trait, however it may affect plant growth either positively at low concentrations or negatively at high concentrations (Arshad and Frankenberg, 1991; Persello-Cartieaux *et al.,* 2003). As already observed by Tarnawski *et al.* (2006), populations able to produce auxin were in this work shown to be less frequent at root proximity. As the detection of auxin in this study was performed *in vitro*, the frequency of isolates which produce it *in situ* could nevertheless be higher at root proximity than in the bulk soil. This indicates a selection of the root-associated populations based on their activity rate.

Surprisingly, siderophore production, which could be a well-known root competence trait (Tarnawski *et al.,* 2006; Delorme, 2001; Lugtenberg and Dekkers, 1999; Glick, 1995; Kloepper *et al.,* 1980), was found to decrease from bulk soil to root. Keeping in mind that the detection

of siderophore activity was here performed in vitro, but that populations would generally not harbour useless abilities, two hypothesis could be formulated to explain the lower proportion of isolates able to produce siderophores found at root proximity: (i) This result could be an indicator of iron non-limiting condition at root vicinity, implicating that siderophore production would not be an advantage either for bacteria or plants, and (ii) This result could also indicate iron-limiting conditions leading to competition between plant- and bacterial-native siderophores, and to the counter-selection of siderophore producing bacteria by plant.

Pseudomonas spp., which are often considered as rhizosphere-competent model populations, present a specific phenotype in the rhizosphere compared to other *Pseudomonas* spp. soil populations. Rhizosphere-competent *Pseudomonas* spp. were characterised by the particular efficiency of their pyoverdine-mediated iron uptake and by their ability to reduce nitrogen oxides (Latour *et al.,* 2003). This supports the hypothesis that soil iron content was not limiting and that competition for iron was low in our experiment.

Some of the most popular root competence traits (auxin and siderophore production) were in this experiment shown not to be the more representative traits of root competent strains, and presented a higher occurence in soil than in root communities. Indeed, in the present study, which includes *Pseudomonas* spp. strains but takes into acount the overall diversity of cultivables heterotrophic bacterial populations, somes other bacterial abilities were identified as more characteristics of root communities. The root competence potential of bacterial abilities probably also depends on the *in situ* activity rate of the population. However this suggests that *Pseudomonas* spp. present traits determinant for root competence, which differ from the determinant traits found here for the global community. Root competence potential of bacterial abilities thus may also differ in regards with the occupied ecological niche, and of the combination of the functional abilities, of the population considered.

A positive correlation has been demonstrated between: (i) NAHL, sulfatase and glucan production, (ii) casein and gelatin hydolysis, and (ii) siderophore production, nitrate reduction and auxin production abilities. This indicates that independently from their ecological niche, strains able to perform one of these function are generally able to perform the others, and suggests that the combination of these abilities could improve population competence.
These results highlight the multifactorial nature of root competence. Therefore some other bacterial abilities, such as production of antibiotics and resistance to toxic compounds, which were not taken into account in these experiment, could also be determinant for root competence.

II.6. CONCLUSIONS AND OUTLOOKS

The complex interactions pattern of the rhizosphere, the diversity of associated organisms and the redundancy of functions which characterise soil community lay down methodological complications and limits the interpretation of the obtained results. The multiplication of the approaches (points of view) seems to be necessary to understand global rhizosphere functioning.

Despite the recognised high genomic and functional divesity of microorganisms in the rhizosphere, studies on root selection processes and bacterial rhizosphere competence up to now rarely included a broad bacterial diversity. Bacterial abilities implicated in root colonisation and rhizosphere competence have frequently been studied on model PGPR or pathogenic groups, such as *Pseudomonadales*, *Rhizobiales* and *Bacillales* (Jjemba and Alexander, 1999; Delorme, 2001; Persello-Cartiaux *et al.,* 2003). The experiments described in this chapter were able to fill partly this gap of knowledges by the characterisation of the genomic and functional structures of the global bacterial root-associated communities .

With the combination of the different approaches used here, some general informations about *L. perenne* rhizosphere functionning were obtained. Moreover, the importance of plant cultivar and development stage impact on the structure of rhizosphere bacterial communities was determined, and the induced functional modifications were characterised.

Total and active communities approaches revealed that plant cultivar and development stage do not have a major effect on population diversity and affect mainly the relative abundance of populations. The selection and identification of metabollicaly active indicative populations will also be necessary for a more accurate characterisation of the changes occuring among these communities.

The most important differences observed between communities associated to the four plant cultivars were found to be linked to N and P and Fe cycling. The main result indicated a higher nitrate content in the rhizosphere of Anaconda cultivar compared to others, leading to a lower competition for N between plant and bacteria in the rhizosphere of this cultivar. The extention of the investigations of root exudates to other component, such as amino-acids and sugars, and the direct study of their impact on bacterial isolates collected in this study will certainly provide additonnal informations.

A comparison between the genetic diversity recovered from total, active, and cultivable communities could also be useful to estimate the level of concordance of the populations recovered with the different approaches.

Many aspects nevertheless have still to be investigated. N-acyl homoserine lactones, glucan, phytase and sulfatase production, and Ca-phosphate solubilisation abilities were shown to be favoured at root proximity. Some clues, such as previous measurement of higher phosphatase and sulfatase activities at root proximity, indicates that at least some of the bacterial abilities determined here as root-related traits correspond to *in situ* activities which are favoured at root proximity. However, some of the most popular root competence-related traits reported for rhizosphere competent model population of *Pseudomonas* were, in this experiment, shown to be more frequent in bulk and rhizosphere soil than in root communities. The main resulting open questions are:

(i) Do the root competence mainly depend on bacterial abilities or on their in situ activity rate ?

(ii) Do the determinants of root competence vary regarding the population taken into account ?

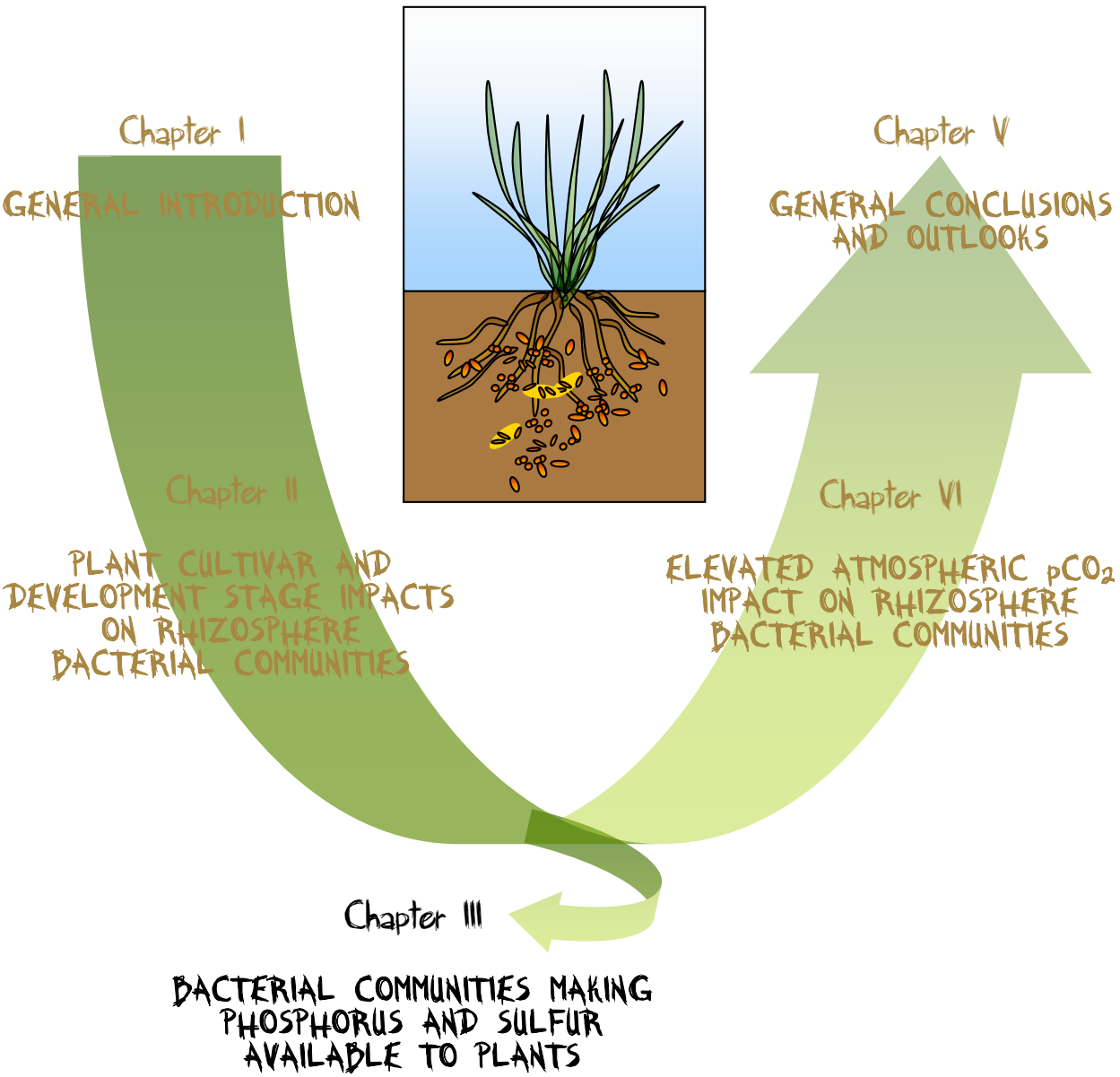
Measurement of *in situ* activities in the root environment, using proteomic or Q-transcriptomic approaches, would therefore be necessary to determine which abilities are really favoured in the plant-soil system studied here.

The second open question could be investigated by the identification of the bacterial isolates characterised in this study in order to know if the profile of potential abilities is linked with the phylogenetical affiliation of the isolates. This aspect as been here studied for phytases and sulfatases producing communities (see chap. III).

Althought experiments conducted in this chapter were performed in a natural soil, the system was reduced and plant were grown under greenhouse conditions. The experiments have thus to be completed with experiments performed in field conditions (see chap. IV).

CHAPTER III :

BACTERIAL COMMUNITIES MAKING PHOSPHORUS AND SULFUR AVAILABLE TO PLANTS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CHAPTER III : BACTERIAL COMMUNITIES MAKING PHOSPHORUS AND SULFUR AVAILABLE TO PLANTS

Overview

III.1. INTRODUCTION

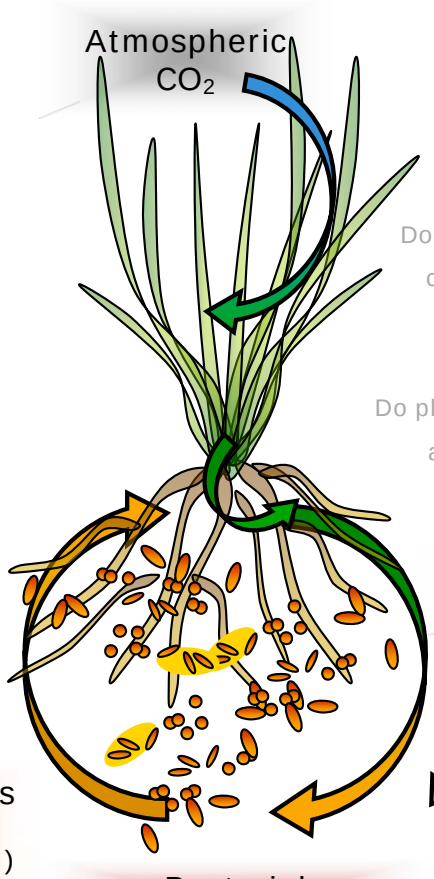
III.1.1. CHAPTER PRESENTATION

This chapter is derivate from the previous experimental part (see chap. II) and focus on two of the studied bacterial functions: phytase and sulfatase productions.

Close-up investigations were performed here for these functions for three main reasons:
(i) In previous experiments, we noted that most of the bacterial isolates able to mineralise organic phosphorus and sulfur by production respectively of phytases and sulfatases were recovered from the root-associated communities.
(ii) The organisms able to perform these activities may be strongly implicated in plant nutrition improvement and could be efficient biofertilizers.
(iii) The knowledge about diversity of microorganisms able to perform these functions are yet still largely incomplete.

The present chapter is then devoted to the study of the frequency and diversity of bacterial populations able to produce phytases (see chap. III.2) and sulfatases (see chap. III.3) in the rhizosphere of four *L. perenne* cultivars at four plant development stages, and in the bulk soil. The relations between these two functions and some other known PGP abilities were also assessed.

A global conclusion, regrouping both functional community studies, ends up this chapter (see chap. III.4).



Bacterial activities :

Which are the active key populations in the rhizosphere ?

Which are the most reactive populations in response to changes mediated by plant rhizodeposition and exudation ?

What is the frequency and taxonomic diversity of bacteria able to make P and S available for plants ?

Plant-mediated influences on rhizosphere environment :

Do plant cultivar influence nutrient content and conditions of their surrounding soil ?

Do plant cultivar have different organic acids and phenolic compounds exudation patterns ?

Are the differences observed in root environment consistant with those observed on bacterial community structure ?

Root selection :

Do plant-mediated influences affect genomic as well as functional bacterial community structures ?

Which bacterial functions are characteristic of root and of soil bacterial communities ?

Which relationship exists between the different bacterial abilities ?

Plant mediated influences on bacterial communities :

How do plant cultivar, plant development stage and elevated atmospheric pCO_2 influence bacterial community structure ?

III.1.2. PHOSPHORUS AND SULFUR AS PLANT GROWTH LIMITING NUTRIENTS

III.1.2.1. IMPLICATION IN BIOLOGICAL FUNCTIONS, AVAILABILITY AND UPTAKE MECHANISMS

Phosphorus is an essential element in a number of biological molecules and functions. There is good evidence that P is the dominant element controlling C and N immobilisation in biological systems (Paul and Clark, 1996). As constituent of essential biological compounds, such as amino acids (i.e. cysteine and methionine), vitamins, enzyme cofactors (i.e. biotin, coenzyme A and thiamine), and Fe-S proteins, sulfur is also an important element in biological functions.

Phosphorus and sulfur are currently known to be broadly present in soils (table I.1, p.16) but only poorly available (Arnou, 1953; Schachtman *et al.,* 1998; Kertesz and Mirleau, 2004). The main forms of these elements found in soil are organic compounds, in particular phytates (penta- and hexa-phosphate esters of myo-inositol) and sulphate esters respectively for phosphorus and sulfur compounds. These organic forms have further to be mineralised to allow nutrient availability for plants.
Moreover, inorganic P and S forms are known to be highly reactive and to be frequently found in soils as precipitated or complexed forms. The little part of inorganic P and S forms remaining directly available for plants and microorganisms is frequently leading to growth-limiting conditions in soils.

Plants do not have direct access to organic forms of minerals and to ions that are precipitated or complexed with metals, and which are not in the soil solution. Mineral ions are transferred through passive diffusion mechanisms from the soil solution to the root, following the concentration gradient, and flow is driven from the soil to the root due to the uptake at root level (Gobat *et al.,* 2004).
The low level of available P and S in soil solution and the necessity for its frequent renewal suggest that organic P and S mineralisation is mainly microbially mediated (Paul and Clark, 1996).

Both plant and microorganisms depend on the soil mineral nutrients. However, microorganisms may be much more efficient than plants in the uptake of these elements (Gobat *et al.,* 2004). Their active transport mechanisms allows the concentration of available nutrients. And their solubilisation and mineralisation abilities, such as secretion of organic acids, protons and specific enzymes, provide nutrients from unavailable forms.

III.1.2.2. FERTILIZATION PROCESSES

The application of chemical fertilizers has been used in order to compensate the reduced fertility of soils due to the intensification of agricultural production, but is currently controversed for several reasons. An important part of chemicals applied in soils are precipitated and remains unavailable for plants, implicating an excessive use of them in order to saturate the soil complex for the obtention of an efficient fertilisation. The remaining available forms are absorbed for plant nutrition or leach into groundwater, in the last case leading to significant environmental degradation.

In addition to the negative ecological impact of this excessive fertilizer application, such an extensive use of them is likely to be restrained in the near future, since mineral elements are not an inexhaustible resource (Vance, 2001).

The main solution will be to replace chemical fertilization with an improvement of solubilisation and mineralisation of precipitated and inaccessible mineral forms (already presents in soils) to allow their absorption by plants and limit environmental perturbations. The use of microorganisms as P and S biofertilizers has been already studied (Richardson *et al.,* 2001b; Gyaneshwar et al., 2002; Kertesz and Mirleau, 2004). However, the diversity and ecology of these populations have to be further investigated to predict their impact and their maintaining in the rhizosphere.

III.2. BACTERIA MAKING P AVAILABLE TO PLANTS

Bacteria making organic phosphate available to plants: Frequency and diversity of cultivable phytate-utilizing and mineralising bacteria in the rhizosphere of *Lolium perenne*.

Jossi M, , Jeanneret N, Roussel-Delif L, Shani N, Aragno M, Tarnawski S

ABSTRACT

Phosphorus is an essential element in a number of biological molecules and functions. In soils, phytate (myo-inositol-hexakisphosphate) is one of the major organic phosphorus compounds. Its mineralisation is likely to provide an available phosphorus source for plants and microorganisms. Phytate hydrolysis in soil is carried out by means of phytases mainly from microbial origin. Some bacterial genera, such as *Pseudomonas*, *Bacillus* and *Burkholderia* are already known to be efficient phytases producers, but the importance and diversity of phytase producing strains in the rhizosphere is still largely unknown.

In this study, a broad collection of bacterial isolates randomly selected on a non-selective agar medium from bulk soil, rhizosphere soil and root of *Lolium perenne*, were tested for phytase activity. More than 80 % of the isolates were able to hydrolyse P bonds of phytate. A low proportion (1.6 %), comprising mainly root-associated strains and a few rhizosphere isolates, was able to completely mineralise phytate releasing all the orthophosphate. All these strains were also able to grow on phytate as sole carbon source. Sequencing of the 16S rDNA gene of phytate mineralising isolates evidenced a broad diversity of strains with a dominance of two main groups: Actinobacteria and Proteobacteria.

Keywords: phytase, dephosphorylation, bacterial communities, plant nutrition, rhizosphere

Submitted to SOIL BIOLOGY AND BIOCHEMISTRY

III.2.1. INTRODUCTION

Phosphate (P) accounts for 1-5% of the plants dry weight (Gobat *et al.,* 2004), and is currently one of the major plant-growth limiting factors due to the low solubility of orthophosphate in soils (Deng *et al.,* 1998; Cassman *et al.,* 1981; Itoh, 1987). The available P concentration in fertile soils is generally not higher than 10 µM, even at pH 6.5 where phosphate solubility is the highest (Arnou, 1953; Schachtman *et al.,* 1998). This low level of available P is mainly due to the precipitation of orthophosphate with calcium (in calcareous soils), iron and aluminium ions (both in acidic soils) (Rodriguez and Fraga, 1999; Sharpley *et al.,* 1984; Zhang *et al.,* 2001), and to the covalent binding of P to stable organic molecules.
Organic phosphorus constitutes 50-80% of total phosphorus in soils (Dalal, 1977). Organic P forms found in soils are phosphoesters, including phospholipids and nucleic acids (Rodriguez and Fraga, 1999), but phytates (myo-inositol-hexakisphosphate $InsP_6$, and intermediate phosphorylated myo-inositol-phosphate $InsP_{1-5}$) are the most abundant (20-50 %) and stable forms identified (Turner *et al.,* 2002). They are synthesized by both microorganisms and plants (Rodriguez and Fraga, 1999), and constitute 1-5% (w/w) of edible legumes, cereals, oil seeds, pollens and nuts (Cheryan, 1980). Phytates are strong chelating agents and, like inorganic P, are mainly found in soils in the form of cation complexes (Jackman and Black, 1951, Turner *et al.,* 2002). They are therefore even less available for plant nutrition (Sanyal and De Datta, 1991; Holford, 1997; Lung and Lim, 2006).

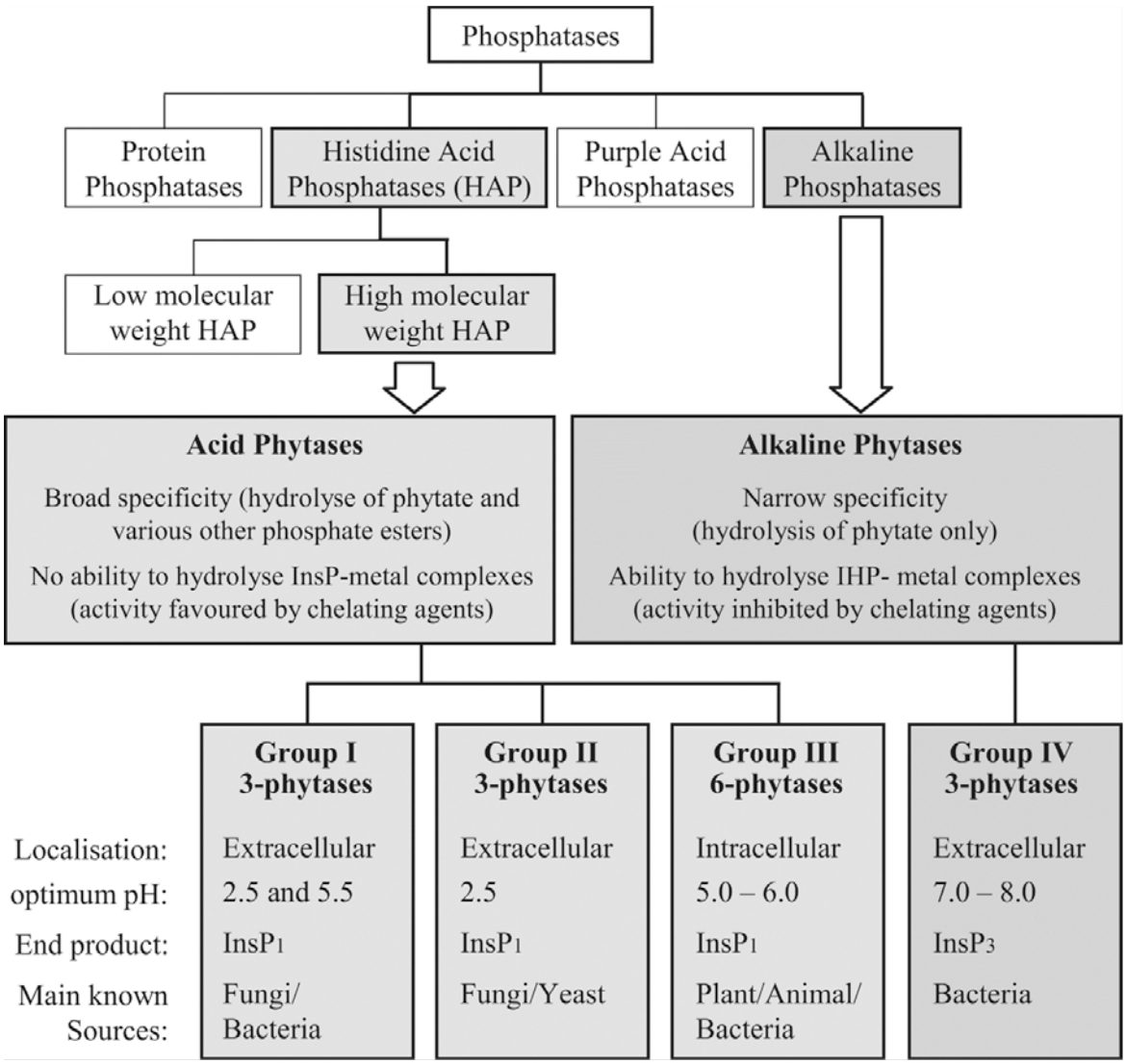
The main microbial functional groups linked to the phosphorus cycle in the rhizosphere are the P-translocating mycorrhizal fungi, as well as other fungi and bacteria mineralising organic P or solubilising inorganic P (Andrade, 2004). The mycorrhizal symbiosis changes the nutritional and physicochemical conditions of the rhizosphere with a direct nutritional flux from the soil to the plant roots, and has a large impact on the functional groups of soil microorganisms (Andrade, 2004; Raiesi, 2006). For instance arbuscular mycorrhizal fungi (AMF) have an elective effect on phosphate-solubilising bacterial populations (Roesti, 2005).

Mineralisation of most organic phosphates is carried out by phosphatases, among which specific phytases are implicated in phytate dephosphorylation (Turner *et al.,* 2002). Phytates are hydrolysed in a stepwise manner by phytases, and dephosphorylation of $InsP_{2-3}$ is considerably slower than the one of $InsP_{4-6}$ (Kemme *et al.,* 1999, Quan *et al.,* 2003). Different types of phytases have been described (figure III.1; Oh *et al.,* 2004; Chu *et al.*, 2004). They differ in their specificity and their final degree of $InsP_6$ dephosphorylation (Konietzny and Greiner, 2002; Quan *et al.,* 2003). In all cases, phytases do not release orthophosphate from $InsP_1$ (Kemme *et*

al., 1999), this final dephosphorylation being achieved by the common myo-inositol monophosphatase (Ganzhorn *et al.,* 1990). Two types of phytases are currently known, based on the specificity of their initial hydrolysis: 3-phytases and 6-phytases. Plants are able to produce intracellular 6-phytases and, in a less extend, extracellular 3-phytases (Pinton *et al.,* 2000; Greiner and Egli, 2003; Lung and Lim, 2006). Some microorganisms also present the ability to use phytate as a source of phosphorus by producing extracellular or ectoparietal 3-, 6- or hybrid phytases (Kerovuo *et al.*, 2000; Richardson *et al.,* 2001a; Vohra and Satyanarayana. 2003, Quan *et al.,* 2003).

Figure III.1. Phytase categories.

Classification, specificity and end products of the different phytases (based on the review of Oh *et al.,* 2004; Vats and Banerjee, 2004).



Previous experiments have shown that phosphatase activity was substantially increased in the rhizosphere (Tarafdar and Junk, 1987), and that the main source of this activity in soil was of microbial origin (Garcia *et al.,* 1992; Xu *et al.,* 1995). In the case of a grassland, 30-48 % of culturable rhizosphere microorganisms utilised phytate as P source (Greaves and Webley, 1965). However, in soil, less than 0.5% of culturable bacteria were able to grow on this substrate as sole C and P source (Richardson and Hadobas, 1997). It was also reported that the nutrition of a plant supplied with phytate was significantly improved when the substrate was inoculated with a cultured population of soil micro-organisms or, in a less extend, with a specific isolate selected for its ability to release phosphate from phytate (Idriss *et al.*, 2002; Richardson *et al.,* 2001b; Unno *et al.,* 2005). Obviously, phytate dephosphorylation catalysed by bacterial extracellular enzymes may allow plants to acquire the resulting orthophosphate.

Strains belonging to various bacterial genera were described as phytase producers (e.g. *Pseudomonas*, *Bacillus*, *Burkholderia*, *Klebsiella*, *E. coli*; Konietzny and Greiner, 2004; Haefner *et al.,* 2005). It was generally expressed that most bacterial phytases are cell associated (ectoparietal), and only strains belonging to the genera *Bacillus*, *Lactobacillus,* and *Enterobacter* were recognised as extracellular phytase producers (Vohra and Satyanarayana, 2003; Konietzny and Greiner, 2004). But in a recent diversity study, Hussin *et al.* (2007) have shown that 83% of isolated strains from Malaysian soil planted with maize produced extracellular phytases, and reported *Bacillus* spp. *Staphylococcus* spp. *Brevibacillus* spp. and *Kocuria* spp. as phytase producers with a high putative activity. Implication of such guild in P cycle is unknown. To our knowledge except this last study, diversity and ecology of phytase producing bacteria in soils are constituting an almost virgin field of research.

In this paper, we report the results of the screening of phytase producing bacteria from a collection of 1750 strains isolated from the rhizosphere of *Lolium perenne*, a perennial grass. This work assessed the frequencies and diversity of bacterial phytase producers in relation with root proximity (from bulk soil to root), plant cultivar (Cavia and Lipresso (2n), Anaconda and Bastion (4n)), and plant development stage (from germination to flowering). A critical comprehension of the results provided by the screening method was proposed, and diversity of bacteria able to completely mineralise phytate was studied.

III.2.2. MATERIALS AND METHODS

III.2.2.1. CULTURE MEDIA.

(A) Modified Angle medium (Angle *et al.*, 1991) contains per litre of nanopure water: 40 ml Tris-HCl 0.5 M (pH 7), 110 µl Fe/EDTA solution (50 % H_2SO_4 36M with Na_2EDTA 0.7 $mg.ml^{-1}$ and $FeSO_4*7H_2O$ 2.5 $mg.ml^{-1}$), 1 ml oligo-elements solution (modified from Aragno and Schlegel, 1992; $ZnSO_4*7H_2O$ 100 $mg.l^{-1}$, $MnCl_2*4H_2O$ 30 $mg.l^{-1}$, H_3BO_3 300 $mg.l^{-1}$, $CoCl_2*6H_2O$ 200 $mg.l^{-1}$, $CuCl_2*2H_2O$ 10 $mg.l^{-1}$, $NiCl_2*6H_2O$ 20 $mg.l^{-1}$, $Na_2MoO_4*2H_2O$ 30 $mg.l^{-1}$), 680 µl KH_2PO_4 0.1 % $^{W}/_{V}$, 500 µl KOH 1 M, 0.2 g NH_4NO_3, 0.406 g $MgCl_2*6\ H_2O$, 7 g KCl, and 15 g Agar-agar. pH of the medium was adjusted to 7.0 with NaOH 1M. Medium was supplemented after sterilisation, with 1 $g.l^{-1}$ glucose. (B) Inorganic P-free medium is a two layers' agar medium: the first layer was composed of (A) medium prepared without KH_2PO_4, and supplemented after sterilisation, with 0.5 $g.l^{-1}$ glucose, 0.52 $g.l^{-1}$ succinate, and 0.6 $ml.l^{-1}$ ethanol; the second layer was supplemented with 10 $g.l^{-1}$ Na-$InsP_6$ (Dodeca sodium salt of inositol hexaphosphoric acid). All the components of the media were obtained at Sigma-Aldrich Co. (Germany).

III.2.2.2. PLANT-SOIL SYSTEM.

Four cultivars of *Lolium perenne*, two diploids (Cavia and Lipresso) and two tetraploids (Anaconda and Bastion), were cultivated in pots containing 700 g of a fertile Eutric Cambisol (FAO classification) sieved at ∅ 2 mm. This soil was collected from field site (topsoil from the first 4 to 30 cm) of the Institute of Plant Science (ETHZ) at Eschikon (8°41'E, 47°26'N, 550 m a.s.l.) and harboured *L. perenne* cv. Bastion since 11 years (Nösberger *et al.,* 2006). This soil contained 28% of clay, 32% of silt, 36% of sand, 2.8-5.1% of organic matter (Lüscher *et al.,* 1998).

III.2.2.3. PLANT GROWTH CONDITIONS AND SAMPLING.

Sterilised seeds (Paterson and Sim, 1999) of the four cultivars were pre-germinated on nutrient agar (Biolife italiana S.r.l., Milan, Italy) to check that they were free of bacteria. Seedlings were grown in pots under greenhouse in semi-controlled conditions with 12h of photoperiod, and without any fertilisation. Samples were collected for the four cultivars at four plant development stages: (i) hypocotyl development (length: ~ 3 cm), (ii) four leaves, (iii) ear development (before flowering) and (iv) ear maturity (after flowering, 7 month of culture). The number

of plants per pot was 10 for the first development stage, 6 for the second one and 3 for the others. Three replicate pot experiments were performed per cultivar and plant development stage, including control soil pot experiments without plant but incubated in same conditions.
Samples from pots were separated in three fractions. Root systems with their adhering soil were collected from planted pots and agitated during 30 minutes in 20 ml sodium phosphate buffer 0.1 M pH 7.0 (SPB). The washed roots were recovered and constitute the rhizoplan-endorhizosphere (R) fraction. The remaining root-adhering soil suspension in SPB corresponds to the rhizosphere soil fraction (RS) fraction. The soil (S) fraction originated from the control soil pots without plant. These three fractions were recovered for each replicate and at each development stage, except for the first stage where plant biomass was low and triplicates were pooled.

III.2.2.4. BACTERIAL COLONY COUNTS AND ISOLATION.

R and S suspension were realized separately by crushing one gram of fresh R or S fractions in a mortar with 10 ml SPB, the 20 ml RS suspensions were also homogenised. Ten-fold serial dilutions of the R, RS and S suspensions were prepared. For each sample 100 µl from the appropriate dilutions were spread in triplicate onto (A) medium supplemented with 1 $g.l^{-1}$ glucose after autoclaving. Numbers of bacterial colonies per g of dry weight ($CFU.g^{-1}$ DW) of R, RS and control S fractions have been determined after 72 hours of incubation at 24°C. At least twenty bacteria per fraction for each cultivar and each plant development stage were randomly isolated from plates presenting 20 to 100 colonies, and purified twice on (A) medium.

III.2.2.5. IN VITRO TEST FOR PHYTASE PRODUCTION.

The (B) medium with Na-$InsP_6$ as sole P source was used for the detection of phytase activity (Richardson et Hadobas, 1997). Precultures (to starve isolates for inorganic P nutrition) and cultures of isolates were performed on (B) medium and incubated one week in the dark at room temperature. Positive isolates for phytase activity were detected following the procedure described in Bae *et al.* (1999). Briefly, the (B) medium was white/opaque and became translucent when Na-$InsP_6$ was (i) solubilised by acidification, (ii) completely dephosphorylated or mineralised. The clearing zone signification was determined, after removing colonies from the agar, with two reagents: (1) a cobalt chloride solution which re-precipitated solublilised InsPn compounds, (2) a molybdo-vanadate coloration solution used to improve contrast. In their procedure, Bae *et al.* (1999) added the cobalt chloride solution to discriminate between a clearing zone due to acidification and to phytate hydrolysis. They considered that reprecipitation was indicative of solubilisation by acidification and not by hydrolysis of the P groups.

After culture incubation in our study, isolates were subsequently classified using different criteria (table III.1), those which were able or not to grow on (B) medium were noted PhyG and PhyNG respectively. Then bacterial cells were removed from the medium plate and presence of a clearing zone in the medium was defined among PhyG isolates. Two new categories were formed: (i) Phy NC, for isolates without clearing zone and (ii) PhyC, for isolates harbouring a clearing zone. PhyC isolates were subdivided in two groups: (iia) PhyCE presented a clearing zone extended around colonies, and (iib) PhyCR presented a clearing zone restricted to the underface of colonies. Isolates harbouring an extended (PhyCE) or a restricted (PhyCR) clearing zone were finally separated in different subgroups: (i) PhyCEd and PhyCRd, corresponding to isolates harbouring a clearing zone which disappeared after cobalt addition, (ii) PhyCEa and PhyCRa, corresponding to isolates harbouring a clearing zone which was attenuated after cobalt addition, and (iii) PhyCEm and PhyCRm, corresponding to isolates harbouring a clearing zone which was maintained after cobalt addition. *Bacillus amyloliquefaciens* DSM 7 (Deutsche Sammlung von Microorganismen) and *Bacillus subtilis* Neu 1 (strains collection of the University of Neuchâtel) were used respectively as PhyCEm positive and PhyNC negative controls.

Table III.1. Behaviour of the isolates on B medium (Phy categories).

Classification of the 1750 isolates based on their ability to grow with phytate as sole P source and to produce a clearing zone.

Growth	Clearing	Zone	Reaction after Co addition	Nb isolates
No (PhyNG)	-	-	-	306
Yes (PhyG)	No (PhyNC)	-	-	656
	Yes (PhyC)	Restricted (PhyCR)	Maintained (PhyCRm)	0
			Attenuated (PhyCRa)	16
			Disappeared (PhyCRd)	76
		Extended (PhyCE)	Maintained (PhyCEm)	28
			Attenuated (PhyCEa)	92
			Disappeared (PhyCEd)	575

All PhyCEm isolates as well as a representative number (n=30) of each PhyG groups were in a second step checked for their ability to grow with phytate as sole C and P source. Isolates were incubated in liquid (B) medium with Na-InsP_6 as sole phosphorus and carbon sources.

The pH influence on the solubility of Na-InsP_6 (15 g.l^{-1}) in (B) medium was also investigated. (B) liquid medium was prepared with pH adjusted to values ranging from 0 to 7 with HCl. After 30 min. agitation, absorbance was measured in the media at 420 nm, and compared to the absorbance of the control medium at pH 7. pH in (B) culture medium was also determined for 30 isolates representative of the different PhyC groups, by adding 25 ml, per litre of medium, of an universal pH indicator (Thymol Blue 66.6 mg.l^{-1}, Methyl Red 333.4 mg.l^{-1}, Bromothymol Blue 800 mg.l^{-1}, Phenolphtalein 800 mg.l^{-1}, prepared in ethanol 70%, pH was adjusted to 7 with NaOH 0.01M). Incubation was performed during one week in the dark at room temperature.

III.2.2.6. FREQUENCY OF PHYTASES PRODUCING BACTERIA.

Proportions of phytase-producing strains were calculated for each fraction, development stage and cultivar. A generalised linear model (*glm*) analysis with a logistic regression model were used to compute the probabilities corresponding to the effects of the fractions, *L. perenne* cultivars and plant development stages on the frequencies of phytase producing strains. Comparison of the isolate proportions in each PhyG groups (table I) between R, RS and S fractions, between *L. perenne* cultivars, and between plant development stages were performed using Tukey Kramer HSD post hoc test which take into account the *glm* results. Calculations were performed using S-plus 6 Statistical Software (Insightful Corporation, Seattle, Washington).

III.2.2.7. MOLECULAR CHARACTERISATION OF PhyCEm ISOLATES.

Strains were grown on liquid (A) medium and DNA was extracted from 2 ml of the culture using the Wizard Genomic DNA purification kit (Promega Co., Madison, WI, USA). PCR amplification of the whole 16S rRNA gene was performed for each isolate using the forward GM3f and the reverse GM4r Bacteria primers (Muyzer and Ramsing, 1995), as described in Jossi *et al.* (2006). 16S rDNA PCR products were purified using Wizard SV Gel and PCR clean-up system (Promega), and cloned in *E. Coli* XL1 by electroporation with pGEM-T Vector System (Promega). Plasmids were extracted and purified using Wizard®Plus SV miniprep System (Promega), and inserts were sequenced by MWG Biotech (Zurich, Switzerland). The phylogenetic affiliation of corresponding organisms was achieved by BLAST analysis (Altschul *et al.,* 1997). Sequences of phytase mineralising strains were deposited at EMBL under the accession numbers AM921621 to AM921648. Phylogenic trees were obtained with ClustalX alignment software (number of bootstrap trial = 1000) and NJplot tree drawing software (Saitou and Nei, 1987; Chenna *et al.*, 2003).

III.2.2.8. SOIL PHOSPHORUS CONTENT DETERMINATION.

Total and available soil phosphorus content was determined on 2.5 g of dried soil before and after growth of each plant cultivar, as well as in uncultured soil incubated in the same conditions that planted pots. For each cultivar, soil samples were collected directly in pots. Total phosphorus was quantified using the mineralisation process described by Kjeldahl (1883) and the colorimetric determination of P by the ascorbic acid-molybdate blue method (Murphy and Riley 1962). Available phosphorus was measured using Olsen method (Olsen *et al.,* 1954). Data obtained from soil before and after growth of each cultivar, and from incubated control soil, were compared using variance analyses (ANOVA). Tukey Kramer HSD post hoc test were used to determine the significance of the observed differences. All calculations were performed with R 2.3.1 (R Development Core Team, 2007).

III.2.3. RESULTS

III.2.3.1. SOIL PH AND PHOSPHORUS CONTENT.

Total and available phosphorus content, $pH_{[H2O]}$ and $pH_{[KCl]}$ measured for initial soil, for control soil after incubation and for soil after growth of the different cultivars, are presented in table III.2. At the end of the experiment, total and available phosphorus contents were not significantly different from the beginning.

Table III.2. Soil pH and phosphorus content.

Total and available phosphorus content ($mg.g^{-1}$), and pH of soil before (initial soil) and after cultivation of the four cultivars of *L. perenne* (Anaconda, Bastion, Cavia, Lipresso), and of control soil incubated without plant.

	Control soil without plant		Planted soil			
	Initial	Incubated	Anaconda	Bastion	Cavia	Lipresso
Incubation time	t = 0	t = 7 month	t = 7 month			
pH [H_2O]	6.63 (±0.04)	7.36 (±0.12)	7.45 (±0.1)	7.44 (±0.04)	7.33 (±0.08)	7.36 (±0.07)
pH [KCl]	5.66 (±0.01)	6.37 (±0.18)	6.46 (±0.1)	6.43 (±0.07)	6.31 (±0.13)	6.37 (±0.08)
Pavailable	0.10 (±0.003)	0.13 (±0.02)	0.09 (±0.004)	0.12 (±0.05)	0.12 (±0.01)	0.11 (±0.02)
Ptotal	0.54 (±0.08)	0.63 (±0.06)	0.51 (±0.04)	0.54 (±0.07)	0.54 (±0.03)	0.52 (±0.03)

III.2.3.2. IN VITRO TEST FOR BACTERIAL ACTIVITY OF PHYTASE PRODUCTION, PH SOLUBILISATION OF $INSP_6$ AND $INSP_6$ USED AS C SOURCE.

According to their growth reaction on (B) medium (figure III.2), isolates were classified in different groups and sub-groups (table III.1). Isolates distribution in each group is presented in figure III.3.

A total of 1750 isolates were collected at the different plant development stages, from R and RS fractions of the four plant cultivars, as well as from S fraction. They were checked for their ability to produce phytase. 82.5 % of the isolates were able to grown on B medium (phyG). Among them 45.4 % did not produce a clearing zone in (B) medium (PhyNC). Among strains presenting a clearing zone diffusing at distance around the colony (PhyCE), cobalt addition resulted in the complete disappearance of this zone in 82.7 % of the cases (PhyCEd), in attenuation in 13.2 % (PhyCEa), and the clearing zone persisted only in 4.1 % (PhyCEm). Among isolates harbouring a clearing zone restricted to the underface of colonies (PhyCR), clearing disappeared upon cobalt addition in 81.7 %, and was attenuated in 18.3 % (PhyCRa), whereas persistence of the clearing zone (PhyCRm) was never observed (figure III.3). No difference was observed between the growth intensities of the different groups of PhyG isolates.

Figure III.2. Clearing zone description.

Example of isolates grown on inorganic P-free medium supplemented with Na-$InsP_6$. Clearing zone type: (A.1) clearing zone restricted to the underface of colonies (PhyCR), (A.2) extended clearing zone (PhyCE). Clearing zone appearance after the addition of the cobalt solution: (B.1) maintained (PhyCm), (B.2) attenuated (PhyCa), and (B.3) disappeared (PhyCd).

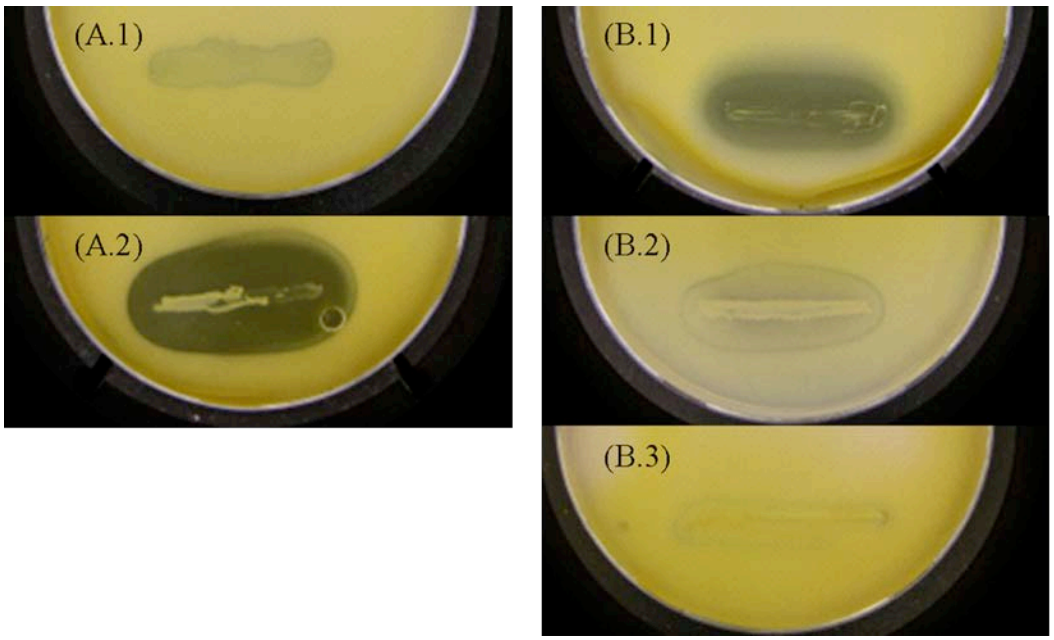
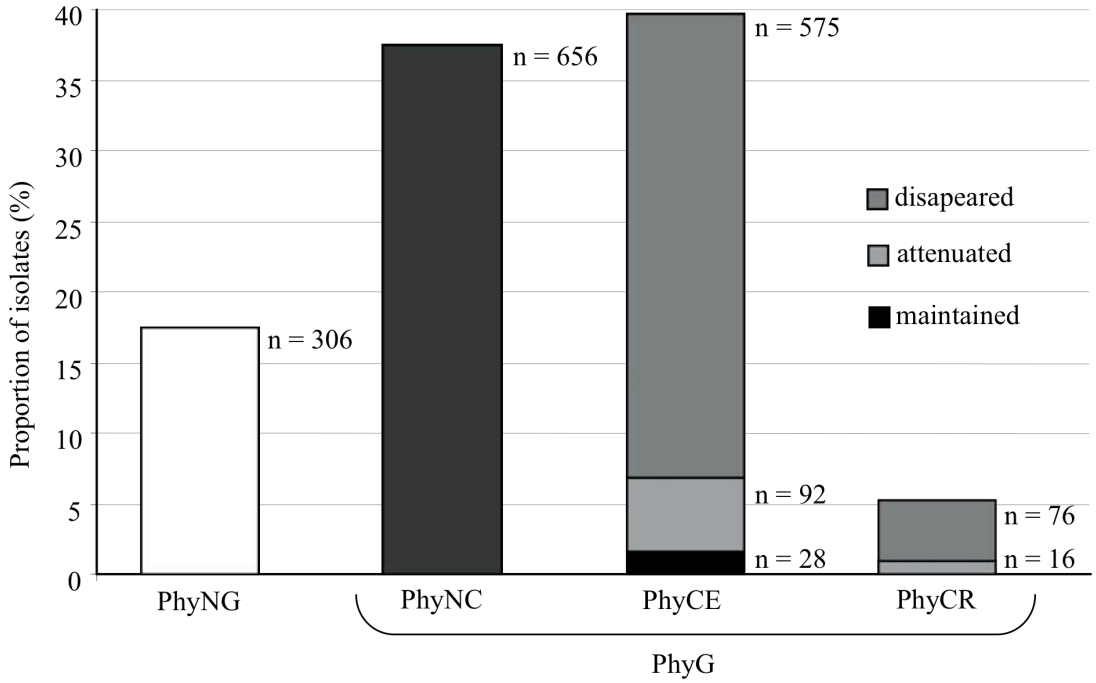


Figure III.3. Distribution of the isolates in the different Phy categories.

Frequencies of isolates growing (PhyG) or not (PhyNG) on Pi-free Angle medium supplemented with Na-$InsP_6$. The proportion of PhyG is detailed according to the different isolate groups, i.e. phyNC, phyCEd/a/m and phyCRa/d (see table I), able or not to provoke a clearing zone on the test culture medium for phytase production.



The effect of acidification on Na-$InsP_6$ solubilisation was tested by measuring absorbance at 420 nm of the liquid (B) medium (inorganic P-free supplemented with Na-$InsP_6$) acidified at pH values ranging from 0 to 7. Complete dissolution of Na-$InsP_6$ was observed only when the medium pH was lower than 5. In most cases, the pH measured around colonies was close to 7, and pH below 6 was never observed (data not shown). The medium pH decreased at 6 with only one PhyCRa isolate among all the PhyG isolates selected, and pH even increased to 8 for two PhyCEa and one PhyCRa isolates. Such result was never observed for PHyCEd or PhyCRd isolates.

The incubation of all PhyCEm isolates (corresponding to phytase producers according to Bae *et al.*, 1999) and of representative numbers of other PhyG groups on liquid (B) medium with Na-$InsP_6$ as sole phosphorus and carbon source showed that all and only PhyCEm strains were able to grow in these conditions, and were therefore able to completely dephosphorylate $InsP_6$ and to use the myo-inositol moiety as C and energy source.

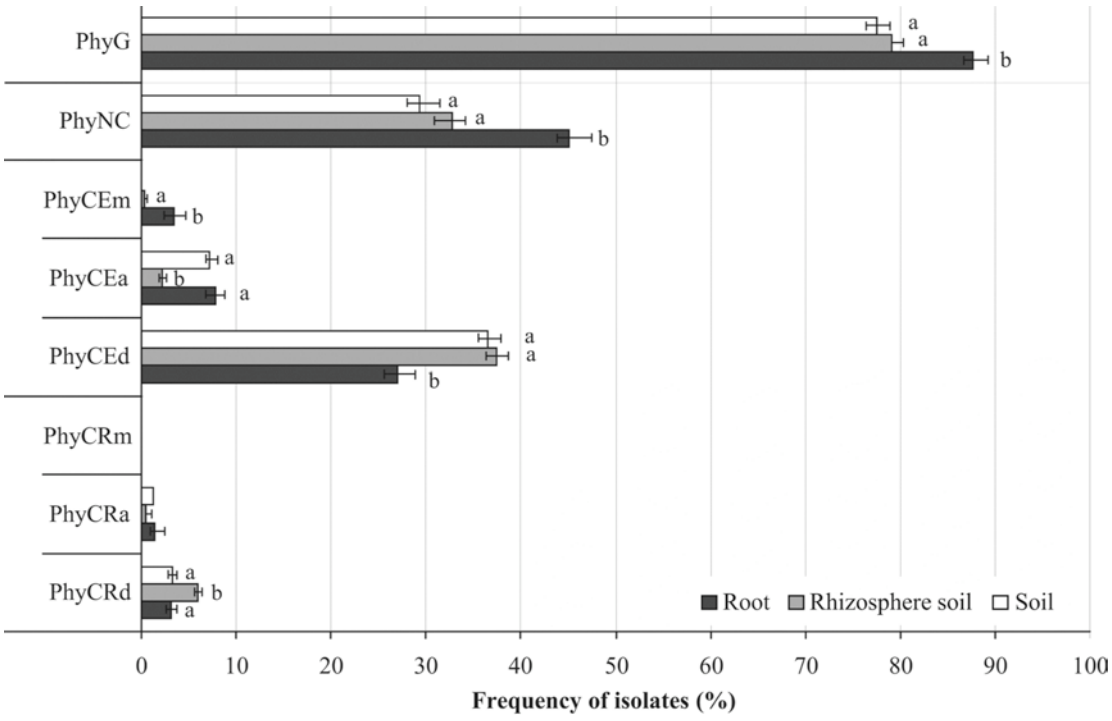
III.2.3.3. BACTERIAL COUNTS AND FREQUENCIES OF PHYTASE PRODUCERS.

The number of bacterial colonies per gram of dry weight ($CFU.g^{-1}$) of root (R), rhizosphere soil (RS) and bulk soil (S) was determined on (A) modified Angle medium plates at each plant development stage (data not shown). Cultivable cell numbers ranged from 2.10^6 to 2.10^7 $CFU.g^{-1}$. No relevant difference was observed between the different cultivars, fractions, and plant development stages.

Among the 1750 collected isolates, frequencies of PhyG strains and of isolates in the different PhyG groups were calculated and compared between fractions, plant cultivars and development stages (figure III.4). No significant differences were observed between PhyG frequencies retrieved from the different cultivars and development stages (data not shown). PhyG isolates was significantly more abundant ($p \leq 0.01$) in R than in RS and S fractions. Among the different PhyG groups, frequency of PhyNC isolates was higher ($p<0.01$) in R than in both other fractions. The proportion of PhyCEa and PhyCRa isolates was lower in RS than in both other fractions. The proportion of PhyCEd strains was lower in R than in RS and S, whereas PhyCRd strains were more abundant in RS than in both other fractions. Finally, proportion of PhyCEm isolates was higher ($p \leq 0.05$) in R than RS fractions (figure III.4), and no such strain was isolated from the control S fraction.

Figure III.4. Frequencies of phytases producing isolates.

Proportion of isolates able to produce phytases (PhyG) from the root, the rhizosphere and the bulk soil fractions (for all the cultivars and development stages). Proportions of isolates in each Phy categories (table III.1) among PhyG are detailed in the second part of the figure.

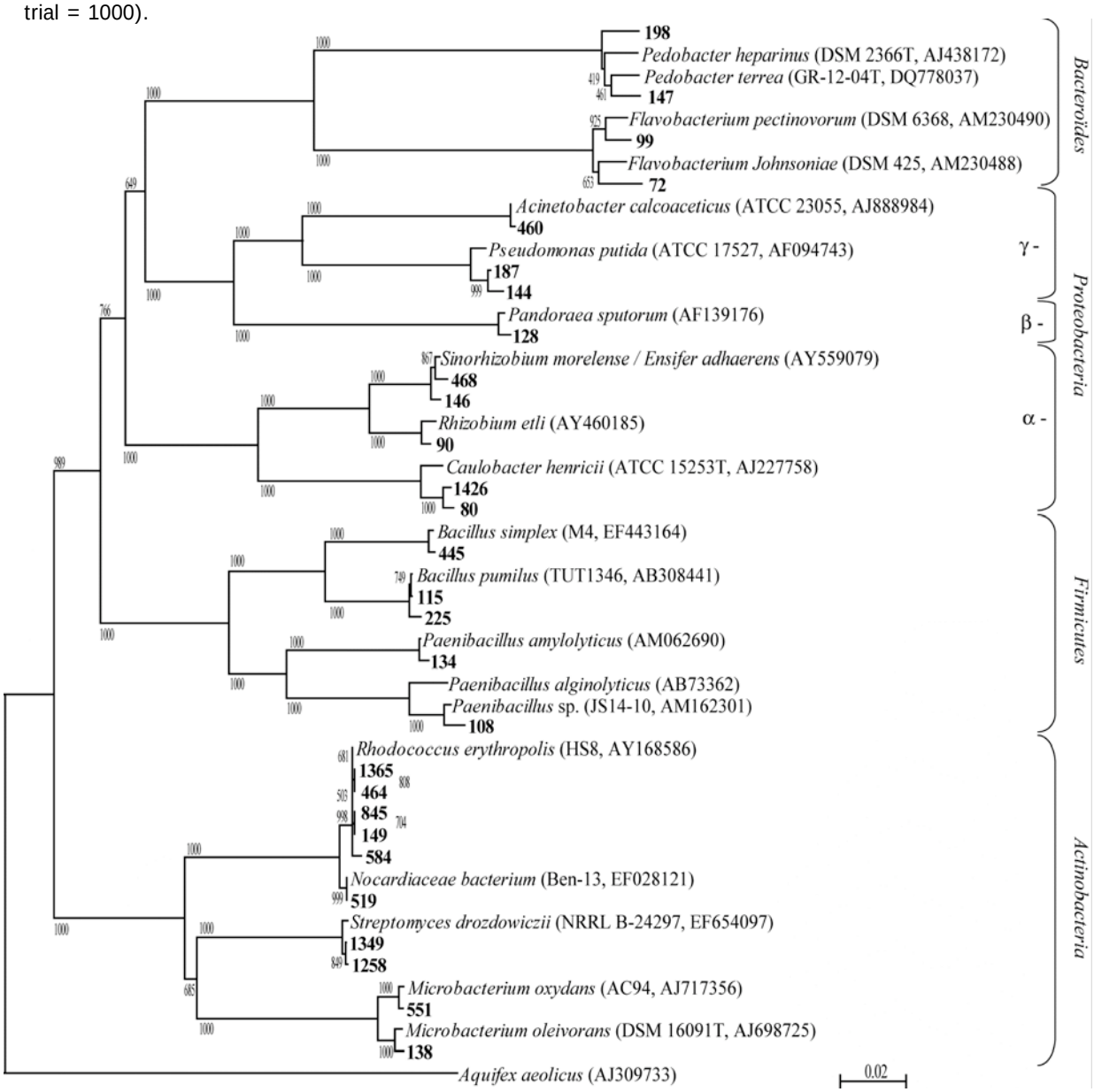


III.2.3.4. PHYLOGENETICAL DIVERSITY OF BACTERIA ABLE TO MINERALISE PHYTATE.

All PhyCEm strains originated from root-related fractions (most from endorhizosphere/root surface) and represented 1.6 % of the total isolates. The sequences of the 16S rDNA retrieved from the 28 PhyCEm isolates were affiliated to different bacterial phylums (figure III.5). Among these, 10 belonged to the Actinobacteria (Actinomycetales), 5 to the Firmicutes (Bacillales), 4 to the Bacteroïdetes (2 Sphingobacteriales, 2 Flavobacteriales), and 9 sequences to the Proteobacteria (α-: 3 Rhizobiales, 2 Caulobacterales, β-: 1 Burkholderiales, γ-: 3 Pseudomonadales).

Figure III.5. Diversity of PhyCEm isolates.

Phylogenetical tree based on 16S rDNA sequences obtained from phytate mineralising isolates (PhyCEm). Sequences were aligned with Clustal-X and tree was drawn with NJplot softwares (number of bootstrap trial = 1000).



III.2.4. DISCUSSION

III.2.4.1. SOIL ANALYSIS.

The planted soil pH was the same that unplanted soils one. The increasing pH, between the beginning and the end of the experiment, are explained by the use of calcareous tap water for irrigation. The absence of significant differences in concentration of total and available P is not surprising. The P content of the grown plant would not allow to measure a significant decrease in the total P of the bulk soil. Regarding available phosphorus, its content is in dynamic equilibrium between plant uptake and dissolution, and its bulk soil concentration would not vary significantly. Moreover, the amount of soil needed for such analysis did not allow to evaluate the P gradient in the rhizosphere at millimetre scale (Hinsinger *et al.*, 2005).

III.2.4.2. BACTERIAL PHYTASE ACTIVITY TEST.

Na-InsP_6 is the most suitable form of InsP_6 for phytase activity detection, and its dephosphorylation leads to the loss of its ability to precipitate with cations (Vats and Banerjee, 2004). However, Na-InsP_6 solubility increased at low pH (Bae *et al.,* 1999) and a chemical, slow and partial, hydrolysis of phytate could be observed in acidic conditions (Turner *et al.,* 2002). Therefore, Bae *et al.* (1999) proposed a test based on the pH-independent precipitation of InsP_6 in the presence of cobalt, which would allow to distinguish between “true” phytate hydrolysis and phytate dissolution through acidification. According to them, isolates presenting a clearing zone on the culture medium, maintained after cobalt addition, are true phytase producers.

In our experiments, such result corresponded to PhyCEm and PhyCRm isolates, which represented only 1.6% of the strains, while 82.5 % of the isolates were able to grow with phytate as sole P-source (PhyG). The presence of free phosphate as contaminant of the phytate used in our assays can be ruled out by the absence of growth of the remaining 17.5 % of the strains (PhyNG). Moreover, the phytase test was performed with inocula precultivated on inorganic P free medium, excluding a contamination by the inoculum. Bae *et al.* (1999) and Fredrikson *et al.,* (2002) considered that the abiotic release of orthophosphate from Na-InsP_6 would be insufficient to promote significant growth. All the strains which grow on Angle medium with Na-InsP_6 as sole P source have thus to be considered as putative phytase producers, even PhyNC which did not cleared the medium.

This interpretation is supported since we determined that to obtain a clearing zone by acidification on angle medium P-free supplemented with Na-InsP_6 15 g.l^{-1}, pH has to be lower than 5. However, such an acidification has never been observed around colonies whatever the

PhyG group tested. This means that clearing zones were not due to acidification, even when re-precipitation with cobalt occurred. We also demonstrated that 100 % of the PhyCEm strains (the "Bae positive" ones), and only them, were able to completely mineralize Na-InsP_6, and to use it as both carbon and phosphorus source for growth.

Three hypotheses could then be formulated concerning the dephosphorylation activity of phytases synthesised by isolates which produce clearing zone disappearing or attenuated (PhyCd and PhyCa), or produce no clearing zone (phyNC) : (i) InsP_6 dephosphorylation could be only partial (Turner *et al.,* 2002; Quan *et al.,* 2003; Vohra and Satyanarayana, 2003; Vats and Banerjee, 2004), and lead to the accumulation of an intermediate InsP_n compound which do not precipitate with Na, but precipitate with cobalt. Therefore, such strains would present a clearing zone, which disappeared after cobalt addition (PhyCd). (ii) Dephosphorylation could also be more advanced, and lead to the accumulation of an intermediate InsP_n compound, which do neither precipitate with Na, nor with cobalt. But regarding the high concentration of Na-InsP_6 in the medium, InsP_6 dephosphorylation could be sufficient to detect a visible solubilisation, while being slow and lead to the precipitation with cobalt of the remaining InsP_6, and of intermediate InsP_n compounds which do not precipitate with Na. A low amount of efficient phytases could also give such a result. Strains producing efficient phytases with slow activity, or in low amounts, would thus present a clearing zone attenuated after cobalt addition (PhyCa). (iii) The absence of a clearing zone in some of the PhyG strains could be explained by a partial dephosphorylation (e.g. InsP_6 to InsP_5) leading to an insoluble Na salt, or by a very low phytase activity, sufficient to provide the necessary P for growth, but undetectable as a clearing zone.

III.2.4.3. FREQUENCY OF PHYTASE-PRODUCING STRAINS.

All phytate-mineralizing isolates (PhyCEm) were retrieved from the root and rhizosphere communities. Their low proportion among all isolated strains (1.6 %) is consistent with results obtained by Richardson and Hadobas (1997), which found that less than 0.5 % of culturable soil bacterial populations were capable of using InsP_6 as a sole C and P source. In the case of a perennial grassland, Greaves and Wembley (1965) showed that 30-48 % of rhizosphere microorganisms were able to grow with phytate as P source in presence of other C-sources. In the present study, the majority of isolates were able to grow on inorganic P-free medium supplemented with Na-InsP_6 (PhyG) and a mix of other carbon sources (D-glucose, succinate, ethanol), but most of them were "phytase negative" according to Bae *et al*. (1999) test. They were nevertheless able to obtain phosphate from phytate, most probably through partial or slow hydrolysis of its phosphoester bounds. PhyG proportions ranged from 87.8 % (root) to 78 % (soil).

The Bae *et al.* (1999) method for phytase detection greatly underestimates the populations able to hydrolyse part or all phosphoester bonds in phytate. Rather, it allows to identify the isolates which completely mineralize phytate, releasing most efficiently all the orthophosphate from phytate.

III.2.4.4. PUTATIVE ECOLOGICAL SIGNIFICANCE OF PHYTASE PRODUCING BACTERIA.

Most of the isolates which present a clearing zone on modified Angle medium with Na-InsP_6 as sole P source harboured a clearing zone extended around the colonies (PhyCE), and were supposed to produce extracellular phytases. Moreover, the presence, in some of the isolates (PhyCR), of clearing zones restricted to the underface of colonies could be indicative of an ectoparietal (cell-wall bound) enzyme. Whatever the fraction considered, the proportions of isolates producing extracellular phytases, which were retrieved in this study, were higher than those of isolates only able to produce ectoparietal phytases. Similar results were reported by Hussin *et al.* (2007) in the rhizosphere of maize.

Ectoparietal enzyme activity would only benefit to bacterial P nutrition, whereas extracellular phytase activity also provides available P to plants and other microorganisms. Additionally, among the different types of phytases produced by bacteria (Turner *et al.,* 2002; Quan *et al.,* 2003; Vohra and Satyanarayana, 2003; Vats and Banerjee, 2004), some are able to release more orthophosphates per phytate molecule than others. Richardson *et al.* (2001b) already reported phytate mineralisation as a plant growth promoting trait. Microorganisms mineralising completely InsP_6 (PhyCEm) will release most of the orthophosphate in their environment while assimilating the inositol moiety of the molecule. This makes most P available for plant and soil organisms.

In this study, isolates able to completely dephosphorylate phytate and to use inositol as C source (PhyCEm), are much more frequent in root than rhizosphere soil, and absent in the bulk soil. They are also all able to solubilise inorganic phosphate (data not shown). This indicates that phytate mineralising bacteria in soil-plant system are favoured at root proximity, and that they might metabolise plant-native phytate (e.g. during cortex autolysis), being therefore involved in the recycling of phytate from root.
In the same way, 1.6 % of the strains isolated from maize root system, by Hussin *et al.* (2007), presented a remarquably high phytase activity, and were mostly endophytic. Such strains could correspond to the phytate mineralising strains (PhyCEm) of the present study.

III.2.4.5. KEY POPULATIONS MAKING P AVAILABLE FOR PLANTS.

Bacterial populations able to use phytate as sole phosphorus and carbon/energy source in *Lolium perenne* rhizosphere presented a broad taxonomic diversity, with Actinobacteria (order *Actinomycetales*) as dominant group. Currently, phytase is mainly known to be synthesised by species of *Pseudomonas*, *Burkholderia* and *Bacillus* (Richardson *et al.*, 1997, Rodriguez and Fraga, 1999; Unno *et al.*, 2005). To our knowledge, no information is available about the implication of Actinobacteria in phytate mineralization in the rhizosphere. *Actinomycetales* are commonly known as active bulk soil organisms but were also recently reported to have an importance in terms of abundance and activity in rhizosphere environments (Jossi *et al.*, 2006, Pereira *et al.*, 2006; Gremion *et al.*, 2003). Conn and Franco (2004) reported that the main group of endophytic Actinobacteria in wheat roots corresponds to *Actinobacteridae*, a subclass including the order *Actinomycetales*.

The use of Actinobacteria as fertilisers would thus be an important field of investigations taking into account their resistance, and the problems related to the conservation and transport of biofertilisers.

III.3. BACTERIA MAKING S AVAILABLE TO PLANTS

Bacteria making organic sulfate available to plants: Frequency and distribution of arylsufatases producing bacteria in the rhizosphere of *Lolium perenne*

Tarnawski S, Jossi M, Aragno M

ABSTRACT

Sulfate is an essential element for biological functions. Preferred source of sulfur for plants and microorganisms is inorganic sulfate, but more than 95% of the soil sulfur are organic forms. Arylsulfatase participates in soil organic S mineralisation and increases soil sulfate content. Arylsulfatase activity was described in the common soil bacteria genus *Pseudomonas* (Kertesz and Mirleau, 2004; Mirleau *et al.*, 2005), but it is not yet clear which organisms realise this function in soil. In this study, 1686 bacterial colonies were randomly isolated on “non-selective” Angle agar medium plate from soil, rhizosphere soil and root of *Lolium perenne* grown in non-fertilized soil pots. These isolates were tested for the sulfatase activity on modified Angle medium supplemented with organic S (X-sulfate) and with or without inorganic S (Na_2SO4). Population structure of sulfatase producing isolates was investigated by 16S rDNA gene RFLP analysis with *Taq*I and *Hae*III restriction enzymes separately. 16S rDNA genes of representative isolates of each restriction profile type (OTU) were currently sequenced. 6% of the isolates presented a sulfatase activity which was not repressed by the presence of inorganic S in the culture medium. Among them, 62.5% were isolated from the root fraction. The 104 sulfatase producing strains were distributed in 23 OTU. Sulfatase-producing bacterial isolates presented a broad taxonomic diversity, however, Proteobacteria (*Rhizobiales*) and Bacteroïdetes (*Flavobacteriales*) were shown to represent the main arylsufatase producing groups.

III.3.1. INTRODUCTION

Sulfur (S) is essential for several biological functions, e.g. in plants, S is constitutive of certain amino acids (as cysteine and methionine), vitamins, enzyme cofactors (as biotin, coenzymeA and thiamine), and Fe-S proteins. Sulfur accounted for 0.5-5 % of the plant dry weight (Gobat *et al.,* 2004), plants nevertheless are only able to uptake sulfur from soil as inorganic sulfate form to synthesise these components (Alves and Lavorenti 2003).

However, most S presents in soil is in organically bounded form, as sulfonate, sulfate-ester, and carbon-bonded sulfur (Kesterz and Mirleau, 2004). In grassland soils, sulfate-esters S are dominant and contributed to 51.5 % of the total available S, sulfonates S to 37.5%, amino acids S to 10%, and remaining inorganic sulfate only to 1% (Freney and Williams 1983; Watwood *et al.,* 1986). As earlier demonstrated for *Sorghum vulgare* and *L. perenne* (Freney *et al.,* 1975; Chapman, 1987), sulfate for plant nutrition, is provided in these systems by S cycling in soil organic matter (Knights *et al.,* 2000), and organic S mineralisation is catalyzed predominantly by microbial activities (Ghani *et al.,* 1992). Lee and Speir (1979) observed also that C-bounded S is not related to plant S uptake, these results indicate that the C-bounded S is of minor importance for the S nutrition of crops than ester sulfate.

The rhizosphere is a key zone with view to the mechanisms of soil nutrient dynamics (Darrah *et al.,* 1993). Bio-physico-chemical processes at the soil-root interface differ considerably from those in the non-rhizosphere soil. The C/S ratio of microbial dry biomass is about 40:1, and in the rhizosphere root exudation provides more easy available C sources enhancing bacterial biomass formation and turnover (Kuzyakov *et al.,* 2000; Whipps, 2001) but also bacterial sulfur requirement compared to bulk soil. In this study we thus assumed that bacteria are in competition with the plant for inorganic S. The soil used in our experiments was planted with *L. perenne* during eleven years. Plant shoots have been regularly harvested, but never received S fertilisation. In these conditions, bacteria able to release inorganic S from organic matter presented a competitive advantage toward other bacteria to colonize the plant rhizosphere. The mineralisation of organic S forms are carried out by means of sulfatases, and in the particular case of sulfate esters, the most rapidly mineralised pool of organic S, by means of arylsufatases (Kertesz and Mirleau, 2004).

Kahnert *et al.* (2002) demonstrated that *sftR* mutant of *Pseudomonas putida* S-313R unable to desulfurize sulfate-esters S badly survived in the rhizosphere than in soil. Thus bacterial ability to produce sulfatase seems lower the competition with the plant and their counter-selection at

root proximity. In the same time the free inorganic S liberated by such bacterial activity should became also available for nutrition of others microorganisms and especially of plants (Freney *et al.,* 1975; Chapman, 1987), conferring to these bacteria a plant growth promotion property.

Limited data are available for the effect of plant growth on the chemical behaviour of S in the rhizosphere. Hu *et al.* (2005) observed that less ester-bounded S was found in the rhizosphere of oilseed rape and rice, and the same was observed with wheat and radish in Hu *et al.* (2002). In relation with the fact that bacterial activity is higher in the rhizosphere due to root exudates used as energy sources, the reason could be a higher arylsulfatase activity in the rhizosphere (Fitzgerald, 1978). Indeed, Han *et al.* (1982) and Vong *et al.* (2003) measured a higher arylsulafatase activity in the rhizosphere of rice, rape and barley than in non-rhizosphere soil. Knauff *et al.* (2003) also reported a higher activity of arylsulfatase in rhizosphere compared to bulk soil of *Sinapsis album*, *L. perenne*, *Triticum aestivum* and *Brassica napus*.

However, Ghani et al. (1992) observed that additions of glucose-C or sulfate-S decreased S mineralisation (whereas N additions slightly enhanced mineralisation in soils). Some study suggest that bacteria preferentially used SO_4^{2-} and that their sulfatase activity is a plus. It is known that in the *Pseudomonas* genus expression of arylsulfatase genes is repressed by the presence of inorganic sulfate (Kahnert *et al.,* 2002; Beil *et al.,* 1995; Kertesz *et al.,* 1993), but currently we did not know how this functions works in other soil bacteria genera. In *Klebsiella aerogenes*, the production of thiamine by the bacteria enables the repression by sulfur compounds of arylsulfatase expression (Azakami *et al.,* 1993), same for *Citrobacter braakii* 69-b (Miura *et al.,* 2006), *Serratia marcescens* IFO 3046 (Murooka *et al.,* 1980). Allowing the release of inorganic S independently from the inorganic S content of the soil, an arylsulfatase function, which would not be repressed by the presence of SO_4^{2-}, might diminish competition for available S and be a real advantage in the rhizosphere.

The present work will further assess the frequency and population diversity of sulfatases-producing bacteria in the bulk soil, the rhizosphere soil and the root of four cultivars of *L. perenne*, in relation with root proximity and plant development stage. The frequency of bacterial isolates able to produce arylsufatases has been determined with and without inorganic S supplementation. As many bacterial functions involved in plant-bacteria interactions are regulated by quorum sensing (Delorme, 2001; Fuqua and Greenberg, 2002), the relation between sulfatase production and N-acyl homoserine lactones production abilities, was also investigated here.

III.3.2. MATERIALS AND METHODS

III.3.2.1. PLANT GROWTH, SAMPLING AND BACTERIA ISOLATION.

Soil bacteria associated with the soil-root systems of two diploid cultivars (Cavia and Lipresso) and two tetraploid cultivars (Bastion and Anaconda) of *L. perenne* were investigated. Seeds of the four *L. perenne* cultivars were disinfected according to Paterson and Sim (1999) protocol, and pre-germinated on nutrient agar (Labolife, Pully, CH) to check that they were free of bacteria. The seedlings were grown in pots (with 700 g of soil sieved at ∅ 2 mm) in greenhouse under semi-controlled conditions (12 h of photoperiod) and without any fertilisation of the soil.

The soil used in this experiment has been planted during 11 years with *L. perenne* cv. bastion (Nösberger *et al.,* 2006), and was collected in 2006 in the Institute of Plant Science field sites (ETHZ, Eschikon: 8°42'E, 47°27'N, 5565 m a.s.l.) at the end of the growing season. Topsoil was collected from the first 4 to 30 cm, and stored at 4°C prior to the experiment. It is a Eutric Cambisol (FAO classification) pH 6.5-7.6, previously described by Hebeisen *et al.* (1997), containing 28% clay, 33% silt and 36% sand and with a high availability of P and K. The organic matter content was between 2.8 and 5.1% of dry soil.

Three replicates pot experiments were performed and sampled for the four cultivars at four plant development stages: (i) hypocotyls' development (3 cm length), (ii) 4 leaves, (iii) ear development (before flowering), and (iv) ear maturity (after flowering). Ten seedlings per pot were planted for the first development stage, 6 per pot for the second one and 3 for the others. Except for the first development stage, where pot replicates were pooled because of the low root biomass, each pot was sampled separately.

For each sample three fractions were retrieved. The rhizosphere soil fraction (RS) recovered by separating the roots from its adhering soil in 20 ml sodium phosphate buffer 0.1 M pH 7.0 (SPB) under agitation during 30 min. The remaining thoroughly SPB washed roots constituted the root fraction (R). The soil fraction (S) came from independent control soil pots without plants but incubated in same conditions than the cultivated pots.

The 20 ml RS suspension, 1 g of fresh material from R or S fractions added with 10 ml of PSB were crushed in a mortar. Ten-fold serial dilutions of R, RS and S suspensions were prepared and 100 ul aliquots from the appropriate dilutions were spread out on Angle agar plates (Angle, *et al.,* 1991) in triplicates. Cultures were incubated 72 h at 24°C. Around twenty bacteria per fraction for each cultivar and each plant development stage were randomly isolated from plates presenting 10 to 100 colonies, and purified twice on Angle medium.

III.3.2.2. BACTERIAL ARYLSULFATASE PRODUCTION ASSAY.

The collection of bacterial isolates was pre-grown during 7 days at room temperature on a sulfur-free medium, adapted from the Angle medium, to exhaust intracellular sulfur. This medium contained the following ingredient per litre of deionised water: 40 ml Tris-HCl 0.5 M (pH 7), 110 µl Fe/EDTA solution (0.05 % (v/v) HCl concentrated, with Na_2EDTA 2.5mM and $FeCl_2*4H_2O$ 12 mM), 1 ml oligo-elements solution (modified from Aragno et Schlegel, 1992; $ZnCl_2$ 350 µM, $MnCl_2*4H_2O$ 150 µM, H_3BO_3 4.85 µM, $CoCl_2*6H_2O$ 840 µM, $CuCl_2*2H_2O$ 60 µM, $NiCl_2*6H_2O$ 80 µM, $Na_2MoO_4*2H_2O$ 125 µM in nanopure water), 500 µl KOH 1 M, 0.2 g NH_4NO_3, 0.59 g $CaCl_2*2\ H_2O$, 0.406 g $MgCl_2*6\ H_2O$, and 1,5 % (w/v) Agarose. pH was adjusted to 7 with NaOH 1M. Glucose 1 g.l-1 was added to the sterile, cooled medium, as a 0.22µm pore size filter sterilized solution.

The ability of the bacterial isolates to mineralise organic sulfur was tested from one sulfate starved colonies on a double layer agarose plate. A first 15 ml layer was constituted by the Angle sulfur-free medium (described above). A second 5 ml layer flowed over, was composed of Angle sulfur-free medium supplemented with a organic and a inorganic sulfur sources at final concentrations of 0.1mM X-sulfate (5-bromo-4-chloro-3-indolyl sulfate, Davies *et al.,* 1992) and with or without 0.5 mM Na_2SO_4. These sulfur sources were prepared in nanopure, deionised water and added to the sterile cooled medium after filtration on 0.22µm pore size filter.
Inoculated medium was incubated 10 days at room temperature. Arylsulfatase activity of the isolates was detected qualitatively by their ability to hydrolyze the chromogenic substrate 5-bromo-4-chloro-3-indolyl sulfate ($X\text{-}SO_4$, turning from white to blue when it is oxidised.
A rhizosphere soil isolate (originated from a garden soil enrichment culture in sulfur-free medium, this study) and the strains *Enterobacter aerogenes* NEU 1036 (strain collection of the University of Neuchâtel) were used respectively as positive and negative control. Arylsulfatase-producing isolates were noted sulf+.

The proportions of sulf+ isolates were analysed using a generalised linear model (glm) analysis with a logistic regression model. This analysis was used to compute the probabilities corresponding to the effects of the soil fractions, *L. perenne* cultivars and plant development stages, on the sulf+ frequencies. Comparison of the proportions between R, RS and S fractions, *L. perenne* cultivars, and plant development stages were performed using Tukey Kramer HSD post hoc test. The tests were performed using S-plus 6 Statistical Software (Insightful Corporation, Seattle, Washington).

III.3.2.3. BACTERIAL N-ACYL HOMOSERINE LACTONES PRODUCTION ASSAYS.

NAHL production was tested for each isolate as describe in Delorme (2001). LB agar plates with 5g.l^{-1} NaCl (Miller, 1972) was used with the biosensor *Chromobacterium violaceum* CV026 (McClean *et al.,* 1997) for detection of oxo- or C_3 non-substituted short carbon chain NAHLs (C_4 to C_8). AB agar plates (Chilton *et al.,* 1974) supplemented with 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal 40 mg.ml^{-1}) were used with the biosensor *Agrobacterium tumefasciens* NTLR4 (Shaw *et al.,* 1997) for detection of C_3 substituted or non-substituted long carbon chain NAHLs (C_6 to C_{12}).

Preculture of each strain was performed on Angle medium (Angle *et al.,* 1991). Incubations were performed in the dark at room temperature for precultures and cultures, and results were noted after a 72h period of incubation. *Pseudomonas syringae* pv. *helianthi* CFBP 2067 was used as positive control for both types of NAHL's. The proportions of NAHL+ isolates were analysed and compared in the same way than sulf+ isolates (see above).

III.3.2.4. MOLECULAR CHARACTERISATION OF ARYLSULFATASE-PRODUCING ISOLATES.

Isolates presenting arylsulfatase activity were grown on 5 ml liquid Angle medium during 24h under agitation. The bacterial culture was centrifuged in a 2 ml tube. The supernatant was discarded and DNA of the cells pellet was extracted using Wizard Genomic DNA purification kit (Promega Co., Madison, WI, USA) according to the manufacturer's protocol.

The whole 16S rDNA gene (1500 pb) was amplified from each isolate DNA extract using the forward GM3f (5'-AGAGTTTGATCMTGGC-3') and the reverse GM4r (5'-TACCTTGTTACGACTT-3') Bacteria primers (Muyzer and Ramsing, 1995), as described in Jossi *et al*. (2006) PCR protocol.

Each PCR product was digested using separately *Hae*III and *Taq*I endonucleases (Promega). Isolates which presented similar restriction profiles of their 16S rDNA sequence for both enzymes, were grouped in a same Operational Taxonomic Unit (OTU).

PCR products of representative isolates of each defined OTU were purified using Wizard SV Gel and PCR clean-up system (Promega), and cloned into pGEM-T Vector (GEM-T Vector System, Promega) and *E. Coli* XL1 electro-competent cells. Transformants with an expected size insert were processed with the Wizard®Plus SV Miniprep System (Promega) for plasmid extraction. The corresponding inserts were sequenced (MWG Biotech, Switzerland). The identification of the corresponding organisms was achieved by BLAST analysis (Altschul *et al*., 1997). These sequences were registered in the EMBL database under the accession numbers AM922175 to AM922195. Phylogenic tree was constructed with ClustalX alignment software (number of bootstrap trial = 1000).

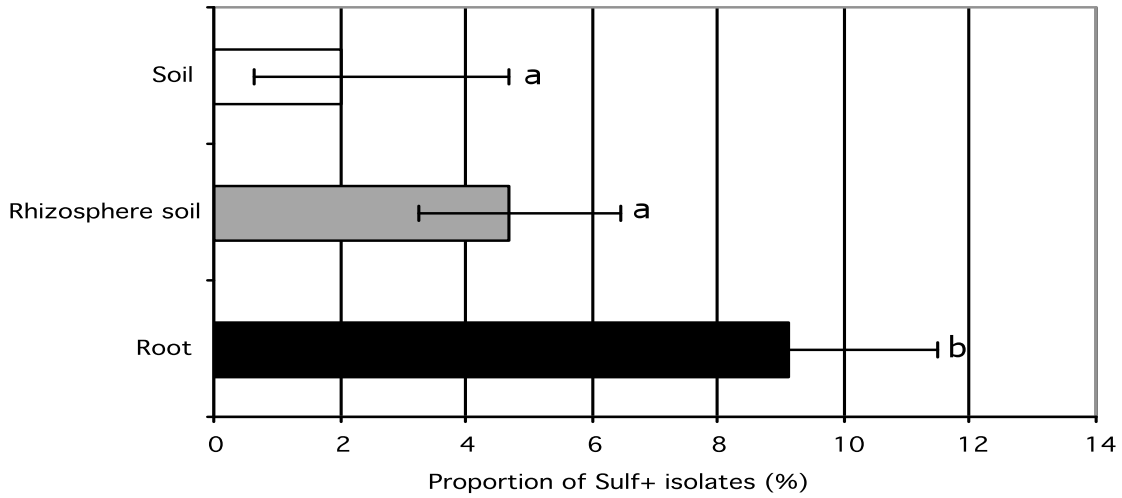
III.3.3. RESULTS

III.3.3.1. FREQUENCIES OF ARYLSULFATASE PRODUCING ISOLATES.

Among the 1686 isolates tested on agar medium with X-sulfate, 104 strains were able to produce arylsulfatases. Frequencies of sulfatases producing isolates were significantly higher in root (9 %) than in bulk soil (2 %) communities, and rhizosphere communities presented an intermediated proportion (figure III.6). No influence of plant cultivar and development stage was observed on the frequency of sulfatase producing isolates.

Figure III.6. Frequencies of arylsufatases producing isolates.

Proportion of sulfatases producing isolates, from the root (black), the rhizosphere (grey), and the bulk soil (white) communities, for all the cultivars and development stages.

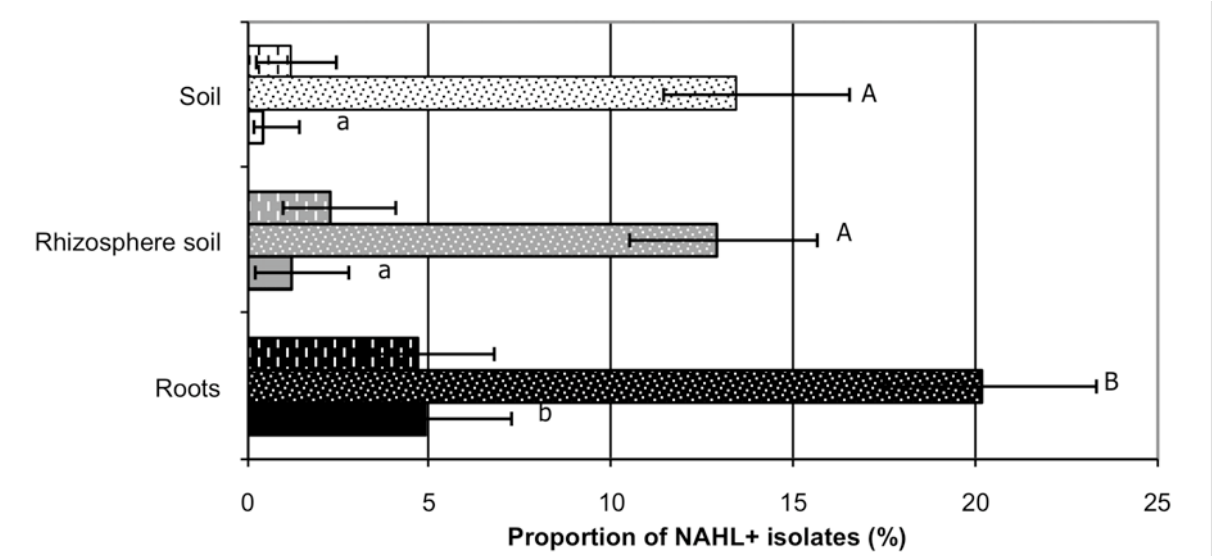


III.3.3.2. FREQUENCIES OF N-ACYL HOMOSERINE LACTONES PRODUCING ISOLATES.

Among the isolates, 2.7 % and 16 % were able to produce respectively only C_4-C_8 or C_6-C_{12} NAHL's, and 3.1 % were able to produce both types of NAHL's. Frequency of C_4-C_8 and C_6-C_{12} NAHL's producing isolates were significantly higher in root than in rhizosphere and bulk soil communities (figure III.7). No differences were observed between the proportions of isolates able to produce both NAHL's retrieved from the different fractions.

Figure III.7. Frequencies of N-acyl homoserine lactones producing isolates.

Proportion of C_4-C_8 NAHL (full color), C_6-C_{12} NAHL (color with dots), and of both NAHL (color with lines) producing isolates, from the root (black), the rhizosphere (grey) and the bulk soil (white) communities, for all the cultivars and development stages.



III.3.3.3. COMMUNITY STRUCTURE OF ARYLSULFATASE PRODUCING ISOLATES.

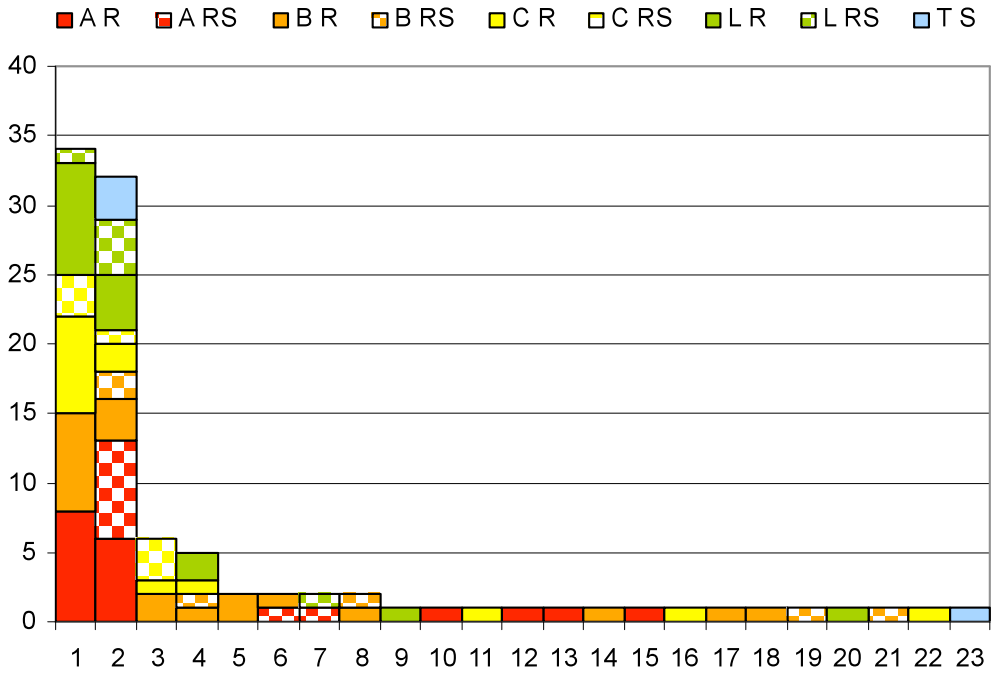
The restriction profiles obtained from 16S rDNA retrieved from the 104 sulfatase producing isolates were distributed in 23 OTU (figure III.8).

Two main OTUs grouped 66% of the isolates. OTU1 contained no strains isolated from the rhizosphere soil of Anaconda (4n) and Bastion (4n) cultivars and no isolates from the bulk soil. OTU2 strains came from bulk soil communities and from root and rhizosphere soil communities whatever the cultivar.

The remaining strains were dispatched in 15 OTUs with only one representative isolate and 6 OTUs with 2 to 6 representatives.

As observed for phytase producing strains sequences, the representatives of each OTU restriction type were affiliated to different bacterial phylum comprising orders frequently found in soil and rhizosphere environment (figure III.9).

Figure III.8. Effectives of the 23 OTU determined based on restriction profiles of 16S rDNA obtained from sulfatase positive isolates collected from the root (R) and the rhizosphere soil (RS) of each cultivars (Anaconda: A, Bastion: B, Cavia: C, Lipresso: L), and from the bulk soil communities (TS).



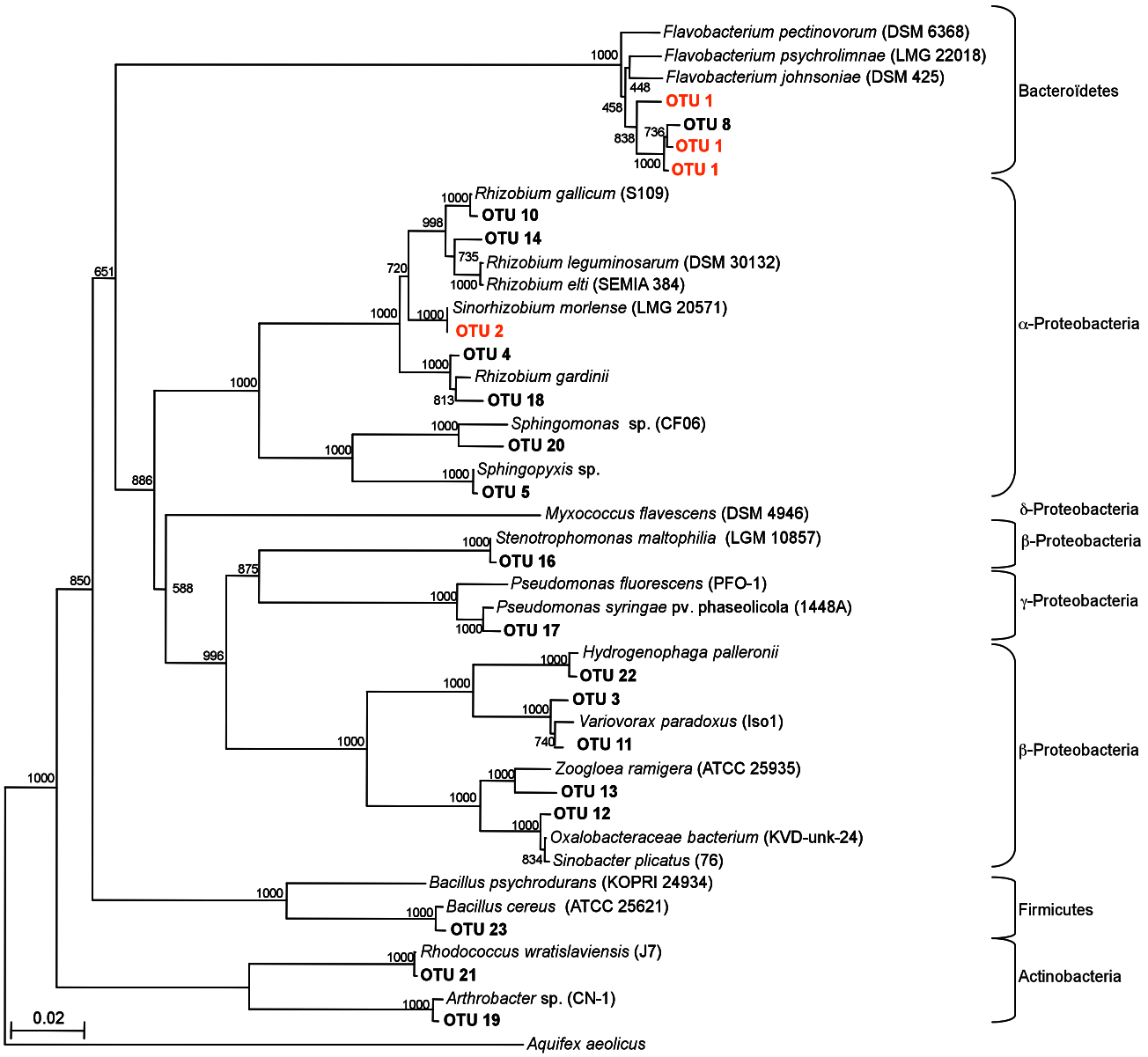
III.3.3.4. DIVERSITY OF ARYLSULFATASE PRODUCING BACTERIA.

Phylogenetical affiliation of the clones' representatives of each OTU is presented in figure III.9. A total of 73 % of the retrieved OTU were affiliated to Proteobacteria, comprising Alphaproteobacteria : 26 % *Rhizobiales*, including OTU2, 11 % *Sphyngomonadales*, Betaproteobacteria : 21 % *Burkholderiales*, 5 % *Rhodocyclales*, and Gammaproteobacteria : 5 % *Pseudomonadales*, 5 % *Xanthomonadales*.

Among the OTU retrieved, 11 %, including OTU1, belong to the Bacteroïdetes (*Flavobacteriales*), 11 % to the Actinobacteria (*Actinomycetales*), and 5 % to the Firmicutes (*Bacillales*).

OTU1 was affiliated to *Flavobacteriales* order, comprising *Flavobacterium* sp., known for plant-parasitic biological control, and OTU2 was affiliated to *Rhizobiales* order, well documented as nodulating nitrogen-fixing bacteria, and which need sulfur as nitrogenase component. Interestingly, only one representative of the *Pseudomonas* genus was retrieved in the minor OTU17.

Figure III.9. Phylogenetic tree (number of bootstrap trial = 1000) based on 16S rDNA sequences obtained from sulfatase producing isolates from each OTU.



III.3.3.5. RELATION BETWEEN ARYLSULFATASE AND N-ACYL HOMOSERINE LACTONES PRODUCTION ABILITIES.

Isolates were classified in relation with their ability to produce arylsulfatase and to produce the two types of N-acyl homoserine lactones (figure III.10). Most of Sulf+ isolates were also able to produce NAHL's. Moreover, the type of NAHL's produced by the different isolates varied in function of the phylogenetic affiliation of their related sulfatase-producing OTU's (figure III.11). Among isolates corresponding to OTU's which were affiliated to the Bacteroïdetes (most of them belong to the OTU 1), 61.1 % were able to produce C_6 to C_{12}, and 5.6 % were able to produce both C_4 to C_8 and C_6 to C_{12} NAHL's. However, among isolates correponding to OTU's which were affiliated to the Alpha-proteobacteria (most of them belong to the OTU 2), 18.6 % were able to produce C_6 to C_{12}, 27.9 % were able to produce C_4 to C_8, and 37.2 % were able to produce both type of NAHL's.

Figure III.10. Relation between sulfatase and NAHL production abilities of the isolates.

Number of isolates able (Sulf+) or not (Sulf-) to grow on inorganic S-free medium supplemented with X-sulfate, and ability of these isolates to produce C_4 to C_8 or C_6 to C_{12} N-Acyl homoserine lactones (Nahl+ C_4-C_8 or Nahl+ C_6-C_{12}).

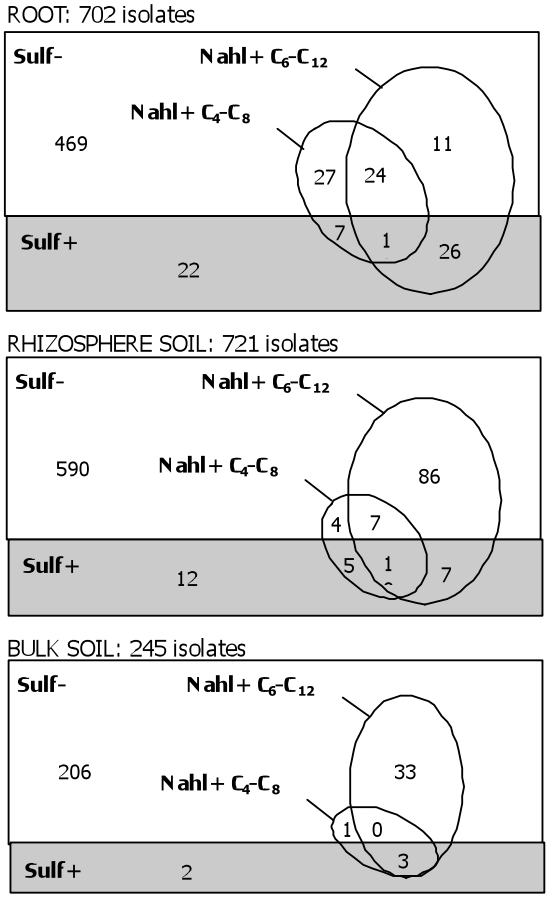
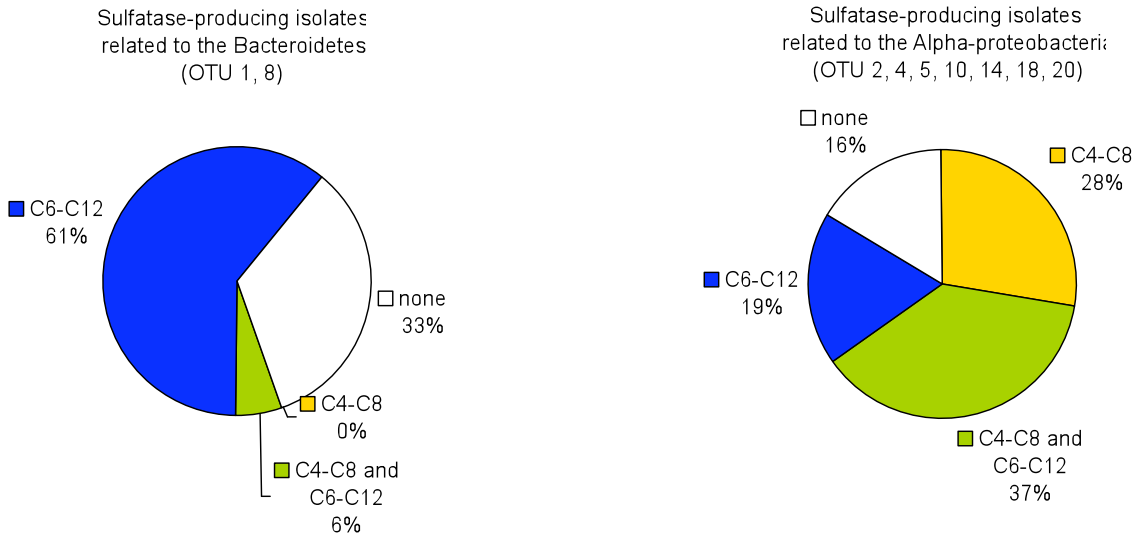


Figure III.11. Relation between the type of NAHL produced, and the phylogenetic affiliation of the isolates.

Proportion of sulfatase producing isolates able to produce C_4 to C_8 , C_6 to C_{12}, none or both types of N-Acyl homoserine lactones, in relation with the phylogenetic affiliation of their corresponding OTU.



III.3.4. DISCUSSION

III.3.4.1. FREQUENCY OF SULFATASE-PRODUCING POPULATIONS.

Among cultivable communities, 9 %, 5 % and 2 % of the isolates were found to be able to produce arylsulfatases respectively in the root, the rhizosphere and the bulk soil fractions. This enhancement of arylsulfatase production at root proximity was already observed by measurement of enzymatic activity in the rhizosphere of several plants (Han *et al.,* 1982; Vong *et al.,* 2003; Knauff *et al.,* 2003). Rhizodeposition, in particular water-soluble carbon, which is used as energy source and increases bacterial activity, favours arylsufatase activity and S mineralisation. It has been shown to have a determinant role in the dynamic of S in soils (Vong *et al.,* 2007). Root productions thus enhance sulfatase-producing populations. The free inorganic S liberated by these populations could in return became available for nutrition of others microorganisms and especially of plants (Freney *et al.,* 1975; Chapman, 1987), conferring to these bacteria a plant growth promotion property. Althought cultivable populations able to produce sulfatases were here favoured at root proximity, their frequency was low.

III.3.4.2. COMMUNITY STRUCTURE AND PHYLOGENETIC DIVERSITY OF SULFATASE-PRODUCING BACTERIA.

The 104 sulfatase-producing isolates were grouped in 23 OTU, based on restriction analysis of their 16S rDNA. Most of the retrieved OTU belong to the Proteobacteria. The two biggest OTU contained 66 % of the isolates and were affiliated to the Bacteroïdetes (OTU 1: *Flavobacteriales*) and to the Alphaproteobacteria (OTU 2: *Rhizobiales*). *Flavobacteriales* are known to belong to the populations stimulated in the rhizosphere due to the presence of root exudates and lysates (Pinton *et al.,* 2000), and *Rhizobacteriales* are well-known nitrogen fixing bacteria which need S as component of the nitrogenase (Paul and Clark, 1996), and also have been shown to produce antibiotics (Werner, 2000). These two groups are frequently found in the rhizosphere but nowadays were not renowned for their implication in organic sulfur mineralisaton. Cregut *et al.* (2007) showed that *Pseudomonas* and *Rhodococcus* were involved in the enhancement of organic S mineralisation in the rhizosphere of rape. Althought *Pseudomonas*, the main genus known to include sulfatase producing strains (Kertesz and Mirleau, 2004; Mirleau *et al.,* 2005, Kahnert *et al.,* 2002), and *Rhodococcus* , were only represented respectively by the minor OTU 17 and OTU 21, the main part of the 23 OTU's retrieved here were nevertheless affiliated to the Proteobacteria group.

III.3.4.3. RELATION BETWEEN ARYLSULFATASE AND N-ACYL HOMOSERINE LACTONES PRODUCTION ABILITIES.

An important part of the sulfatase-producing isolates recovered here were also able to produce N-acyl homoserine lactones. This could indicate that quorum sensing may be involved in the regulation of sulfatase activity. Moreover, the type of NAHL produced was shown to vary in function of the phylogenetic affiliation of the sulfatase-producing isolates.

III.3.4.4. REPRESSION OF ARYLSULFATASE ACTIVITY BY SULFATE.

The knowledges about the regulation of sulfatase production among Bacteria are still incomplete. It is however known that in the *Pseudomonas* genus expression of arylsulfatase genes are repressed by the presence of inorganic sulfate (Kahnert *et al.,* 2002; Beil *et al.,* 1995; Kertesz *et al.,* 1993). In *Klebsiella aerogenes* production of thiamine enables the repression by inorganic sulfate of arylsulfatase expression (Azakami *et al.,* 1993), same for *Citrobacter braakii* 69-b (Miura *et al.,* 2006), *Serratia marcescens* IFO 3046 (Murooka *et al.,* 1980).

In our study, all the arylsulfatase-producing isolates recovered were able to perform this function in presence or absence of inorganic sulfate. Moreover, frequency of these isolates was higher at root contact than in bulk soil.

Allowing the release of inorganic S independently from its content in soil, bacterial populations harbouring an arylsulfatase function, which is not repressed by the presence of inorganic sulfate, might improve soil inorganic S pool, diminish plant-bacteria competition for S, and thus be a real advantage in the rhizosphere. Cregut *et al.* (2007) documented that to satisfy its S demand, plant could affect arylsulfatase activity and structure of functional bacterial community capable to mineralise sulfate esters.

Another hypothesis could be formulated concerning this absence of a repression by sulfate. It may also be linked with a remaining of inorganic S in the S-free culture medium, which could explain that all the sulfatase-producing isolates recovered in this study harboured a sulfatase activity which was not affected by the addition of sulfate. In these conditions, isolates harbouring a sulfatase production repressed by sulfate could not grow. This may certainly lead to an underestimation of the proportion of sulfatase-producing isolates. However, the strains collected here correspond to populations likely to have the highest plant growth promotion potential.

III.4. CONCLUSIONS AND OUTLOOKS

The biochemical mechanisms implicated in microbial mineralisation of organic phosphorus and sulfur, and the impact of these activities on plant growth, are currently studied. However, there is still a lack of knowledge about the regulation of these activities and the diversity and population structure of the performing microorganisms. The experiments described in this chapter aimed to evaluate the occurrence and structure of bacterial populations able to mineralise organic phosphate or organic sulfate, and likely to be able to improve plant growth by these P and S nutrient supply. Focus was put on bacterial diversity of phytases and sulfatases producing populations, which may present the highest PGP potential: (i) phytate-mineralising strains were chosen among phytases producers. Indeed, the complete dephosphorylation of phytate linked to the consumption of inositol as C source was a less frequent ability than its partial dephosphorylation. However, phytate mineralisation leads to a higher ortho-phosphate release, and may increase its availability for plants. (ii) accent was put on the overall set of sulfatase producing isolates recovered here. All of them presented an arylsufatase activity, which was not inhibited by sulfate, and therefore could lead to an improvement of the soil inorganic S pool.

Phytate and sulfate esters hydrolysis were found to be more frequent in root than rhizosphere and bulk soil communities, indicating a positive selection by roots of strains presenting these abilities. No relationship was observed between siderophore production and Ca-phosphate solubilisation or phytate hydrolysis, suggesting that while phytate dephosphorylation and Ca-phosphate solubilisation could be favoured by siderophore chelatation activity, the possession of both abilities is nevertheless not an important advantage for bacteria in soil. Most of the arylsulfatase producing isolates were also able to produce N-acyl homoserine lactones, suggesting that quorum sensing is implicated in the regulation of arylsulfatase production.

Phytate and sulfate-esters hydrolysing bacterial populations presented a broad taxonomic diversity. Strains from the same phylum, commonly found in soil and rhizosphere, were retrieved for both functional communities, but the represented genera were different.

The Actinobacteria and Firmicutes Gram positive bacteria phylum were shown as likely to be important actors in the mineralisation of organic phosphorus, in particular regarding some members of the *Actinomycetales* order, which presents interesting characteristics for a use as biofertiliser. Their sporulation ability in particular improves their resistance and might be an important advantage for their conservation and transport.

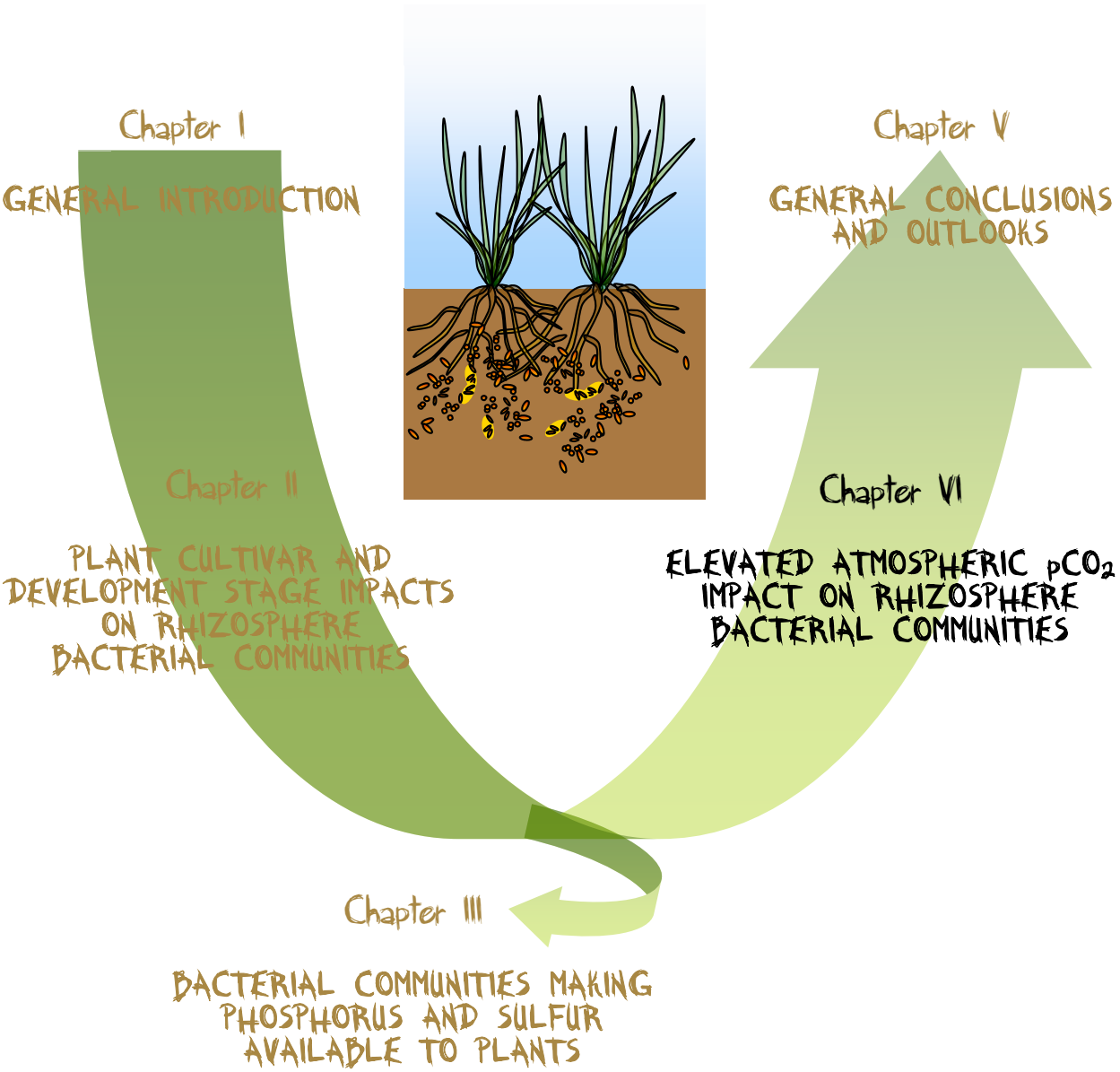
On the other hand, the Gram negative phylums Proteobacteria (*Rhizobiales*) and Bacteroïdetes (*Flavobacteriales*) were shown to be the main arylsufatase producing groups.

Moreover the dominant groups able to mineralise phytate (*Actinomycetales* and *Bacilliales*) and to hydrolyse sulfate esters (*Rhizobiales* and *Flavobacteriales*), which were retrieved here from the rhizosphere of *L. perenne*, were not the most known for these abilities. Interestingly the well-known *Pseudomonas* PGPR group was poorly represented in both phytase and sulfatase producing communities.

Functional molecular approach would be necessary to control the *in situ* activity of these populations and their impact on P and S mineralisation. And inoculation experiments have to be performed to test the efficiency of plant growth promotion activity of these strains. Anyway, in addition to their contribution to the fundamental knowledge of the rhizosphere, the information retrieved here may be important for the development of efficient biofertilisers.

CHAPTER IV :

ELEVATED ATMOSPHERIC pCO_2 IMPACT ON RHIZOSPHERE BACTERIAL COMMUNITIES


Chapter I
GENERAL INTRODUCTION
Chapter II
PLANT CULTIVAR AND DEVELOPMENT STAGE IMPACTS ON RHIZOSPHERE BACTERIAL COMMUNITIES
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BACTERIAL COMMUNITIES MAKING PHOSPHORUS AND SULFUR AVAILABLE TO PLANTS
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ELEVATED ATMOSPHERIC pCO2 IMPACT ON RHIZOSPHERE BACTERIAL COMMUNITIES

CHAPTER IV : ELEVATED ATMOSPHERIC pCO_2 IMPACT ON RHIZOSPHERE BACTERIAL COMMUNITIES

Overview

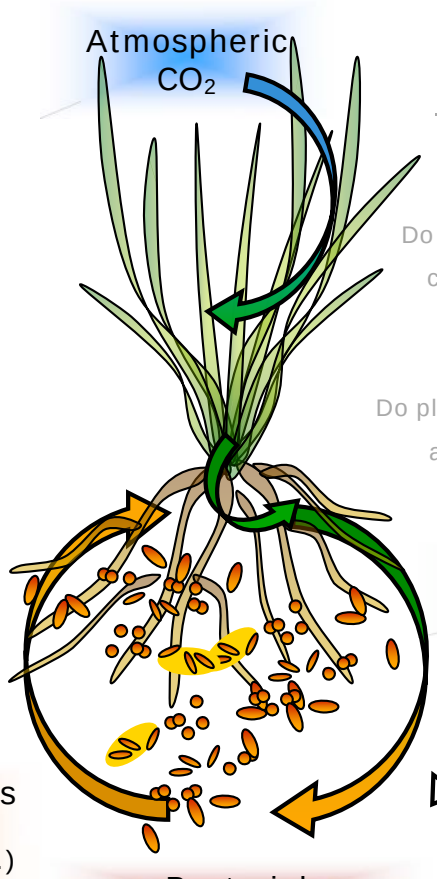
IV.1. INTRODUCTION

IV.1.1. CHAPTER PRESENTATION

This part of the work was dealing with the effect of an increase in atmospheric CO_2 content on the bacterial populations and communities in the rhizosphere of perennial grasses (*L. perenne* and *M. coerulea*). These researches benefited from the Swiss FACE (Free Air CO_2 Enrichment) system in Eschikon (Zurich), in collaboration with the Institute of Plant Science (ETHZ). As an extention of the previous experiments presented in this study, in which rhizosphere functionning was invesitgated in controlled or semi-controlled plant growth conditions, this part of the investigations was carried out in field conditions.

Under enriched CO_2 atmosphere, the plant production and rhizodeposition is enhanced and its composition may be altered. Moreover, the availability of nutrients is decreased, because of higher plant demand and of enhanced microbial activities. Rhizosphere bacterial communities selection process is therefore likely to be enhanced under elevated pCO_2. For a complete review of the impact of elevated pCO_2 on managed ecosystems, see Nösberger *et al.* (2006), and in particular Tarnawski and Aragno (2006) for impact on bacterial communities.

The objectives in this work were to determine the impact of atmospheric pCO_2 elevation on total and metabollicaly active bacterial communities structuration, and to identify indicative populations responsive to these plant-mediated changes.



Bacterial activities :

Which are the active key populations in the rhizosphere ?

Which are the most reactive populations in response to changes mediated by plant rhizodeposition and exudation ?

What is the frequency and taxonomic diversity of bacteria able to make P and S available for plants ?

Plant-mediated influences on rhizosphere environment :

Do plant cultivar influence nutrient content and conditions of their surrounding soil ?

Do plant cultivar have different organic acids and phenolic compounds exudation patterns ?

Are the differences observed in root environment consistant with those observed on bacterial community structure ?

Root selection :

Do plant-mediated influences affect genomic as well as functional bacterial community structures ?

Which bacterial functions are characteristic of root and of soil bacterial communities ?

Which relationship exists between the different bacterial abilities ?

Plant mediated influences on bacterial communities :

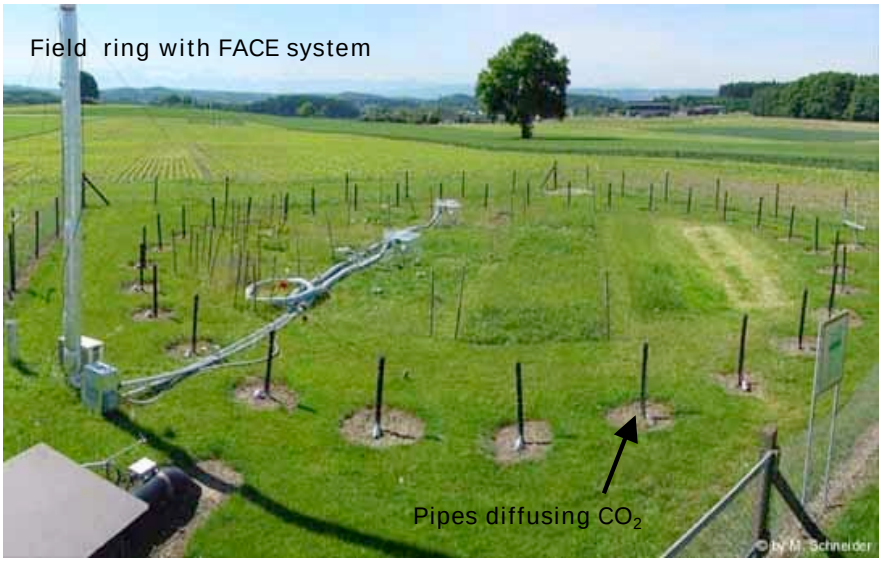
How do plant cultivar, plant development stage and elevated atmospheric pCO_2 influence bacterial community structure ?

IV.1.2. FREE-AIR CARBON DIOXIDE ENRICHMENT FACILITIES

Free-air carbon dioxide enrichment (FACE) offers a technology allowing an experimental setup, which do not change the microclimate, and which is large enough to permit a reasonable field sampling. The FACE experiments are not only useful to predict the impact of an elevated pCO_2 on ecosystems, but are also designed to investigate fundamental mechanisms that drives ecosystem structure and function, and to test ecological concepts (Nösberger *et al.,* 2006).

The site of the facilities was located at Eschikon (8°41'E, 47°27'N, 550 m a.s.l.). The experiment consited of rings of 18 m diameter with or without (control) CO_2 enrichment pipes installations (Nösberger *et al.,* 2006). Pairs of one FACE and one control ring were placed on fields with the same crop history. The Swiss FACE experiment offered the opportunity to investigate the impact of environmental perturbations on grassland ecosystems for a relatively long period of 10 years (from 1993 to 2003).

FACE facilities (Eschikon)



IV.2. ELEVATED pCO_2 IMPACT ON RHIZOSPHERE BACTERIAL COMMUNITIES

How elevated pCO_2 modifies total and metabollically active bacterial communities in the rhizosphere of two perennial grasses grown under field conditions ?

Maryline Jossi , Nathalie Fromin , Sonia Tarnawski , Florian Kohler , François Gillet , Michel Aragno, and Jérôme Hamelin

ABSTRACT

The response of total (DNA-based analysis) and active (RNA-based analysis) bacterial communities to a pCO_2 increase under field conditions was assessed using two perennial grasses: the nitrophilic *Lolium perenne* and the oligonitrophilic *Molinia coerulea*. PCR- and reverse transcriptase-PCR denaturing gradient gel electrophoresis analysis f 16S rRNA genes generated contrasting profiles. The pCO_2 increase influenced mainly the active and root-associated component of the bacterial community. Bacterial groups responsive to the pCO_2 increase were identified by sequencing of corresponding denaturing gradient gel electrophoresis bands. About 50 % of retrieved sequences were affiliated to Proteobacteria. Our data suggest that Actinobacteria in soil and Myxococcales (Deltaproteobacteria) in root are stimulated under elevated pCO_2.

For full text see FEMS MICROBIOLOGY ECOLOGY 55 (2006) 339-350

How elevated pCO_2 modifies total and metabolically active bacterial communities in the rhizosphere of two perennial grasses grown under field conditions

Maryline Jossi[1], Nathalie Fromin[1,2], Sonia Tarnawski[1], Florian Kohler[3,4], François Gillet[3,5], Michel Aragno[1] & Jérôme Hamelin[1,6]

[1]Microbiology Laboratory, University of Neuchâtel, Neuchâtel, Switzerland; [2]CEFE-CNRS, Montpellier Cedex, France; [3]Plant Ecology Laboratory, University of Neuchâtel, Neuchâtel, Switzerland; [4]WSL, Swiss Federal Research Institute, Antenne Romande, Lausanne, Switzerland; [5]Laboratory of Ecological Systems, EPFL-ECOS, Swiss Federal Institute of Technology, Lausanne, Switzerland; and [6]Laboratory of Environmental Biotechnology, INRA, Avenue des Etangs, Narbonne, France

Correspondence: Maryline Jossi, Microbiology Laboratory, University of Neuchâtel, PO Box 2, CH-2007 Neuchâtel, Switzerland. Tel.: +41 32 718 23 34; fax: +41 32 718 22 31; e-mail: maryline.jossi@unine.ch

Received 28 June 2005; revised 2 September 2005; accepted 6 September 2005.
First published online 5 January 2006.

doi:10.1111/j.1574-6941.2005.00040.x

Editor: James Prosser

Keywords
Bacterial community; 16S rRNA; DGGE; global change; carbon dioxide; FACE.

Abstract

The response of total (DNA-based analysis) and active (RNA-based analysis) bacterial communities to a pCO_2 increase under field conditions was assessed using two perennial grasses: the nitrophilic *Lolium perenne* and the oligonitrophilic *Molinia coerulea*. PCR- and reverse transcriptase-PCR denaturing gradient gel electrophoresis analysis of 16S rRNA genes generated contrasting profiles. The pCO_2 increase influenced mainly the active and root-associated component of the bacterial community. Bacterial groups responsive to the pCO_2 increase were identified by sequencing of corresponding denaturing gradient gel electrophoresis bands. About 50% of retrieved sequences were affiliated to *Proteobacteria*. Our data suggest that *Actinobacteria* in soil and Myxococcales (*Deltaproteobacteria*) in root are stimulated under elevated pCO_2.

Introduction

Since the beginning of the industrial revolution, the atmospheric CO_2 concentration (pCO_2) has been rapidly increasing, affecting the global climate and the functioning of oceanic and terrestrial ecosystems (Bazzaz & Sombroek, 1999; Fuhrer, 2003). Much research has focused on the consequences of elevated pCO_2 on plant physiology and growth, as well as on vegetation structure. Elevated pCO_2 enhances the net photosynthesis, the shoot and root biomass, and the litter input relative to ambient pCO_2 condition (Sowerby *et al.*, 2000; Zak *et al.*, 2000; Ainsworth *et al.*, 2003), particularly in C3 plants (Long *et al.*, 2004).

Under current ambient atmospheric conditions, up to 50% of the assimilated carbon is translocated to the belowground (Kuzyakov & Domanski, 2000) providing carbon and energy sources easily available for soil biota. Under elevated pCO_2, greater input (Darrah, 1996) and qualitative changes (Hodge *et al.*, 1998) in carbon released into the rhizosphere are likely to impact the soil microflora (Jones *et al.*, 1998). For instance, the effects of a pCO_2 increase were described on arbuscular mycorrhizal fungi (Gamper *et al.*, 2004), on relative frequency (Marilley *et al.*, 1999) and on phenotypic structure of *Pseudomonas* (Roussel-Delif *et al.*, 2005; Tarnawski *et al.*, in press). Firstly, CO_2-induced alterations in carbon supply could modify microbial processes that are directly dependant on carbon input, particularly decomposition and nutrient cycling (Hu *et al.*, 1999). Secondly, elevated pCO_2 could alter the structure of the microbial community due to qualitative changes in carbon supply under these conditions. In turn, the selection or counterselection of plant-deleterious (Chakraborty *et al.*, 2000) or plant-beneficial microorganisms (Gamper *et al.*, 2004; Tarnawski *et al.*, in press) would have feedback effects on plant growth and physiology, because of a shift in microbial balance. In particular, this might enhance plant growth by increasing nutrient acquisition from previously unavailable pools (Hu *et al.*, 1999).

In order to understand how soil–plant systems respond to elevated pCO_2, the response of the microbial community has to be characterized and the populations involved in this response have to be identified. As most microbes are in an 'inactive' state in soils (Hu *et al.*, 1999), whole community parameters (i.e. DNA- and fatty acid-based analyses) are probably less sensitive than those measuring some

component of the active microflora. Recently developed molecular approaches such as stable isotope probing (Radajewski *et al.*, 2000) or RNA-based analysis (Felske & Akkermans, 1998; [19]Koizumi *et al.*, 2003) may be appropriate.

The development of molecular techniques in microbial ecology, including those based on the small subunit ribosomal RNA gene sequence as molecular marker, provides a significant advantage in studying microbial communities in terms of richness and structure, allowing monitoring of changes in microbial communities in a large number of samples (Muyzer *et al.*, 1993; [20]Fromin *et al.*, 2002). Molecular fingerprinting techniques such as denaturing gradient gel electrophoresis (DGGE) generate snapshots of the bacterial community, displayed as patterns related to the presence of dominant populations. Moreover 16S rRNA transcripts can be targeted. As the ribosome content of cells depends on their activity level, profiles obtained after reverse transcriptase (RT)-PCR on environmental 16S rRNA are therefore weighted according to the actual activity of the related populations (Wagner, 1994). By comparing DNA- and RNA-based profiles, it is then possible to highlight the dominant active members of the community (Felske *et al.*, 1998). These fingerprinting analyses generate a large amount of data, which should benefit from the development of numerical ecology (Legendre & Legendre, 1998; Fromin *et al.*, 2002). Ordination methods can be used to compare DGGE patterns with each other as multivariate responses to environmental variables (Ter Braak, 1986; Borcard *et al.*, 1992).

The aims of the present study were to investigate whether elevated pCO_2 influence the bacterial community and to highlight and identify the most affected populations and their potential metabolic role in these soils. The response of soil and root-associated microflora to high atmospheric pCO_2 content was assessed by molecular fingerprinting of total and active bacterial communities. Two hemicryptophytic perennial grasses were used as model plants: *Lolium perenne* and *Molinia coerulea*. These plants have different trophic requirements (nitrophilic and oligonitrophilic Vazquez de Aldana & Berendse, 1997), allowing testing of the importance of the functional type regarding the plant response to an elevation of atmospheric pCO_2 (Lüscher *et al.*, 1998). A shift in the bacterial community structure under high pCO_2 was revealed by DGGE profiling after direct PCR (total) and RT-PCR (active community) amplification of 16S rRNA genes from soil and root samples. Multivariate statistical analyses were used to highlight responsive bacterial groups.

Materials and methods

Study site and plant material

Lolium perenne and *Molinia coerulea* were grown under field conditions and current ambient (36 Pa, C for control) vs. elevated pCO_2 (60 Pa, T for treated) in the Free Air CO_2 Enrichment (FACE) facilities at Eschikon, Switzerland (Hebeisen *et al.*, 1997). The atmosphere of treated plots was enriched with CO_2 during the growing season and daytime only. *Lolium perenne* cv Bastion (L.) was grown as a monoculture on three control (LC) and three CO_2-treated (LT) replicate plots from May 1993. The plants were grown on the autochthonous soil, a fertile Eutric Cambisol (FAO classification). The shoots were harvested four times a year. The LC and LT plots received $14\,g\,m^{-2}\,year^{-1}$ N as NH_4NO_3, at the beginning of the season, and then after each cut, except the last (this amount was demonstrated to be limiting for plant growth during the FACE experiment; Daepp *et al.*, 2000). *Molinia coerulea* plants (M) originated from a littoral meadow on the south shore of Lake Neuchâtel (Cudrefin, Switzerland). The local soil, a Gleysol, Typic Haplaquoll, contained about 4.7% clay, 9.5% silt and 85.8% sand, with a pH[H_2O] value of 8.4 (Hamelin *et al.*, 2002). Plants with undisturbed root systems were taken with their surrounding and underlying soil, and transferred to the FACE facilities in September 1999. About $0.7\,m^2$ of littoral meadow with reconstructed soil profile below the root horizon (total depth: 35 cm) was installed in one control plot (MC) and one CO_2-treated plot (MT). The plants were neither cut nor fertilized.

Sampling

Sampling was performed at three sampling dates (21 June 2001, 7 May 2002 and 15 July 2002). Two subsamples were collected, one for DNA extraction and another for RNA extraction (RNase-free sampling conditions). For *L. perenne*, one to three control (C) and CO_2-treated (T) plots were sampled at each date (Table 1). For *M. coerulea*, only technical replicates could be sampled as only one plot per pCO_2 condition was available. Sampling of *L. perenne* was always performed just before a cut. For each sampling, three soil cores (about 3 cm diameter, 10–12 cm depth) including root systems, were taken and pooled for analysis. Two fractions were recovered: the non-adhering soil, obtained by shaking roots (S) and the root itself (R, for rhizoplane-endorhizosphere) after thorough washing of root-adhering soil. Soil and root samples were immediately placed in FastRNA™ matrix tubes for RNA and FastDNA™ matrix tubes for DNA (Bio101, QBiogene, Inc., Basel, Switzerland) and instantaneously frozen in liquid nitrogen.

DNA extraction and purification

DNA extraction and purification were performed on about 0.5 g of fresh root or soil material. A bead-beating apparatus (FP120 FastPrep™ cell disruptor, Savant Instruments, Inc.,

Table 1. DNA and RNA yields ($\mu g\, g^{-1}$ soil or root fresh weight) obtained from soil and root samples of *Lolium perenne* and *Molinia coerulea* growing under ambient or elevated pCO_2 content

				Average yield ± SD (no. replicates)	
Sampling date	pCO_2 treatment	Type of sample	Nucleic acid type	*Lolium perenne*	*Molinia coerulea*
21 June 2001	Control	Soil	DNA	2.5	6.2
			RNA	14.1	9.2
		Root	DNA	3.8	2.9
			RNA	7.8 ± 0.5 (3)	8.8
	Treated	Soil	DNA	6.7	4.0
			RNA	6.5	4.7
		Root	DNA	5.9	7.5
			RNA	8.5 ± 0.2 (3)	7.4
7 May 2002	Control	Soil	DNA	10.3	4.4
			RNA	8.0 ± 0.8 (2)	9.8 ± 5.7 (2)
		Root	DNA	12.3	8.4
			RNA	29.2	10.5 ± 0.7 (2)
	Treated	Soil	DNA	10.8	5.1
			RNA	8.9 ± 7.5 (2)	7.9 ± 2.4 (2)
		Root	DNA	4.2	10.2
			RNA	28.8 ± 3.2 (2)	9.8 ± 1.5 (2)
15 July 2002	Control	Soil	DNA	6.2 ± 1.0 (3)	5.8 ± 1.2 (2)
			RNA	8.3 ± 1.5 (3)	7.2 ± 0.2 (2)
		Root	DNA	6.3 ± 2.8 (3)	7.6 ± 1.0 (2)
			RNA	9.3 ± 1.4 (3)	8.9 ± 0.2 (2)
	Treated	Soil	DNA	3.3 ± 0.8 (3)	6.6 ± 0.4 (2)
			RNA	6.9 ± 3.0 (3)	6.1 ± 2.3 (2)
		Root	DNA	7.7 ± 1.4 (3)	8.2 ± 1.8 (2)
			RNA	5.6 ± 1.2 (3)	11.7 ± 2.1 (2)

Standard deviation (± SD) is indicated when replications were performed. The number of replicates is indicated in parentheses.

Hotbrook, NY) was used in combination with the FastDNA Spin Kit for Soil (Bio101) according to Borneman *et al.* (1996), except that 500 μL of DNA lysate were purified using 500 μL of Binding Matrix (Bio101). The final DNA extracts were quantified using GeneQuant RNA/DNA calculator (Amersham Pharmacia Biotech, Cambridge, UK) and stored at −20 °C before use.

RNA extraction and purification

From sampling until cDNA synthesis, all RNA handling was performed under RNase-free conditions. Aqueous solutions were treated with 0.1% diethyl pyrocarbonate (DEPC). Glassware was heated to 200 °C overnight and plastic material soaked overnight in a 0.1 N NaOH/1 mM EDTA solution, before rinsing with RNase-free water. The working area and materials reserved for RNA handling were treated with RNase-AWAY solution (Molecular BioProducts Inc., San Diego, CA).

Total RNA was extracted and purified using a combination of FastRNA™ tubes with Green Caps (Bio101) and RNeasy® Plant Kit (Qiagen AG, Basel, Switzerland). The samples were put on ice between the extraction steps. In each FastRNA™ tube containing about 150–500 mg of frozen sample, 450 μL of RLT Buffer (Qiagen) were added. The mixture was shaken for 10 s at $6\, m\, s^{-1}$ using the FastPrep™ cell disruptor. This step was repeated once after cooling tubes for 5 min on ice. Borneman & Triplett (1997) found this 20-s period of bead beating to be optimal for maximum cell lysis and minimum RNA shearing. The tubes were then centrifuged for 5 min at 13000 ***g*** and the supernatant was loaded on QIAshredder Spin Columns (Qiagen) and then processed as recommended by the manufacturer. DNA was removed using DNase (Qiagen) according to the manufacturer's protocol. The final RNA extracts were eluted in 100 μL 10-mM Tris pH 7.0, quantified using GeneQuant (Amersham Pharmacia), and stored at −80 °C before use. PCR amplification and DGGE were performed directly on each RNA extract to detect DNA contamination. In a few cases the presence of DNA was detected in the RNA extract, in which case the corresponding band positions were then discarded for further analysis.

Reverse transcription of total RNA

Reverse transcription reactions were performed using ImProm-II™ Reverse Transcription System (Promega Corp.,

Madison, WI) with random hexamer primers in a thermocycler model PTC-200 (MJ Research Inc., Watertown, MA). A total of 3.5 μL of RNA extract (55–70 ng depending on the sample) was mixed with 1 μL of primers (10 mM), and 0.5 μL of RNasin® Ribonuclease Inhibitor. This mixture was incubated at 70 °C for 5 min for an optimal contact between RNA and primers, and chilled on ice until the reverse transcription mix was added. This mix was then combined with (final concentrations) 1× ImProm-II™ Reaction Buffer, 0.05 U μL^{-1} RNasin, 6 mM $MgCl_2$, 0.5 mM each dNTP, 5% (v/v) ImProm-II™ Reverse Transcriptase and DEPC-treated nanopure water in a final volume of 20 μL. The reaction consisted of annealing at 25 °C for 5 min, extension at 42 °C for 1 h and inactivation of reverse transcriptase at 70 °C for 15 min. The resulting cDNA was used immediately for PCR or stored at −20 °C. Positive and negative control reactions were performed as recommended by the manufacturer.

PCR amplification

PCR amplification of the V3 region of 16S rRNA gene was performed in two steps. The whole 16S rRNA gene was first amplified using the forward GM3f (5′-AGAGTTTGATCMTGGC-3′) and the reverse GM4r (5′-TACCTTGTTACGACTT-3′) *Bacteria* primers (Muyzer & Ramsing, 1995). The PCR reaction mix contained (final concentrations) 1X Thermophilic DNA Buffer, 3 mM $MgCl_2$, 0.25 mM dNTPs, 0.25 μM of each primer (MWG Biotech AG, Ebersberg, Germany), and 0.05 U μL^{-1} of Taq DNA polymerase (Promega). A total of 2 μL of DNA or cDNA extract were added as template for the PCR. The final reaction volume was adjusted to 20 μL. The reaction mixtures were subjected to 26 amplification cycles in a thermo-cycler. The first heat denaturation step was performed at 94 °C for 4 min 30 s. Cycles consisted of heat denaturation at 94 °C for 1 min, primer annealing at 56 °C for 30 s with a touchdown of 1 °C every 2 cycles for a total of ten cycles, and extension at 74 °C for 1 min. The mixture was maintained at 74 °C for 10 min for the final extension. The forward 338f (5′-ACTCCTACGGGAGGCAGCAG-3′) and reverse 520r (5′-ATTACCGCGGCTGCTGG-3′) universal primers (Ovreas *et al.*, 1997) were used for nested amplification of the V3 region of the 16S rRNA gene to increase the amplification yield and to obtain a fragment size suitable for DGGE analysis. A 40-bp GC-clamp (Muyzer *et al.*, 1993) was added on the forward primer for DGGE analysis. The nested-PCR mix was prepared as for the first PCR except 5 μL of PCR-amplified 16S rRNA were added as template and the final volume was adjusted to 50 μL. The first heat denaturation step was performed at 94 °C for 5 min. The reaction mixtures were then subjected to 31 amplification cycles. Cycles consisted of heat denaturation at 94 °C for 1 min, primer annealing at 65 °C for 30 s with a touchdown of 1 °C per cycle for ten cycles, and extension at 74 °C for 1 min. The mixture was maintained at 74 °C for 10 min for the final extension. The PCR products were checked for size and yield on 1% agarose gels in comparison to the Low DNA Mass Ladder (Invitrogen).

DGGE analysis

Denaturing gradient gel electrophoresis analysis of 16S rRNA genes and cDNA amplicons was performed using the D-code electrophoresis system (Bio-Rad Inc., Hercules, CA). About 600–800 ng of PCR products were loaded directly on a 8% (w/v) polyacrylamide gel (acrylamide-bisacrylamide 37.5 : 1) with a linear gradient from 30% to 60% denaturants (100% correspond to 40% formamide plus 7 M urea). The strains used to build the reference DGGE pattern were ordered as follows after migration (Fig. 1): *Pseudomonas fluorescens* ATCC 27663, *Bacillus subtilis* ATCC 14893, *Flavobacterium capsulatum* DSM 30196, *Rhizobium meliloti* DSM 1981, *Arthrobacter globiformis* DSM 20124 and *Thermus filiformis* NCIMB 12588. The gels were run at 60 °C and 150 V for 5 h in 1× TAE buffer. They were stained with

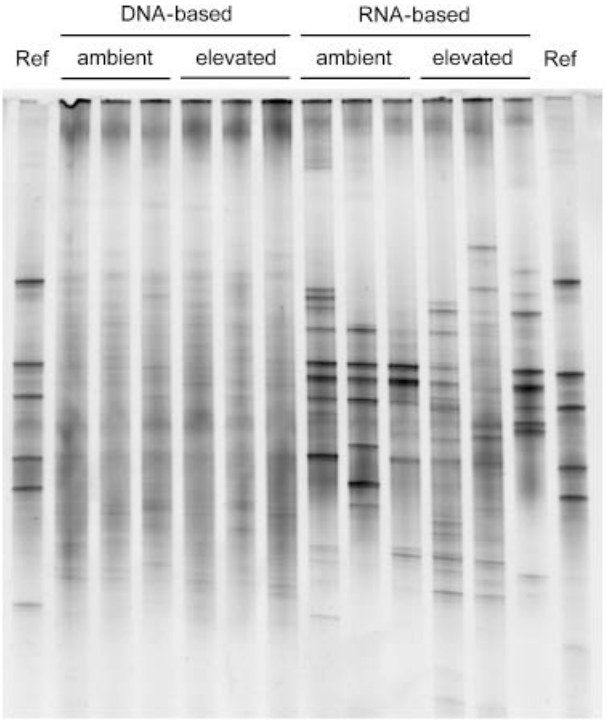


Fig. 1. Example of 16S rRNA gene-based denaturing gradient gel electrophoresis profiles obtained from soil of *Lolium perenne* plots cultivated under ambient and elevated pCO_2. The first six patterns are rDNA-based profiles and the last six are rRNA-based profiles from three replicate plots for each pCO_2 condition for the third sampling date. Ref stands for the reference pattern.

0.01% SYBR Green (Molecular Probes, Leiden, the Netherlands) in 1× TAE at 4 °C in the dark for 20 min, then UV photographed with the Multi-Analyst package (Bio-Rad).

Data handling and statistical analysis of DGGE patterns

The gel images were normalized regarding the band positions of the reference patterns for each gel, then the sample patterns were compared using GelCompar software (Applied Maths, Kortrijk, Belgium). The fingerprints obtained were codified in terms of migration length and relative intensity (p_i) of each band (i) within the profile (total intensity of the profile $\Sigma p_i = 1$). Two datasets were obtained: (1) DNA data matrix, containing relative intensity of bands for DNA-based profiles and (2) RNA data matrix containing relative intensity of bands for RNA-based profiles. For both plants, co-amplification of amyloplastic DNA and RNA was often observed for root samples (the position of plant 16S rRNA fragment after DGGE analysis was checked using plant axenic DNA extract as PCR template). Corresponding bands were discarded for further analyses.

The percentage of similarity between DNA- and RNA-based profiles obtained from both pCO_2 conditions (ambient/treated) was calculated using the Steinhaus coefficient. It was computed from the relative intensity of DGGE bands (set between 0 to 1), by the sum of the lower frequency observed for each pair of common bands (Legendre & Legendre, 1998). It gives more weight to intense common bands than to weak common bands. All calculations were done using the R 1.9.0 package (R Development Core Team, 2004).

The data matrices were then transformed for ordination analyses. Bands appearing only in one DNA- or RNA-based profile were discarded from dataset due to their low representation within profiles to avoid a rare species effect. Band intensities from the remaining data (64 bands for RNA matrix and 55 bands for DNA matrix) were normalized using the log-transformation: $p_i' = \ln(p_i + 1)$.

Canonical Correspondence Analysis (CCA) was applied on both transformed data matrices to represent the influence of explanatory variables on the one hand, and to evidence the most 'impacted' bands on the other hand. Data were initially submitted to variation partitioning analysis (Borcard *et al.*, 1992) using a series of partial CCA to display variability of the patterns constrained by factors of interest. Three sets of explanatory variables were employed: (1) sampling date (environmental conditions at sampling time) and replication; (2) plant–soil system (*M. coerulea*/*L. perenne*); and (3) root presence combined with pCO_2 treatment (ambient/elevated). The significance of the results was tested with the Monte Carlo permutation test. The whole process was based on computations made with R 1.9.1 (R Development Core Team, 2004). In a second step, in order to focus on the effects of pCO_2 and roots on DGGE profile variability, part of the variability explained by sampling date and replication was subtracted by using these as co-variables for partial CCA. Data obtained from *L. perenne* and from *M. coerulea* were separated to distinguish key DGGE bands for each plant–soil system.

Selection of indicative bands

Various bands were selected according to the number of profiles in which they were detected (frequent bands were preferred to rare ones) and their position on CCA plots (bands distant from the graphic axes origin were preferred to others). Among these bands, we considered that those which were close to the centroïds of the explanatory variables corresponded to indicative populations that were most influenced by these factors. Five indicative bands (corresponding to position 225 for *L. perenne* samples and 151, 224, 246 and 249 for *M. coerulea* samples; Table 2) were excised from different DGGE patterns to check the corresponding sequence homogeneity.

Identification of the responsive populations

Selected DGGE bands were excised. Corresponding DNA was eluted overnight at 4 °C with 15 µL nuclease-free water and the electrophoresis – excision – elution cycle was repeated to ensure that the recovered DNA corresponded to a given DGGE band. Then the recovered DNA was amplified with a 338f – 520r primer set using 15 amplification cycles (heat denaturation at 94 °C for 1 min, primer annealing at 65 °C for 30 s and extension at 74 °C for 1 min). PCR products were purified and cloned into pGEM-T and introduced into *Escherichia coli* XL1 by electroporation. The sequence homogeneity within a single DGGE band was checked on obtained clones with *Hae*III restriction patterns of T7-SP6 PCR amplicons. For each band, one clone was sequenced for each restriction profile type. The phylogenetic affiliation of corresponding organisms was achieved by BLAST analysis (Altschul *et al.*, 1997). Sequences were deposited at EMBL under the accession numbers AJ851090 to AJ851151.

Results

Extraction yields and extracts purity

The average DNA and RNA extraction yields were respectively 6.0 (SD ± 2.5) and 8.1 (SD ± 2.4) µg g^{-1} fresh weight for soil and 7.1 (SD ± 2.7) and 12.2 (SD ± 8) µg g^{-1} fresh weight for root samples (Table 1). The quality and quantity of the extracts were always sufficient for PCR and RT-PCR reactions, irrespective of the plant-soil system and the pCO_2 treatment.

Table 2. Characterisation of bands obtained from 20 and 11 DNA- and 39 and 27 RNA-based denaturing gradient gel electrophoresis (DGGE) profiles for *Lolium perenne* and *Myxococcales coerulea* respectively. The bands were selected for their variation in intensity within profiles according to the origin of the sample, to the pCO_2 content and to the nucleic acid type. The total length of the DGGE gel corresponds to 500 pixels. The representativity of a given band was expressed as the percentage of DNA- or RNA-based profiles in which the band was detected. Changes in band intensities induced by pCO_2 elevation are expressed as increase (+%) or decrease (−%) in band intensity. For each band, several clones were checked, the number of clone affiliated to the same restriction type is mentioned in parentheses. One clone of each type has been sequenced, accession numbers of the sequences are indicated after the affiliation group. An asterisk was added next to the DGGE band name when the sequences was retrieved from RNA profile.

DGGE band description							Corresponding populations	
			Representativity among profiles (%)		Effect of pCO_2 elevation on band relative intensities (%)		Closest phylogenetic group (affiliation based on Blast)	
Name	Position (pixels)	Origin	DNA	RNA	Root	Soil	Restriction type A	Restriction type B
Lolium perenne								
L1*	101	Soil	0	13	−100	+100	*Gammaprot.*/Pseudomonadales (4/8); AJ851090	Actinobacteria (4/8); AJ851091
L2*	112	Root	5	18	−100	U	unaffiliated (3/3); AJ851092	
L3	128	Root	20	28	−11	+100	Bacteroidetes (7/7); AJ851093	
L4	133	Root	40	41	+82	–15	*Betaprot.*/Burkholderiales (1/4); AJ851094	*Gammaprot.*/Enterobacteriales (3/4); AJ851095
L5*	164	Root	20	15	+81	+444	*Betaprot.*/Burkholderiales (3/8); AJ851096	*Deltaprot.*/Myxococcales (5/8); AJ851097
L6*	168	Root	20	36	+35	−3	*Gammaprot.*/Enterobacteriales (1/8); AJ851098	*Deltaprot.*/Myxococcales (7/8); AJ851099
L7	179	Soil	20	18	−13	+12	Bacteroidetes (2/7); AJ851100	Bacteroidetes (5/7); AJ851101
L8*	190	Root	25	26	−86	+48	*Alphaprot.*/Rhizobiales (2/5); AJ851102	*Deltaprot.*/Myxococcales (3/5); AJ851103
L9	225	Soil	50	62	+136	+1021	unaffiliated (4/4); AJ851104	
L10*	225	Root	50	62	+136	+1021	unaffiliated (8/8); AJ851105	
L11*	225	Soil	50	62	+136	+1021	Actinobacteria (6/6); AJ851106	
L12*	225	Root	50	62	+136	+1021	*Alphaprot.* (8/8); AJ851107	
L13*	231	Root	15	26	+1264	−1	*Deltaprot.*/Myxococcales (5/5); AJ851108	
L14*	237	Soil	25	33	+197	+100	Actinobacteria (1/4); AJ851109	unaffiliated (3/4); AJ851110
L15*	290	Root	25	36	−54	+4	*Deltaprot.*/Desulfurellales (3/6); AJ851111	*Deltaprot.*/Myxococcales (3/6); AJ851112
L16	293	Soil	30	13	+74	–58	unaffiliated (1/8); AJ851113	Actinobacteria (7/8); AJ851114
L17	298	Soil	5	8	U	–56	unaffiliated (1/3); AJ851115	*Alphaprot.* (2/3); AJ851116
Myxococcales coerulea								
M1*	98	Soil	0	11	U	+96	Bacteroidetes (3/8); AJ851117	unaffiliated (5/8); AJ851118
M2	140	Root	46	11	+82	+317	unaffiliated (4/4); AJ851119	
M3	145	Root	36	44	+61	+32	unaffiliated (1/8); AJ851120	Actinobacteria (7/8); AJ851121
M4	151	Root	73	37	+105	−66	*Gammaprot.*/Enterobacteriales (1/5); AJ851122	Firmicute/Bacillales (4/5); AJ851123
M5*	151	Root	73	37	+105	−66	*Gammaprot.*/Enterobacteriales (2/3); AJ851125	*Deltaprot.*/Myxococcales (1/3); AJ851124
M6*	172	Soil	55	44	+121	+297	unaffiliated (3/8); AJ851126	*Betaprot.*/Burkholderiales (5/8); AJ851127
M7*	207	Soil	9	26	−100	−38	*Betaprot.*/Burkholderiales (3/5); AJ851128	*Betaprot.*/Burkholderiales (2/5); AJ851129
M8*	224	Soil	55	41	+635	−70	*Betaprot.*/Burkholderiales (3/8); AJ851130	Actinobacteria (5/8); AJ851131
M9*	224	Root	55	41	+635	−70	Bacteroidetes (6/8); AJ851132	unaffiliated (2/8); AJ851133
M10*	233	Soil	27	41	+4	−53	Firmicutes (1/3); AJ851134	*Deltaprot.*/Myxococcales (2/3) ; AJ851135
M11	237	Root	9	7	+100	+100	*Deltaprot.*/Myxococcales (1/6); AJ851136	*Alphaprot.* (5/6); AJ851137
M12	246	Root	18	15	−35	−100	*Deltaprot.*/Myxococcales (3/7); AJ851138	*Deltaprot.*/Myxococcales (4/7); AJ851139
M13*	246	Root	18	15	−35	−100	unaffiliated (1/8); AJ851140	*Deltaprot.*/Myxococcales (7/8); AJ851141
M14	249	Root	27	37	+267	+1740	unaffiliated (1/3); AJ851142	*Alphaprot.*/Rhodospirillales (2/3); AJ851143
M15*	249	Soil	27	37	+267	+1740	unaffiliated (1/4); AJ851144	*Deltaprot.*/Myxococcales (3/4); AJ851145
M16*	265	Root	27	26	−100	−97	Chloroflexi (1/4); AJ851146	*Alphaprot.*/Rhodospirillales (3/4); AJ851147
M17*	303	Soil	18	33	+30	+549	*Alphaprot.*/ Rhodospirillales (2/4); AJ851148	unaffiliated (2/4); AJ851149
M18*	332	Soil	18	19	+100	+241	Chloroflexi (1/5); AJ851150	Actinobacteria (4/5); AJ85115

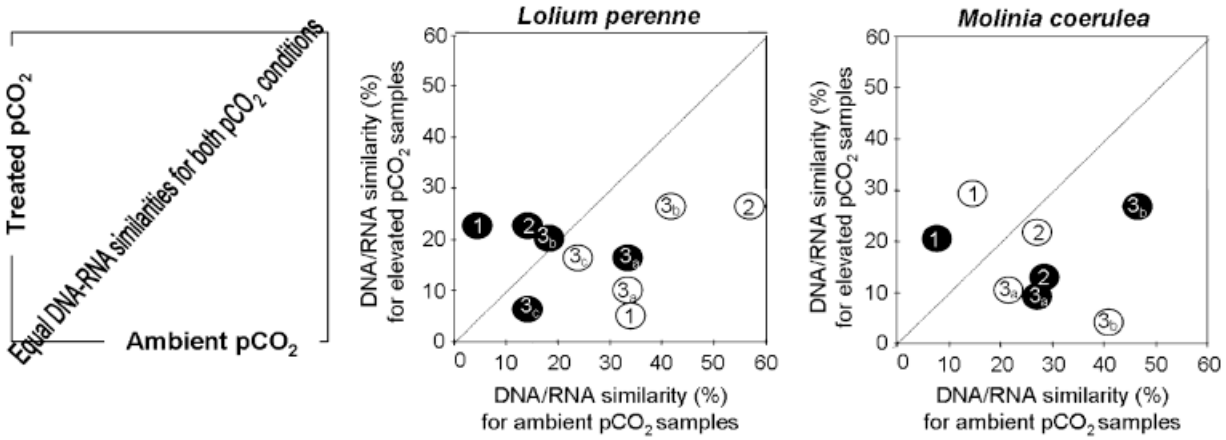


Fig. 2. DNA-/RNA-based profile similarity compared for ambient and elevated pCO_2 conditions (calculated with Steinhaus coefficient). (•) Soil samples; (○) Root samples. Sampling dates are indicated with numbers (1 for June 2001, 2 for May 2001 and 3 for July 2002) and replicates with small letters (a; b; c). Samples scattered on the left upper part of the plot indicate that their DNA and RNA patterns are more similar for treated plots compared to control plots. Samples scattered on the right lower part of the plot indicate that DNA and RNA patterns are more similar for ambient plots than for treated plots.

DGGE pattern description

DNA and RNA extracts obtained from the same sample, after PCR and RT-PCR amplification, generated contrasting DGGE profiles (Fig. 1). Band intensity was more uniform within DNA-based patterns, whereas RNA profiles displayed a clear dominance of a few bands. Soil patterns displayed smeared areas, probably representing clusters of low intensity bands, whereas root profiles often presented sharp bands.

For both plant–soil systems, the similarities between DNA- and RNA-based profiles for a given sample (Fig. 2) were generally below 50%. The DNA and RNA profile similarities were higher for ambient than for elevated pCO_2 plots (eight out of nine for root fraction, and five out of nine for soil fraction), indicating an influence of pCO_2 on community profiles.

Sources of the DGGE profile variability

The variation partitioning analysis (Fig. 3) allowed the relative influence of: (1) replicate plots and sampling date; (2) plant–soil system; and (3) elevated pCO_2 and root proximity on total (DNA-based) and active (RNA-based) bacterial community profiles to be shown. This analysis first revealed the high percentage of unexplained variance (71–79%, Fig. 3). The remaining 21% and 29% of the variance were significantly explained by each of the identified descriptors. The descriptors displaying the highest part of explained profile variability were the sampling date and plots. Globally, 15.1% of DNA- and 11.9% of RNA-based pattern variation were attributed to these descriptors, which integrate numerous environmental conditions (e.g. temperature, soil water content) varying in time and space. The plant–soil system explained 8.8% (DNA-) and 6.3% (RNA-

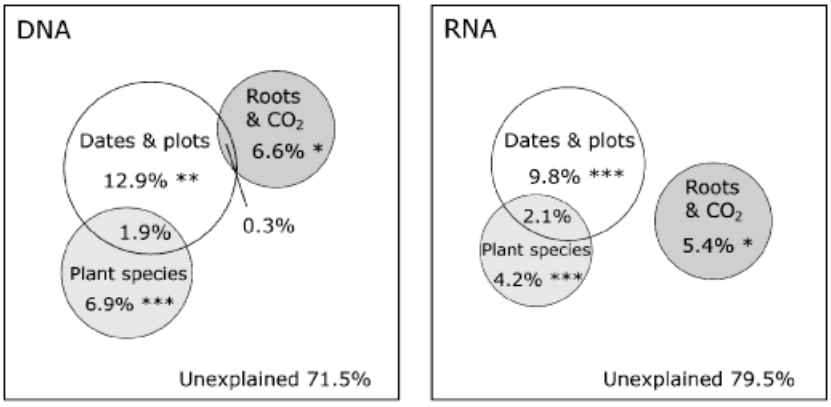


Fig. 3. Variation partitioning for data obtained from DNA- and RNA-based denaturing gradient gel electrophoresis patterns. The variation partitioning was tested for significance with 999 permutations using the Monte Carlo test for each set of descriptors: (1) plant–soil system; (2) pCO_2 treatment and root proximity; and (3) sampling date and replicate plots (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

based patterns) of the variability. The pCO_2 treatment alone did not influence microbial fingerprints; however, the combined influence of root vicinity and pCO_2 treatment significantly accounted for DNA- (6.9%) and for RNA-based (5.4%) profile variation, suggesting that elevated pCO_2 impacts bacterial communities through the roots.

Canonical Correspondence Analysis (Fig. 4) allowed us to ordinate response variables in a single ordination plane, constrained only by root and pCO_2 treatment after removing part of the pattern variability explained by date and plot replication, and by the soil-plant system. Bands influenced by elevated pCO_2 condition were generally associated with the root fraction. This is in agreement with data from similarity coefficients (Fig. 2). Root influence (as shown by strong correlation of the corresponding centroïd to CCA axis 1) was more important than pCO_2 influence (more correlated to CCA axis 2) (Fig. 4). Changes induced by pCO_2 increase were observed for both DNA- ($P = 0.021$) and RNA-based ($P = 0.026$) community profiles for *M. coerulea*. A similar trend was observed for metabolically active communities associated with *L. perenne* ($P = 0.087$).

Choice of characteristic bands and affiliation of corresponding populations

A total of 17 and 18 characteristic bands were selected for *L. perenne* and *M. coerulea*, respectively, based on the DGGE bands representativity (relative intensity and frequency of occurrence among all DNA- and RNA-based profiles, Table 2) and on CCA graphic representation (Fig. 4). On average, the intensity of all selected bands within a given profile represented 26% of the total intensity (data not shown). Twenty-seven (*L. perenne*) and 35 (*M. coerulea*) sequences were retrieved from these selected bands. Clones obtained

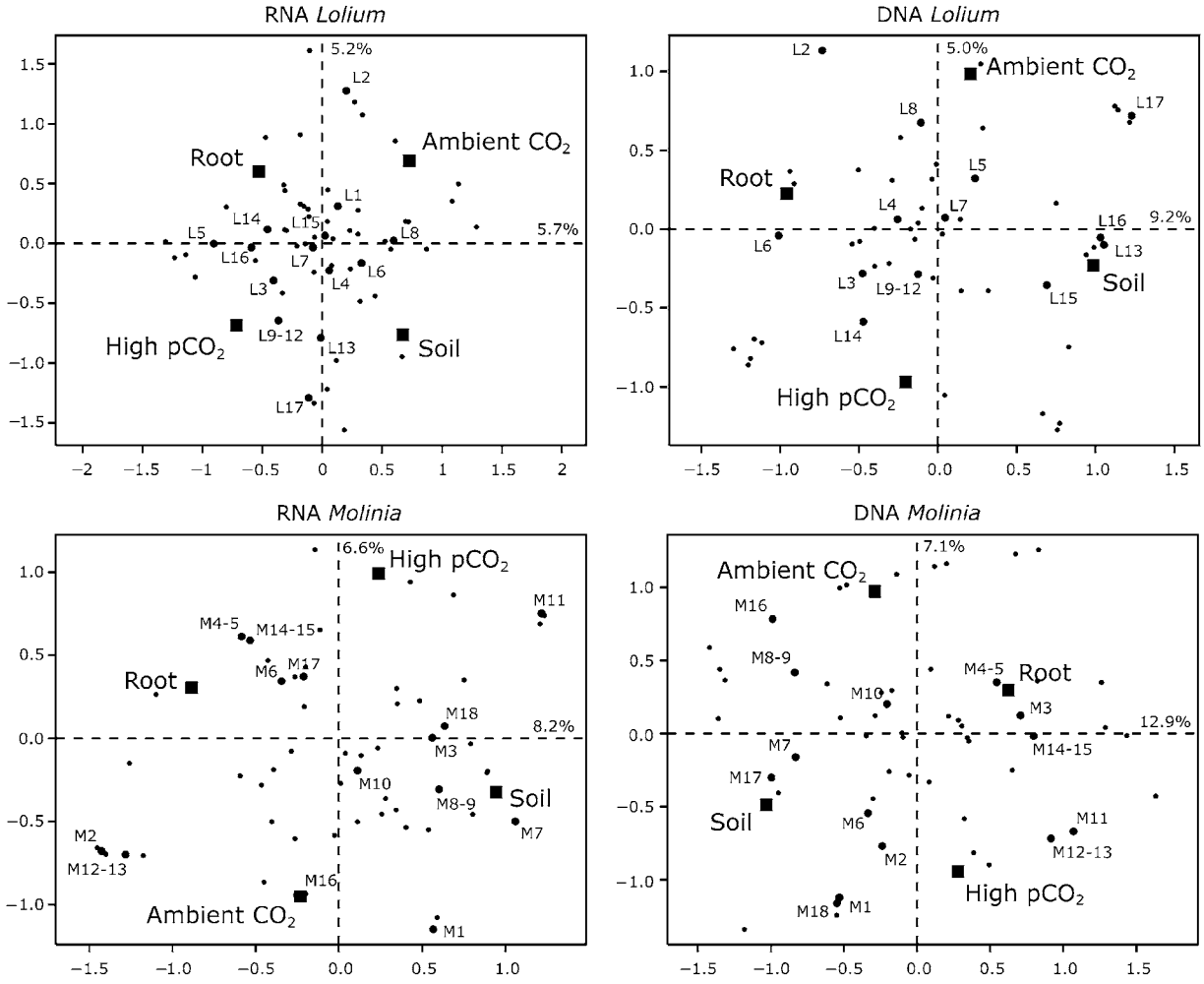


Fig. 4. Biplots of partial Canonical Correspondence Analysis of data obtained from DNA- and RNA-based denaturing gradient gel electrophoresis (DGGE) patterns for *Lolium perenne* and *Molinia coerulea datasets*. Constrained axes 1 and 2 were used for graphic representation. DGGE bands selected for sequencing are indicated with a letter (M or L).

from the same excised band corresponded to one or two restriction types. In most cases, sequences from a single DGGE band were affiliated to the same group or to related groups (e.g. bands L7, M7 and M12, Table 2). However sometimes sequences from a single band were affiliated to different phylogenetic groups (e.g. bands L1 and M8). Some sequences obtained from co-migrating bands for different samples were affiliated to related groups (bands M12 and M13), whereas some others were affiliated differently (bands L11 and L12). Generally, sequences obtained from both plant–soil systems displayed similar affiliations. Among the 27 sequences retrieved for *L. perenne*, 52% were affiliated to *Proteobacteria* (*Alpha-*, *Beta-*, *Gamma-*, *Delta-*), of which 36% were related to Myxococcales (*Deltaproteobacteria*). Other sequences were affiliated to *Actinobacteria* (15%), *Bacteroidetes* (11%), and others were unaffiliated. Similar proportions were observed for the 35 sequences obtained from *M. coerulea*. The average intensity of selected bands doubled under elevated compared to ambient pCO_2 (Table 2). The average intensity of bands corresponding to *Actinobacteria* increased under elevated pCO_2 (root: +77%, soil: +291% for *L. perenne*; root: +265%, soil: +68% for *M. coerulea*). Myxococcales-related bands displayed higher intensities under elevated pCO_2, particularly in root (+248%) compared to soil (+79%) for *L. perenne*, and in soil (+254%) compared to root (+67%) for *M. coerulea*.

Discussion

Comparison between total and active 16S rRNA gene community profiles

Whereas RNA-based profiles highlight active bacterial populations at the time of sampling, DNA-based profiles display the most abundant bacterial populations, independently of their current activity. DNA- and RNA-based profiles for a given sample generally shared less than 50% of pattern similarity (Fig. 2), as previously observed (Muyzer & Smalla, 1998; Kowalchuk *et al.*, 1999; Duineveld *et al.*, 2001).

Field-induced variation

The FACE system currently provides the most realistic way to estimate how plants will respond to elevated pCO_2 in their native environment, avoiding the modification of natural air flow induced by other CO_2 enrichment systems (Long *et al.*, 2004). However, field experiments imply large variations in environmental conditions within time and space (e.g. soil heterogeneity, root distribution, temperature, precipitation). A high unexplained variation was observed, as in most ecological studies (Borcard *et al.*, 1992; Ritz *et al.*, 2004). The largest part of the profiles variability was explained by time of sampling and plots (Fig. 3). Such a high percentage of fingerprint variability between sampling dates is consistent with the short-term response of microbial populations to environmental changes.

Plant–soil system influences

Bacterial communities associated with the ecologically contrasting perennial grasses *L. perenne* and *M. coerulea* (Vazquez de Aldana & Berendse, 1997) were different: 6.3% (RNA) and 8.8% (DNA) of the variability of community profiles could be significantly explained by the plant–soil system (Fig. 3). The soil characteristics are different for *L. perenne* and *M. coerulea* swards: they allow different bacterial communities to settle (Latour *et al.*, 1996).

We expected the response of soil microbial communities to elevated pCO_2 to be dependent on the plant type, through rhizodeposition (Wardle *et al.*, 2004), whereas the same bacterial groups were found to be influenced by pCO_2 in the rhizosphere of both plants. This suggests that bacterial communities associated with the two plants (both being perennial hemicryptophytic grasses) responded similarly to the pCO_2 increase, despite the functional differences between the two host plants (nitrophilic for *L. perenne* vs. oligonitrophilic for *M. coerulea*).

Influence of elevated pCO_2

The direct influence of an atmospheric pCO_2 increase on soil bacterial communities is probably negligible because of the naturally high pCO_2 concentrations in the soil atmosphere (200–3500 Pa) (Gobat *et al.*, 2004). However, bacterial communities were significantly modified by the combined effect of pCO_2 treatment and root vicinity (Fig. 3). The effect of pCO_2 enrichment on soil bacterial communities is likely mediated by the plant through quantitative and qualitative changes in rhizodeposition (Paterson *et al.*, 1996; Hodge *et al.*, 1998).

Changes induced by high pCO_2 were more pronounced on active than on total bacterial communities at root vicinity. Similarities between DNA- and RNA-based profiles were higher under ambient than under elevated pCO_2, except for *L. perenne* soil samples (Fig. 2). This could reflect a more stable state of the bacterial community under ambient pCO_2, whereas low similarity between total and active communities under high pCO_2 would reflect a shifting state of the bacterial community due to fluctuations in the metabolic activity of specific populations (Montealegre *et al.*, 2000), because of root-mediated modification in trophic fluxes due to higher pCO_2 (Hodge *et al.*, 1998).

Total community analysis frequently failed to indicate pCO_2-induced changes (Griffiths *et al.*, 1998; Jones *et al.*, 1998; Insam *et al.*, 1999; Ebersberger *et al.*, 2004) and soil

microbial biomass often seemed unaffected under elevated pCO_2 (Zak *et al.*, 2000). By contrast, pCO_2-induced changes could be observed when targeting specific functional or taxonomic groups (Jones *et al.*, 1998; Ronn *et al.*, 2003; Roussel-Delif *et al.*, 2005; Tarnawski *et al.*, in press). The activity of some functional groups such as fungal cellulose decomposers (Jones *et al.*, 1998) and simple carbohydrate consumers (Hodge *et al.*, 1998) was shown to be enhanced under elevated pCO_2. Belowground responses of microbial communities to global change could generate feedback effects on aboveground biota, such as plant physiology and diversity (Van der Heijden *et al.*, 1998; Jackson *et al.*, 2002). The identification of responsive groups is necessary to understand putative feedbacks on the functioning of soil-plant systems.

Key populations

The bacterial groups responding to root vicinity and pCO_2 increase were highlighted by sequencing selected DGGE bands from total or metabolically active communities. A high proportion (11%) of the selected bands corresponded to sequences affiliated to *Actinobacteria*. They were generally retrieved from the active fraction of the soil bacterial community, regardless of the plant-soil system studied. *Actinobacteria* are known to be soil engineers using soil organic matter as their main carbon source (Ensign, 1992). Their dependence on plant exudates is therefore weak and *Actinobacteria* may be less affected by root-mediated perturbations. The importance of *Actinobacteria* in terms of abundance and activity in soils was demonstrated (Gremion *et al.*, 2003), including under elevated pCO_2 (Billings & Ziegler, 2005).

Myxococcales were identified as the most responsive group to either pCO_2 increase or root influence (19% of retrieved sequences). The relative intensity of corresponding bands increased under elevated pCO_2. Myxococcales are known to be cellulolytic organisms (Reichenbach & Dworkin, 1992). Cellulolytic fungi were shown to be favoured under elevated pCO_2 conditions (Jones *et al.*, 1998), as shown for Myxococcales in the present study. Root growth and exudation are increased under elevated pCO_2 (Zak *et al.*, 2000), leading to a greater availability of cellulose (Robinson *et al.*, 1997), and a stimulation of cellulolytic organisms.

Currently, a major challenge to understand better the role of microbial communities in plant-soil system functioning is to link taxonomic diversity and functions. This requires a prior identification of organisms and their corresponding functions. Some bacterial groups (*Actinobacteria*, Myxococcales) were identified as key organisms in the response of soil-plant systems to elevated pCO_2. This study will be useful further to identify bacterial functions involved in the response of ecosystems to global changes.

Acknowledgements

This research was supported by the Swiss National Science Foundation (grants number 3100-055899.98 and 31-68208.02). We are also grateful to the Swiss National Centre of Competence in Research (NCCR) 'Plant Survival'. We thank to Jacob Zopfi for the English corrections.

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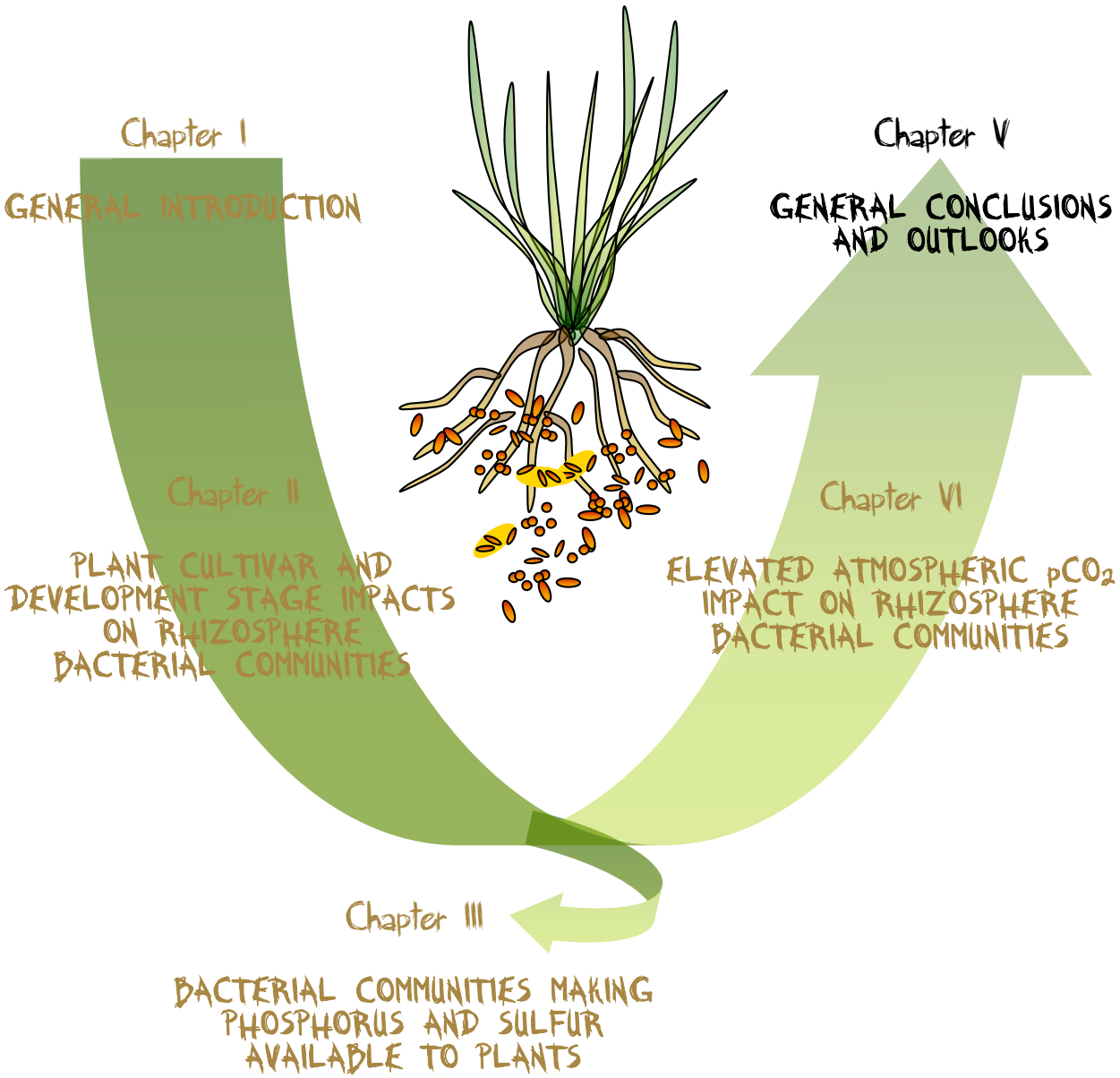
IV.3. CONCLUSIONS AND OUTLOOKS

The impact of elevated atmospheric pCO_2 on soil bacterial communities is mediated by the plant (Paterson *et al.,* 1996; Hodge *et al.,* 1998). Qualitative changes of root input were observed (Hodge *et al.,* 1998), and rhizodeposition was shown to increase under elevated pCO_2, providing increased C sources (Darrah, 1996). In this chapter, total and metabollicaly active communities were characterised in the rhizosphere of two perenial grass. Some bacterial groups poorly studied as root competent populations (*Actinomycetales*, *Myxococcales*), but mainly known as soil saprophic populations were identified in this study as key organisms in the response of plant-soil system to pCO_2. Actinobacteria were shown to be important active members of the rhizosphere communities whatever the pCO_2 conditions, indicating that they are only slightly affected by root-mediated perturbations. Delta-proteobacteria (*Myxococcales*) were, on the contrary, shown to be the main group responsive to pCO_2 elevation and to root-mediated influences.

These results will be useful for further identification of bacterial functions involved in the response of ecosystems to global changes, and are also a gain of knowledges for the investigation of rhizosphere functionning.

CHAPTER V :

GENERAL CONCLUSIONS AND OUTLOOKS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Chapter II
PLANT CULTIVAR AND DEVELOPMENT STAGE IMPACTS ON RHIZOSPHERE BACTERIAL COMMUNITIES
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CHAPTER V : GENERAL CONCLUSIONS AND OUTLOOKS

Overview

V.1. MAIN REALISATIONS

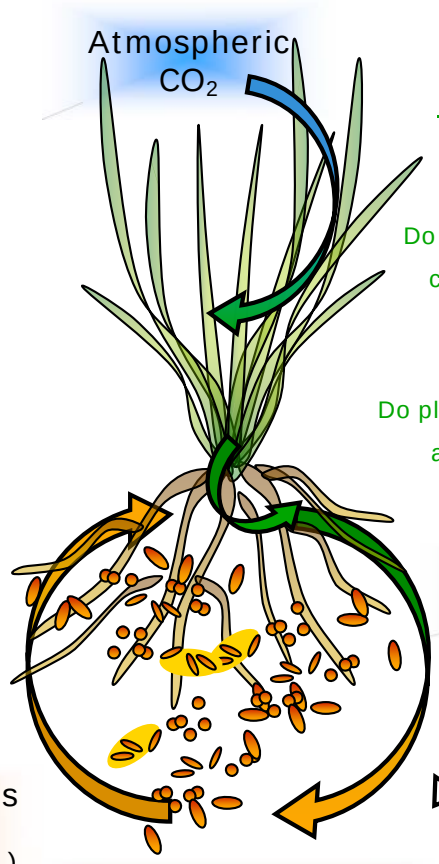
Throughout the experiments conducted in this work, we aimed to gain additional knowledge about rhizosphere functioning, and especially about root selection and plant-mediated influences on bacterial communities, and bacterial activities related to the element cycles. Investigations were performed taking into account the different temporal scales related to rhizosphere functioning (e.g. plant development, dynamic of bacterial populations during plant growth, and short term nutrient cycling), and the spatial gradient of root influence existing from endorhizosphere to bare soil.

Total, active and cultivable rhizosphere bacterial communities were characterised, in field and microcosm experiments, and the influences of plant-soil system, cultivar, development stage, and atmospheric pCO_2 were investigated in root (endorhizosphere-rhizoplan), rhizosphere soil (attached to the root) and bulk soil (without plant) fractions. Although methodological approaches used to characterise bacterial communities were different, experimental systems were complementary. This gives us the opportunity to take into account a large bacterial diversity, and to analyse plant-bacteria interactions from different perspectives. All the investigations were conducted in the same agricultural soil in order to conserve the natural soil contributions in plant-bacteria interactions.

As an enhancement of root influence on the surrounding soil is linked to plants that maintain from year to year at the same location, focus was here put on perennial meadow. *Lolium perenne*, was chosen as model plant because of its abundance and its agricultural importance. It also presents the advantage to grow fast and was used as model plant in many other studies, including previous researches conducted in our laboratory (see chap. I.6). As plant type and species are commonly known to influence bacterial community structure (Westover *et al.*, 1997; Smalla *et al.*, 2001; Marschner *et al.*, 2005; Patra *et al.*, 2006; Costa *et al.*, 2006), cultivar impact is currently less studied (Dalmastri *et al.*, 1999; Varma *et al.*, 2004). We choose different *L. perenne* cultivars to investigate the impact of minor plant variability on bacterial communities and to evaluate the response sensitivity of bacterial communities. Rhizosphere bacterial communities were characterised before cultivation and during plant development for each cultivar and for soil incubated without plant. This allowed to assess the general evolution of bacterial community structure during plant development, and the temporal evolution of the cultivar influences on these communities. It also allowed the determination of the phenotypic structure of bacterial communities along root-soil gradient and to evidence bacterial activities which are favoured, or inhibited, in each environment.

We thereafter focused on two root-associated functional communities (phytase and aryl-sulfatase producers) likely to make organic phosphate and sulfate available to plants (Richardson *et al.*, 2001b; Gyaneshwar *et al.*, 2002; Mirleau *et al.*, 2004), and having high economical importance. This was achieved in order to evaluate the frequency of these organisms, their distribution and their genomic diversity, which are still largely unknown in the rhizosphere.

The elevation of atmospheric pCO_2 is commonly known to modify qualitatively and to enhance root exudation (Long *et al.*, 2004; Ainthworth *et al.*, 2003; Hodge *et al.*, 1998; Darrah, 1996). We therefore investigated, in field conditions, the impact of elevated pCO_2 on a large bacterial diversity in order to target, on one hand the stable and active soil bacterial populations, and on the other hand the populations responsive to perturbation related to the C cycle.



Bacterial activities :

Which are the active key populations in the rhizosphere ?

Which are the most reactive populations in response to changes mediated by plant rhizodeposition and exudation ?

What is the frequency and taxonomic diversity of bacteria able to make P and S available for plants ?

Plant-mediated influences on rhizosphere environment :

Do plant cultivar influence nutrient content and conditions of their surrounding soil ?

Do plant cultivar have different organic acids and phenolic compounds exudation patterns ?

Are the differences observed in root environment consistant with those observed on bacterial community structure ?

Root selection :

Do plant-mediated influences affect genomic as well as functional bacterial community structures ?

Which bacterial functions are characteristic of root and of soil bacterial communities ?

Which relationship exists between the different bacterial abilities ?

Plant mediated influences on bacterial communities :

How do plant cultivar, plant development stage and elevated atmospheric pCO_2 influence bacterial community structure ?

V.2. ROOT SELECTION AND PLANT-MEDIATED INFLUENCES

Independently from the used approach and wathever the perturbations studied, the differentiation of root communities compared to rhizosphere and bare soil communities was always observed. As reported by Weisskopf (2005) for lupin and wheat, only slight differentiation was detected between rhizosphere and bulk soil communities of *L. perenne*.

Whatever it was performed in field or in greenhouse experiments, genomic approach of community structure revealed that the differentiation from soil to root was more important for active communities than total communities. It also revealed that population richness was poorly affected by root selection. Taking into account the saturation state, which could occur when using diversity index calculations, and the methodological limitations linked with metagenomic approaches, we are not able to affirm that plant-mediated influences have no impact on population's richness of rhizosphere communities. But our results nevertheless shows that plant did not exert a major influence on the overall bacterial diversity, and mainly affects the activity and abundance of specific populations, as already reported in the rhizosphere of *Thlaspi caerulescens* (Gremion, 2003), maize and soybean (Buyer *et al.,* 2002).
Independently from the plant cultivar, the phenotypic differentiation of bacterial communities along the root-soil gradient was attenuated after flowering (when root exudates become sparser), and root-associated communities tended to get closer to bare soil communities.
As already suggested by Tscherko *et al*. (2004), our results indicate that soil bacterial communities act as reservoir, and that living plant roots enhance or inhibit growth and activities of specific bacterial populations.

Plant-soil system, cultivar, development stage and atmospheric pCO_2 content were shown to influence the structure of root as well as rhizosphere soil bacterial communities. Most of changes were detected at population level when regarding general activity of bacterial communities (RNA-based approach) and functional abilities of cultivable communities.

The soil used for our experiments was an agricultural soil cultivated since 11 years with *L. perenne* cv. Bastion. The pre-adaptation of the soil to a define cultivar seems to have no significant impact on the structure of root-associated bacterial communities. No particular differentiation was observed between Bastion-associated community structures and those associated with other cultivars. However, among the four cultivars studied, Bastion presented the higher differentiation between its root and rhizosphere soil communities. It might signify that root selection was more advanced for this cultivar, and that soil communities faster adapted to Bastion roots.

Most of the results show a particular differentiation of bacterial communities associated to Anaconda cultivar (4n) compared to others (Bastion, 4n and Cavia and Lipresso, 2n).
This differentiation was visible at different levels:
(i) Genotypic structure of bacterial communities: Active and, in a less extend, total communities were affected (inhibition/enhancement of numerous specific populations). Globally, the differentiation between rhizosphere (including root) communities and bulk soil communities tend to be lower for Anaconda than for other cultivars.
(ii) Phenotypic structure of cultivable bacterial communities: Five of the nine enzymatic activities investigated were affected by plant cultivar. In particular, we found a higher frequency of nitrate-reducing strains, and a lower frequency of protease-producing strains, in the rhizosphere of Anaconda cultivar. Both results indicate a higher availability of nitrate in the rhizosphere of this cultivar compared to others. Soil nitrate content seemed also to be higher when planted with this cultivar compared to others. Considering the nitrophilic nature of *L. perenne* and the fertility problems encountered in soils, this is an important founding. Three of the seven secretion activities investigated were also affected by cultivar type. Proportion of siderophore-producing isolates was in particular higher in the rhizosphere of Anaconda cultivar. This enhancement could be linked to the absence of citric and acetic acids in its root exudates. Indeed, in the present study, these two organic acids implicated in P and Fe solubilisation were not detected only in the root exudates of this cultivar.

We revealed that even when plant effects are slight, and correspond to variations at the lower level of the vegetal systematic, and when these effects seems to have few impact on bacterial richness, the derived modifications of rhizosphere bacterial community structure may be significant and come in large numbers.

V.3. DETERMINANTS OF ROOT COMPETENCE

Functional approach of cultivable root, rhizosphere and bulk soil communities (see chap. II) allowed the determination of the frequency of 16 bacterial traits among a large bacterial diversity. The investigated functions correspond to enzymatic or secretion activities known to be involved in plant-bacteria interactions. As hypothesised by Delorme (2001) for *Pseudomonas* populations, the bacterial traits, which discriminate the root-associated populations from those of the bulk soil, were here considered as putative root competence or plant growth promoting traits.

Some of the most popular root competence traits, reported to be particularly efficient among *Pseudomonas* model populations (i.e. siderophore, auxin productions), were shown in our experiment to be more frequent among bare and rhizosphere soil than among root communities. Some other activities, such as N-acyl-homoserine lactones, glucan, phytase or sulfatase productions were, as already reported in other studies, shown to be favoured at root proximity. Our results therefore suggest that the determinant functions for root competence can vary regarding the bacterial population or genus taken into account. We could also suppose that root competence not only depend on population abilities but also on their *in situ* activity rate. *In vitro* quantitative measurement of strains activities and *in situ* global activity measurements in our soil and would thus be a further step to complete our data.

Based on literature and on our phenotypic description of root (endorhizosphere-rhizoplan), rhizosphere and bare soil bacterial communities, a conceptual global scheme of the putative ecological significance of the functional groups highlighted in our study is proposed in figure V.1.

(i) Cell adhesion facilitates root colonisation (Bianciotto *et al.*, 2001; Varma *et al.,* 2004), and exopolysaccharides producers are more frequent at root proximity. Bacterial mucilage also provides physical protection and facilitates soil exploration by the roots. Moreover EPS production might be favoured by plant soluble organic carbon input in soil.

(ii) Ca-phosphate solubilisers are also favoured at root proximity and release inorganic P, which could benefit to plant and soil microorganisms (Rodriguez and Fraga, 1999; Roesti, 2005).

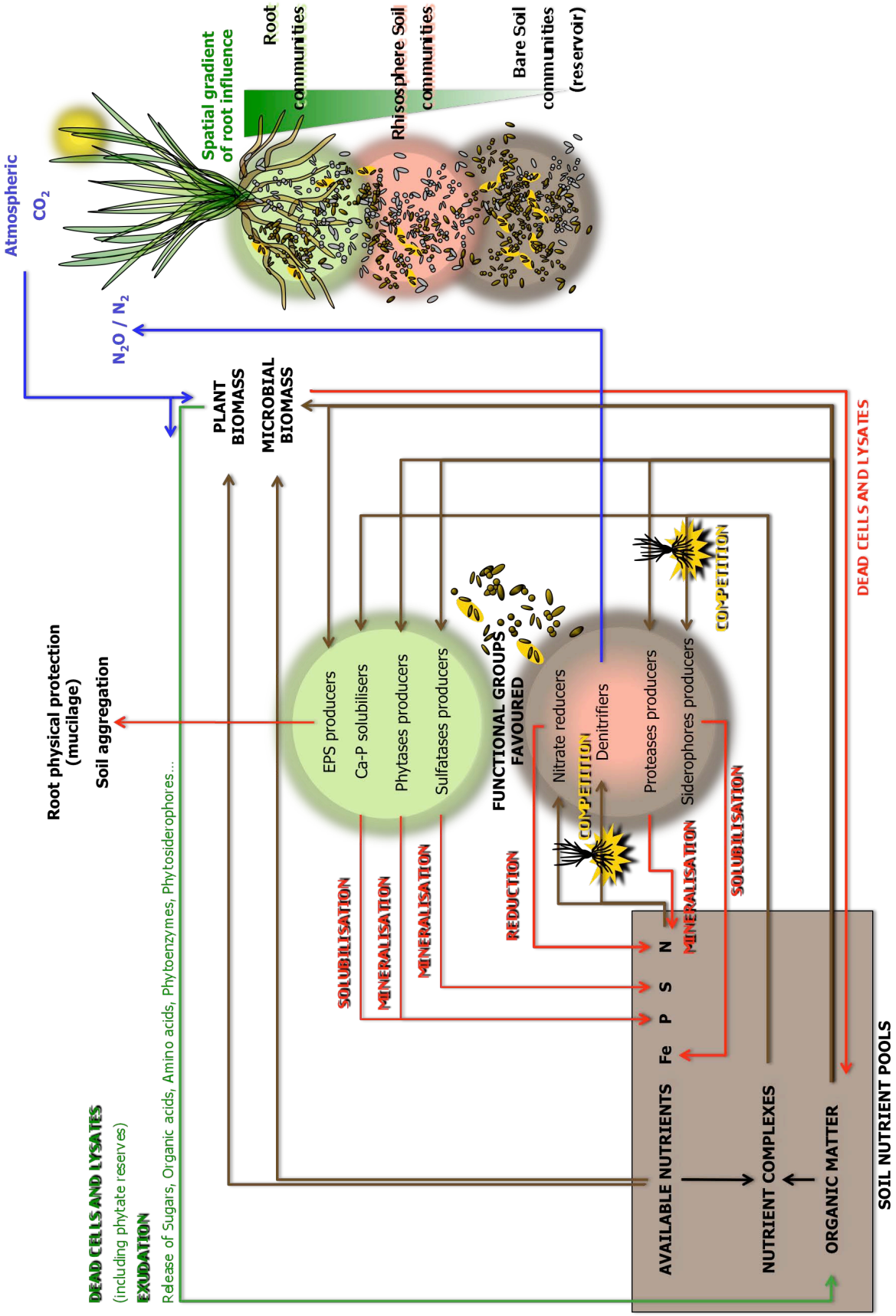
(iii) Sulfatases and phytases producers are favoured at root proximity. They grow on organic S and P, releasing inorganic forms which could benefit to plant and soil microorganisms (for S-related publications see Kertesz, 2004; and for P-related publications see Richardson and Hadobas, 2001b; Unno *et al.,* 2005).

(iv) Nitrate reducers, especially denitrifiers, are inhibited at root proximity and compete with plant for nitrate (Fromin *et al.*, 2005).

(v) Protease producers could be inhibited at root proximity due to a deleterious effect on plant-native proteins, especially extracellular phytoenzymes, and have been reported not to be necessary for root colonisation (O'Sullivan *et al.*, 1991). Some studies however reported a higher proportion of protease producers in the rhizosphere than in the bulk soil (Marcote *et al.,* 2001; Johanson and Binnerup, 2002).

(vi) Siderophore producers are less frequent in root communities, may be some of them compete with plant for Fe (phytosiderophores). Siderophore production is a well-known plant growth-promoting trait among *Pseudomonas* populations (Kloepper *et al.*, 1980; Lugtenberg and Dekkers, 1999; Delorme, 2001; Tarnawski *et al.*, 2006). Some siderophores could nevertheless lead to the unavailability of the bounded iron to native microflora and plant (Kloepper et al, 1980). This inhibition could also be due to Fe non-limiting conditions in our soil, since siderophore production would in this case not be a competitive advantage for root colonisation.

Figure V.1. Conceptual representation of plant-bacteria interactions related to nutrient cycles in the rhizosphere. Bacterial abilities favoured at root proximity are grouped in the green circle, and those favoured in rhizosphere and bulk soil are grouped in the pink-brown circle. The impacts of microbial and plant productions on soil nutrient compartment are represented respectively with red and green arrows. Uptakes of soil constituents are represented with brown arrows.



V.4. CLOSE-UP ON PHYTASE AND ARYL-SULFATASE PRODUCING BACTERIA

The phenotypic approach of bacterial communities performed here allowed targeting some important functions strongly related to root, such as sulfatase and phytase production (see chap. II). Several studies reported a higher phosphatase and sulfatase *in situ* activity in the rhizosphere environment, and the importance of bacteria in these processes (Tarafdar and Junk, 1987; Garcia *et al.,* 1992; Xu *et al.*, 1995; Ftzgerald, 1978; Han *et al.*, 1982; Vong *et al.*, 2003; Knauff *et al.*, 2003; Dedourge *et al.*, 2003).
Limitation of P and S forms available to plants is frequently encountered in soils. Organic P and S, and in particular phytate ($InsP_6$) and sulfate esters, are the most abundant P and S forms in grassland soils (Freney and Williams, 1983; Watwood *et al.*, 1986; Turner *et al.*, 2002; Kertesz and Mirleau, 2004). Phytase and aryl-sulfatase bacterial activities are thus likely to promote plant nutrition by releasing available P and S. These two functions were in the present study investigated in order to evaluate the diversity of the performing bacterial populations, and to identify the populations likely to make P and S available to plants (see chap. III).
Strains from the same phylum, commonly found in soil and rhizosphere, were retrieved for both functional communities, but the dominant populations were different for the two abilities.

Some studies reported an aryl-sulfatase activity for strains belonging to the Pseudomonas (Kertesz *et al.*, 1993; Beil *et al.*, 1995; Kahnert *et al.*, 2002; Mirleau *et al.*, 2005), *Klebsiella* (Azakami *et al.*, 1993), *Citrobacter* (Miura *et al.*, 2006) and *Serratia* (Murooka *et al.*, 1980), but the frequency and diversity of sulfatase producers in the rhizosphere is still largely unknown (Kertesz and Mirleau, 2004). In the present study, we determined the proportion of arylsulfatase producers, and their genomic diversity. Proportion of isolates able to grow on X-sulfate as sole S source was globally low (<10 %), and higher in root than in rhizosphere and bulk soil communities. Isolates were grouped in 23 OTU's, and the Gram negative phylum Proteobacteria (*Rhizobiales*) and Bacteroïdetes (*Flavobacteriales*) appeared as the main aryl-sufatase producing groups. We also evidence a link between aryl-sulfatase and N-acyl-homoserine lactones production. Aryl-sulfatase producing isolates belonging to the *Flavobacteriales* preferentially produce C_3 substituted or non-substituted long carbon chain N-acyl-homoserine lactones (C_6 to C_{12} NAHL), and few of them was also able to produce oxo- or C_3 non-substituted short carbon chain N-acyl-homoserine lactones (C_4 to C_8). On the other hand, aryl-sulfatase producing isolates belonging to the *Rhizobiales* preferentially produce C_4 to C_8 NAHL, and most of them was also able to produce C_6 to C_{12} NAHL. These results suggest that sulfatase production could be dependant of cell density and regulated by quorum sensing mechanisms. The type of NAHL produced vary regarding to the phylogenetic affiliation of the

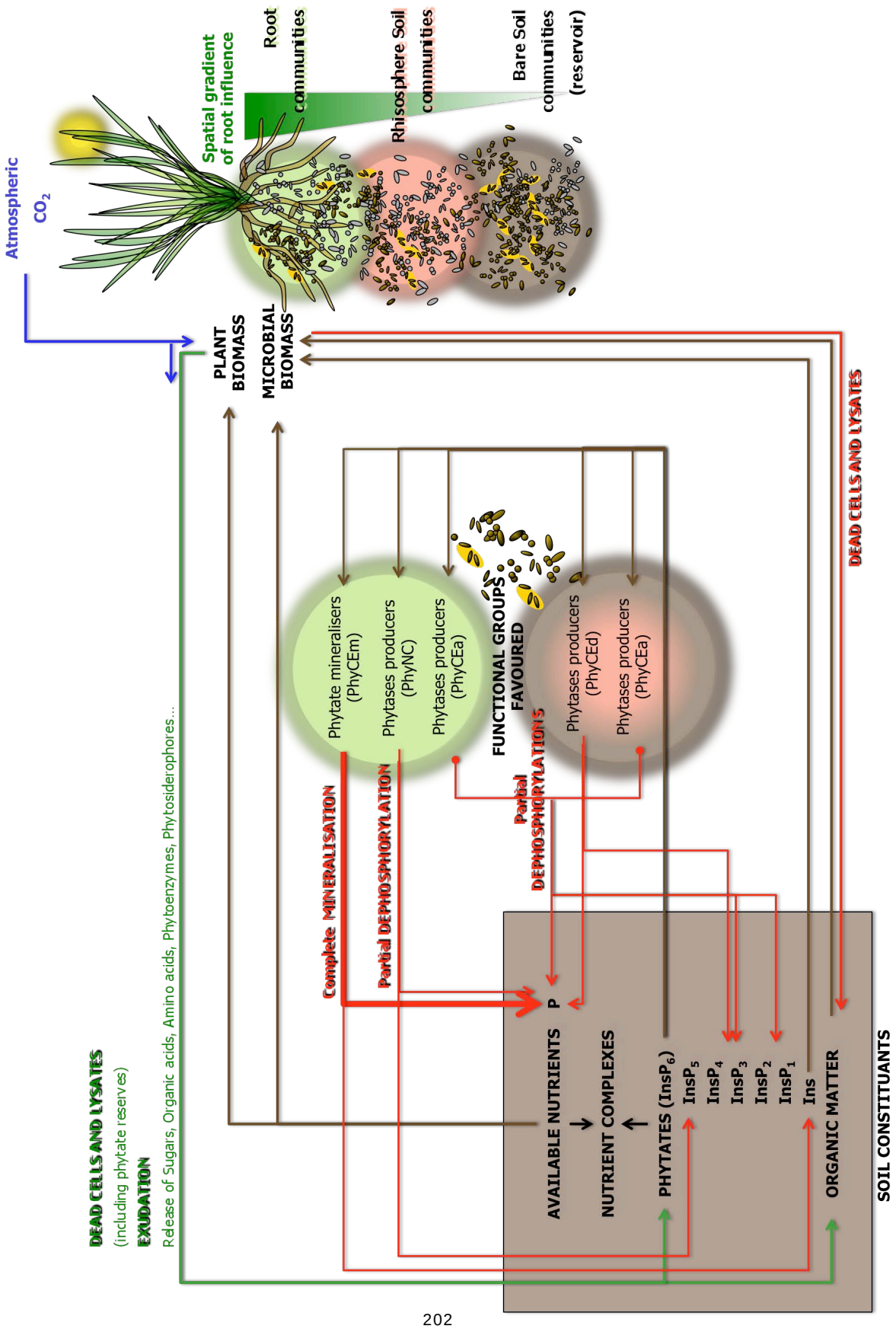
arylsufatase-producing populations. Unfortunately we do not found available data to corroborate the link between these two functions. *In vitro* test of aryl-sulfatase production in presence and absence of NAHL in the culture media would be necessary to confirm our hypothesis.

Our study furthermore revealed that the main part of root, rhizosphere and bulk soil cultivable populations was able to use phytate as P source (see chap. III.2). It allows the definition of different categories of isolates presenting the ability to grow with phytate as sole P source (PhyG isolates), including PhyNC, PhyCEd, PhyCEa, PhyCEm, and PhyCRd, PhyCRa). Groups were based on the degree of phytate dephosphorylation and the localisation of the produced enzymes (extracellular, ectoparietal). A conceptual representation of plant-bacteria interactions related to phytate hydrolysis in the rhizosphere is presented in figure V.2. Ectoparietal enzyme activity would only benefit to bacterial P nutrition, whereas extracellular phytase activity also provides available P to plants and other microorganisms. The conceptual representation therefore not focuses on bacterial populations only able to produce ectoparietal phytases (PhyCR).

Isolates able to completely dephosphorylate phytate (PhyCEm) produced extracellular phytases, and 93 % of them were found in the rhizoplane-endorhizosphere fraction. We also evidence that all of them use inositol as C source. This indicates that these populations are responsible for the recycling of plant-native phytate (figure V.2). As one of the main P reserve forms in plants, phytate is released from lysate and dead cells. Phytate pool might be increased at root contact by plant inputs. Since PhyCEm isolates might be mainly interested by $InsP_6$ as C source, and completely dephosphorylate phytate, they release orthophosphate which would not be necessary for them, and which could therefore be available and benefit to plant and soil microorganisms.

Taking theses result into account, we focused on phytate mineralisers for further investigation of population diversity. The Actinobacteria (*Actinomycetales*) and Firmicutes Gram positive bacteria phylums were shown to be important actors in the mineralisation of organic phosphorus. Presenting a particular resistance to environmental perturbations, mainly due to their sporulation ability, the groups highlighted here could be good candidates for the development of biofertilisers more suitable for transport and conservation.

Figure V.2. Conceptual representation of plant-bacteria interactions related to organic P (phytates) hydrolysis in the rhizosphere. Extracellular phytase-producers favoured at root proximity are grouped in the green circle, and those favoured in rhizosphere and bulk soil are grouped in the pink-brown circle. The impacts of microbial and plant productions on soil nutrient compartment are represented respectively with red and green arrows. Uptakes of soil constituents are represented with brown arrows.



V.5. KEY POPULATIONS OF THE RHIZOSPHERE

Regarding the functional diversity and redundancy encountered among bacterial activities, and the complexity of root selection processes, we choose to gain additional information using a global approach, based on general metabolic activity and abundance, of a large bacterial diversity.

Identification of the characteristic populations, which are maintained or fluctuate under the plant-mediated influences, has been here performed (based on 16S RNA genomic approach) for rhizosphere bacterial communities submitted to an elevation of atmospheric pCO_2, which has been previously reported to increase and modify rhizodeposition (see chap. IV).

Actinobacteria were shown to be important active members of the rhizosphere communities whatever the pCO_2 conditions, indicating that they are only slightly affected by root-mediated perturbations, especially by C cycle perturbations.

Delta-Proteobacteria (*Myxococcales*) was, on the contrary, shown to be the main group responsive to pCO_2 elevation and to root-mediated influences.

Similar investigations of the populations responsive to plant-mediated perturbations, among communities associated to the different plant cultivars and development stages studied here (see chap. II), would be a further step to confirm these results, and to highlight some other bacterial groups responsive to plant-mediated changes.

V.6. IMPORTANCE OF *PSEUDOMONAS* POPULATIONS IN THE RHIZOSPHERE

The well-known *Pseudomonas* group, which is currently used as model rhizosphere population (Lemanceau *et al.* 1995; Rainey, 1999; Latour *et al.*, 2003; Lucy *et al.*, 2004), has been reported to be favoured in the root environment of *L. perenne* (Marilley *et al.,* 1999; Tarnawski *et al.,* 2003). In the present study, this group was nevertheless poorly represented among populations responsive to root-mediated perturbations related to pCO_2 elevation, as well as among phytase and arylsulfatase producing isolates.

It does not want to say that *Pseudomonas* populations are insignificant, but it indicates that some other populations could be well adapted to the rhizosphere environment and have also to be taken into account.

V.7. IMPORTANCE OF ACTINOBACTERIA POPULATIONS IN THE RHIZOSPHERE

Actinobacteria, which are k-strategists commonly known to be adapted to the poor nutrient status of the soil (Ensign, 1992), were shown to take place in the active rhizosphere populations, even after a perturbation such as an increased carbon input (see chap. IV). They also were shown to be the main potential phytate mineralisers, under limiting inorganic P conditions and in presence of C sources (see chap. III), two characteristics of the rhizosphere environment (Arnou, 1953; Schachtman *et al.,* 1998; Deubel *et al.,* 2000; Uren, 2000; Kuzyakov, 2001).

We highlighted some *Actinomycetales* (*Actinobacteridae*) as important members of the root-associated phytate mineralising populations, and as active populations of the root and soil communities, resistant to C cycle related perturbations. Conn and Franco (2004), reported that the main group of endophytic Actinobacteria in wheat roots correspond to *Actinobacteridae*. Gremion (2003) and Pereira *et al.* (2006) showed that the main groups of Actinobacteria in the rhizosphere soil of the perennial plant *Thlaspi caerulescens*, and of tomato, beans and corn, belong to the *Rubrobacteridae*. Among Actinobacteria, the subclass *Actinobacterideae*, and especially the *Actinomycetales* populations, seems to be an interesting functional group adapted to the endorhizosphere and rhizoplan as well as to the rhizosphere soil.

The capacity of Actinobacteria for polymers degradation, antibiotic production, resistance to dessication, and their relative insensivity to toxic compounds may give these organisms a selective advantage in the rhizosphere. The use of Actinobacteria, and in particular of *Actinomycetales*, as biofertilisers would thus be an important field of investigations.

The strains collection recovered in this study could be used for plant inoculation experiments in order to confirm an eventual plant growth promoting activity of these populations. Moreover, the importance of Actinobacteria could be assessed by the investigation, based on the total DNA and RNA samples recovered, of genomic diversity and abundance of these populations, using Q-PCR and cloning-sequencing approaches.

V.8. CONCLUDING REMARKS

This study present the advantage to take into account a large bacterial diversity from different perspectives, which is a necessary complement to model populations investigations, when studying rhizosphere processes or interaction between organisms. Of course, working with a broad diversity of microorganisms in a complex system of interactions practically limits many aspects of the investigations and several close up questions remains.

This work however allows to:

(i) Characterise and differentiate rhizosphere communities associated to four cultivars of *L. perenne*, at the level of their genomic and phenotypic structures. Most of our results indicate that Anaconda cultivar harboured especially specific bacterial communities, and had a particular exudation pattern compared to other cultivars.

(ii) Gain information about rhizosphere competence, and about frequency of different bacterial abilities and relations between theses activities. Differentiation of the phenotype of bacterial communities during plant development, and distribution of the different abilities from the endorhizosphere to the bulk soil, were also determined.

(iii) Target bacterial groups likely to have a high rhizosphere competence potential, and likely to take an important place in the rhizosphere functionning and the promotion of plant growth: Actinobacteria (*Actinomycetales*) were highlighted as stable active populations, and as efficient root-associated phytate mineralisers, *Bacillales* as root-associated phytate mineralisers, *Rhizobiales* and *Flavobacteriales* as aryl-sulfatase root-associated producers, and Delta-Proteobacteria as populations responsive to plant-mediated changes related to carbon cycle.

The acquired knowledge could be usefull for further investigations of plant-bacteria interactions in soils, and hopefully in the long term, might allow the improvement of soil management and agricultural practices. Results related to phytate and sulfate mineralising communities could, especially help increasing the efficience of biofertilisers, in particular regarding the conservation and resistance of the bioinoculants.

This study thus gives an insight of the complexity and sensivity of plant-bacteria interactions, and root selection processes in soil. It also allows to highlight some bacterial groups which were untill now not considered as important players of the rhizosphere.

L'esprit qui invente est toujours mécontent de ses progrès, parce qu'il voit au-delà.
[Jean le Rond d'Alembert]

On ne fait jamais attention à ce qui a été fait ; on ne voit que ce qui reste à faire.
[Marie Curie]

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ANNEXES

Richness, diversity and Evenness of DNA and RNA based DGGE profiles

Cultivar	Fraction	Stage	DNA samples	Richness	Shannon-Weaner index	Evenness index	RNA samples	Richness	Shannon-Weaner index	Evenness index
A	R	1	1	29	3.3	0.68	1	17	2.7	0.67
A	R	2	2	24	3.1	0.68	2	18	2.8	0.67
A	R	2	3	25	3.1	0.68	3	15	2.7	0.68
A	R	2	4	28	3.2	0.67	4	15	2.7	0.68
A	R	3	5	29	3.3	0.68	5	21	3.0	0.69
A	R	3	6	27	3.2	0.68	6	25	3.2	0.69
A	R	3	7	27	3.2	0.67	7	15	2.7	0.68
A	R	4	8	26	3.2	0.67	8	18	2.9	0.69
A	R	4	9	30	3.4	0.69	9	20	3.0	0.69
A	R	4	10	26	3.2	0.68	10	21	3.0	0.69
A	RS	1	11	28	3.3	0.68	11	17	2.8	0.68
A	RS	2	12	27	3.2	0.68	12	18	2.8	0.68
A	RS	2	13	28	3.2	0.68	13	18	2.9	0.68
A	RS	2	14	28	3.3	0.68	14	16	2.7	0.69
A	RS	3	15	23	3.0	0.67	15	19	2.9	0.68
A	RS	3	16	34	3.7	0.73	16	19	2.9	0.69
A	RS	3	17	31	3.4	0.68	17	20	2.9	0.68
A	RS	4	18	37	3.6	0.70	18	17	2.8	0.69
A	RS	4	19	30	3.3	0.67	19	18	2.8	0.67
A	RS	4	20	28	3.2	0.67	20	19	2.9	0.68
B	R	1	21	19	2.9	0.69	21	22	3.0	0.68
B	R	2	22	23	3.1	0.69	22	20	2.9	0.67
B	R	2	23	20	3.0	0.69	23	18	2.8	0.68
B	R	2	24	17	2.8	0.69	24	19	2.9	0.67
B	R	3	25	24	3.1	0.68	25	20	2.9	0.67
B	R	3	26	18	2.8	0.68	26	15	2.6	0.67
B	R	3	27	18	2.8	0.66	27	15	2.7	0.68
B	R	4	28	19	2.7	0.63	28	23	3.1	0.68
B	R	4	29	21	3.0	0.69	29	22	3.0	0.68
B	R	4	30	22	3.1	0.69	30	23	3.1	0.68
B	RS	1	31	28	3.3	0.68	31	22	3.0	0.68
B	RS	2	32	25	3.2	0.68	32	18	2.8	0.68
B	RS	2	33	29	3.3	0.68	33	19	2.9	0.68
B	RS	2	34	29	3.3	0.67	34	23	3.1	0.68
B	RS	3	35	28	3.2	0.67	35	16	2.7	0.68
B	RS	3	36	28	3.3	0.68	36	19	2.9	0.69
B	RS	3	37	34	3.4	0.68	37	18	2.8	0.68
B	RS	4	38	28	3.2	0.67	38	18	2.8	0.68
B	RS	4	39	28	3.2	0.67	39	16	2.7	0.68
B	RS	4	40	29	3.3	0.67	40	17	2.8	0.69

Cultivar	Fraction	Stage	DNA samples	Richness	Shannon-Weaner index	Evenness index	RNA samples	Richness	Shannon-Weaner index	Evenness index
C	R	1	41	26	3.2	0.68	41	16	2.7	0.68
C	R	2	42	25	3.2	0.68	42	23	3.1	0.69
C	R	2	43	23	3.1	0.68	43	15	2.7	0.68
C	R	2	44	24	3.1	0.68	44	18	2.8	0.68
C	R	3	45	26	3.2	0.67	45	15	2.6	0.67
C	R	3	46	22	3.0	0.68	46	14	2.5	0.66
C	R	3	47	24	3.1	0.68	47	11	2.3	0.68
C	R	4	48	24	3.1	0.68	48	19	2.9	0.69
C	R	4	49	25	3.2	0.68	49	23	3.1	0.68
C	R	4	50	24	3.1	0.68	50	11	1.9	0.55
C	RS	1	51	30	3.3	0.67	51	25	3.1	0.68
C	RS	2	52	43	3.7	0.68	52	21	3.0	0.68
C	RS	2	53	39	3.6	0.68	53	20	2.9	0.68
C	RS	2	54	36	3.5	0.68	54	19	2.8	0.67
C	RS	3	55	32	3.4	0.69	55	18	2.8	0.67
C	RS	3	56	32	3.4	0.68	56	19	2.9	0.67
C	RS	3	57	34	3.5	0.68	57	19	2.9	0.68
C	RS	4	58	39	3.6	0.68	58	23	3.1	0.68
C	RS	4	59	31	3.4	0.68	59	20	2.9	0.68
C	RS	4	60	33	3.4	0.68	60	18	2.8	0.68
L	R	1	61	25	3.2	0.68	61	19	2.9	0.69
L	R	2	62	22	3.0	0.68	62	24	3.1	0.68
L	R	2	63	24	3.2	0.69	63	19	2.9	0.68
L	R	2	64	24	3.1	0.68	64	18	2.9	0.69
L	R	3	65	24	3.1	0.68	65	24	3.2	0.69
L	R	3	66	22	3.0	0.68	66	20	2.9	0.68
L	R	3	67	33	3.5	0.69	67	24	3.1	0.69
L	R	4	68	27	3.2	0.68	68	18	2.8	0.68
L	R	4	69	26	3.2	0.68	69	14	2.6	0.69
L	R	4	70	31	3.4	0.68	70	14	2.6	0.68
L	RS	1	71	27	3.2	0.67	71	18	2.8	0.68
L	RS	2	72	33	3.4	0.68	72	18	2.8	0.67
L	RS	2	73	23	3.1	0.68	73	16	2.8	0.69
L	RS	2	74	35	3.5	0.67	74	16	2.7	0.67
L	RS	3	75	30	3.3	0.68	75	17	2.8	0.68
L	RS	3	76	32	3.4	0.69	76	15	2.6	0.68
L	RS	3	77	32	3.4	0.68	77	18	2.9	0.69
L	RS	4	78	28	3.3	0.68	78	23	3.0	0.67
L	RS	4	79	22	3.0	0.68	79	15	2.6	0.68
L	RS	4	80	32	3.4	0.68	80	16	2.7	0.68

Cultivar	Fraction	Stage	DNA samples	Richness	Shannon-Weaner index	Evenness index	RNA samples	Richness	Shannon-Weaner index	Evenness index
T	S	1	81	30	3.4	0.69	81	22	3.0	0.68
T	S	1	82	29	3.4	0.69	82	17	2.8	0.67
T	S	1	83	28	3.3	0.69	83	16	2.7	0.66
T	S	2	84	31	3.4	0.69	84	11	2.3	0.67
T	S	2	85	34	3.5	0.69	85	11	2.3	0.66
T	S	2	86	33	3.5	0.69	86	12	2.4	0.67
T	S	3	87	28	3.3	0.69	87	14	2.6	0.68
T	S	3	88	31	3.4	0.69	88	13	2.5	0.67
T	S	3	89	31	3.4	0.69	89	18	2.8	0.68
T	S	4	90	32	3.5	0.69	90	17	2.8	0.68
T	S	4	91	33	3.5	0.69	91	18	2.3	0.56
T	S	4	92	32	3.4	0.69	92	26	3.2	0.68

Isolates description : Colony morphology

souche	cv	stade	repl.	fract.	forme	couleur	élévation	pourtour	surface	halo
1	A	1	1	R	régulière	blanche	convexe	régulier	lisse / brillante	
2	A	1	1	R	régulière / petite	jaune	convexe	régulier	lisse / brillante	
3	A	1	1	R	régulière	blanche	convexe	régulier	lisse / brillante	
4	A	1	1	R	régulière	jaune	légèrement convexe	régulier	lisse / brillante	
5	A	1	1	R	régulière / petite	blanche / translucide	plate	régulier	lisse / brillante	
6	A	1	1	R	régulière	jaune	convexe	régulier	lisse / brillante	
7	A	1	1	R	régulière	blanche	plate	régulier	lisse / brillante	
8	A	1	1	R	régulière	jaune - orangé	légèrement convexe	régulier	enfoncée au centre	
9	A	1	1	R	régulière	crème	légèrement convexe	régulier	lisse / brillante	
10	A	1	1	R	régulière / petite	blanche	convexe	régulier	lisse / brillante	
11	A	1	1	R	régulière	jaune	légèrement convexe	régulier	lisse / brillante	
12	A	1	1	R	régulière	jaune - orangé	convexe	régulier	lisse / brillante	
13	A	1	1	R	régulière	crème	légèrement convexe	régulier	ombiliquée	
14	A	1	1	R	type bacillus	-	-	-	-	
15	A	1	1	R	régulière / petite	blanche	convexe	régulier	lisse / brillante	
16	A	1	1	R	régulière	jaune	plate	régulier	lisse / brillante	
17	A	1	1	R	régulière / petite	blanche	plate	régulier	lisse / brillante	
18	A	1	1	R	régulière / petite	jaune / translucide	plate	régulier	lisse / brillante	
19	A	1	1	R	irrégulière	jaune	plate	régulier	lisse / brillante	
20	A	1	1	R	régulière / petite	crème	plate	régulier	lisse / brillante	
21	A	1	1	R	irrégulière	jaune	plate	régulier	lisse / brillante	
22	A	1	1	R	régulière	crème	convexe	régulier	enfoncée au centre	
23	A	1	1	R	régulière	saumon	convexe	régulier	lisse / brillante	
24	A	1	1	R	régulière	crème	légèrement convexe	type actino	rugueuse	
25	A	1	1	R	régulière	crème	convexe	régulier	enfoncée au centre	
26	A	1	1	R	régulière	blanche	plate	régulier	rugueuse	
27	A	1	1	R	type bacillus	-	-	-	-	
28	A	1	1	R	régulière	jaune pâle	plate	régulier	lisse / brillante	
29	A	1	1	R	régulière	blanche	plate	régulier	rugueuse	
30	A	1	1	R	régulière	crème	légèrement convexe	type actino	duveteuse blanche	
31	A	2	1	R	régulière / petite	rose	plate	régulier	lisse / brillante	
32	A	2	1	R	régulière	rose	plate	régulier	lisse / brillante	
33	A	2	1	R	régulière	blanche	plate	régulier	lisse / brillante	
34	A	2	1	R	régulière	jaune	légèrement convexe	diffus	lisse / brillante	
35	A	2	1	R	régulière	blanche	plate	régulier	lisse / brillante	
36	A	2	1	R	régulière	blanche	plate	régulier	lisse / brillante	
37	A	2	1	R	régulière	blanche	plate	régulier	lisse / brillante	
38	A	2	1	R	régulière / petite	blanche	plate	régulier	lisse / brillante	
39	A	2	1	R	régulière	jaune pâle	plate	régulier	lisse / brillante	
40	A	2	1	R	régulière / petite	orange	plate	régulier	lisse / brillante	
41	A	2	1	R	régulière	jaune	plate	régulier	rugueuse	
42	A	2	1	R	irrégulière	jaune pâle	plate	diffus	lisse / brillante	
43	A	2	1	R	régulière	jaune	plate	régulier	lisse / brillante	

44	A	2	1	R	régulière	jaune	plate	régulier	lisse / brillante	
45	A	2	1	R	irrégulière	jaune	plate	diffus	lisse / brillante	
46	A	2	1	R	régulière	jaune / translucide	légèrement convexe	régulier	lisse / brillante	
47	A	2	1	R	régulière	jaune	légèrement convexe	diffus	lisse / brillante	
48	A	2	1	R	régulière	jaune	légèrement convexe	diffus	lisse / brillante	
49	A	2	1	R	régulière	jaune / translucide	plate	régulier	lisse / brillante	
50	A	2	1	R	régulière	crème	plate	régulier	lisse / brillante	
51	A	2	2	R	irrégulière	jaune / translucide	plate	diffus	rugueuse	
52	A	2	2	R	régulière	jaune pâle	plate	diffus	lisse / brillante	
53	A	2	2	R	régulière	jaune	légèrement convexe	régulier	lisse / brillante	
54	A	2	2	R	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
55	A	2	2	R	irrégulière	jaune / translucide	plate	diffus	rugueuse	
56	A	2	2	R	irrégulière	jaune	plate	diffus	lisse / brillante	
57	A	2	2	R	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
58	A	2	2	R	régulière	jaune	légèrement convexe	diffus	lisse / brillante	
59	A	2	2	R	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
60	A	2	2	R	régulière	jaune pâle	plate	diffus	lisse / brillante	
61	A	2	2	R	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
62	A	2	2	R	irrégulière	jaune / translucide	plate	diffus	rugueuse	
63	A	2	2	R	irrégulière	jaune / translucide	plate	diffus	rugueuse	
64	A	2	2	R	irrégulière	jaune / translucide	plate	diffus	rugueuse	
65	A	2	2	R	régulière	jaune	légèrement convexe	régulier	lisse / brillante	
66	A	2	2	R	irrégulière	jaune	plate	diffus	lisse / brillante	
67	A	2	2	R	régulière	jaune	légèrement convexe	régulier	lisse / brillante	
68	A	2	2	R	irrégulière	jaune / translucide	plate	diffus	rugueuse	
69	A	2	2	R	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
70	A	2	2	R	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
71	A	2	3	R	régulière	crème	convexe	régulier	lisse / brillante	
72	A	2	3	R	régulière	jaune	plate	régulier	lisse / brillante	
73	A	2	3	R	irrégulière	blanche	plate	diffus	lisse / brillante	jaune
74	A	2	3	R	régulière	blanche	plate	régulier	lisse / brillante	
75	A	2	3	R	irrégulière	jaune	plate	régulier	lisse / brillante	
76	A	2	3	R	régulière	jaune	convexe	régulier	lisse / brillante	
77	A	2	3	R	régulière	jaune	légèrement convexe	régulier	lisse / brillante	
78	A	2	3	R	régulière	blanche	plate	régulier	lisse / brillante	
79	A	2	3	R	régulière	jaune / translucide	légèrement convexe	régulier	lisse / brillante	
80	A	2	3	R	régulière	jaune	légèrement convexe	régulier	lisse / brillante	
81	A	2	3	R	régulière	blanche	plate	régulier	lisse / brillante	
82	A	2	3	R	régulière / petite	rose / translucide	plate	régulier	lisse / brillante	
83	A	2	3	R	régulière	jaune / translucide	plate	diffus	rugueuse / brillante	
84	A	2	3	R	régulière	crème	convexe	régulier	lisse / brillante	
85	A	2	3	R	irrégulière	jaune / translucide	plate	diffus	rugueuse / brillante	
86	A	2	3	R	régulière	jaune	légèrement convexe	régulier	lisse / brillante	
87	A	2	3	R	régulière	jaune	légèrement convexe	régulier	lisse / brillante	
88	A	2	3	R	régulière	jaune	légèrement convexe	régulier	lisse / brillante	
89	A	2	3	R	régulière	jaune / translucide	plate	régulier	lisse / brillante	
90	A	2	3	R	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
91	A	4	1	R	régulière	crème - jaunâtre	convexe	régulier	lisse / brillante	

92	A	4	1	R	régulière	crème / translucide	plate	ondulé	lisse / brillante	
93	A	4	1	R	irrégulière	orange	plate	diffus	lisse / brillante	
94	A	4	1	R	irrégulière	orange / translucide	plate	diffus	rugueuse	
95	A	4	1	R	régulière / petite	blanche / translucide	plate	diffus	lisse / brillante	
96	A	4	1	R	régulière / petite	orange	légèrement convexe	régulier	lisse / brillante	
97	A	4	1	R	régulière	orange	légèrement convexe	diffus	lisse / brillante	
98	A	4	1	R	régulière	crème	plate	diffus	rugueuse	
99	A	4	1	R	irrégulière	orange	plate	diffus	lisse / brillante	
100	A	4	1	R	régulière	crème	convexe	régulier	lisse / brillante	
101	A	4	1	R	régulière	crème - jaunâtre	convexe	type actino	rugueuse	
102	A	4	1	R	régulière / petite	jaune	convexe	régulier	lisse / brillante	
103	A	4	1	R	irrégulière	crème	plate	ondulé / diffus	rugueuse	
104	A	4	1	R	irrégulière / petite	jaune pâle / translucide	plate	diffus	lisse / brillante	
105	A	4	1	R	régulière	crème	convexe	régulier	cercles concentriques	
106	A	4	1	R	irrégulière	orange	plate	diffus	lisse / brillante	
107	A	4	1	R	irrégulière	blanchâtre / translucide	plate	diffus	rugueuse	
108	A	4	1	R	régulière	crème	légèrement convexe	diffus	lisse / brillante	
109	A	4	1	R	irrégulière	crème / translucide	plate	régulier	lisse / brillante	
110	A	4	1	R	irrégulière	orange	plate	diffus	lisse / brillante	
111	A	4	2	R	régulière / petite	crème	convexe	régulier	lisse / brillante	
112	A	4	2	R	régulière	crème	plate	régulier	lisse / brillante	
113	A	4	2	R	irrégulière	transparente	plate	diffus	rugueuse	
114	A	4	2	R	régulière	transparente	plate	diffus	rugueuse	
115	A	4	2	R	régulière	crème	plate	envahissant	lisse	
116	A	4	2	R	régulière	crème	plate	diffus	lisse	
117	A	4	2	R	régulière	jaune pâle	plate	régulier	lisse / brillante	
118	A	4	2	R	régulière	transparente	plate	diffus	rugueuse	
119	A	4	2	R	régulière	transparente	plate	diffus	rugueuse	
120	A	4	2	R	régulière	crème	plate	régulier	lisse / brillante	
121	A	4	2	R	régulière	crème	plate	régulier	lisse / brillante	
122	A	4	2	R	irrégulière	transparente	plate	diffus	rugueuse	
123	A	4	2	R	irrégulière	jaune pâle / translucide	plate	envahissant	rugueuse	
124	A	4	2	R	régulière	crème	plate	régulier	lisse	
125	A	4	2	R	régulière	crème	légèrement convexe	régulier	lisse / brillante	
126	A	4	2	R	régulière	crème	plate	régulier	opaque	
127	A	4	2	R	irrégulière	crème / translucide	plate	envahissant	rugueuse	
128	A	4	2	R	régulière	crème	plate	envahissant	lisse / brillante	
129	A	4	2	R	régulière / petite	jaune / translucide	légèrement convexe	régulier	lisse / brillante	
130	A	4	2	R	irrégulière	jaune pâle / translucide	plate	ondulé	rugueuse	
131	A	4	3	R	régulière	orange	plate	régulier	lisse / brillante	
132	A	4	3	R	régulière / petite	jaune pâle / translucide	légèrement convexe	régulier	lisse / brillante	
133	A	4	3	R	régulière	jaune / translucide	plate	régulier	lisse / brillante	
134	A	4	3	R	régulière	crème	plate	régulier	cercles concentriques	
135	A	4	3	R	irrégulière	jaune-orangé / translucide	plate	envahissant	rugueuse / brillante	
136	A	4	3	R	régulière	orange	plate	régulier	lisse / brillante	
137	A	4	3	R	irrégulière	jaune pâle / translucide	plate	envahissant	lisse / brillante	
138	A	4	3	R	irrégulière	saumon	plate	régulier	lisse / brillante	
139	A	4	3	R	régulière	jaune-orangé	légèrement convexe	régulier	lisse / brillante	

140	A	4	3	R	irrégulière	transparente - jaunâtre	plate	envahissant	rugueuse / brillante	
141	A	4	3	R	régulière	orange	plate	régulier	lisse / brillante	
142	A	4	3	R	régulière	crème	plate	régulier	enfoncée au centre	
143	A	4	3	R	régulière	jaune / translucide	plate	envahissant	lisse / brillante	
144	A	4	3	R	régulière	jaune pâle / translucide	plate	régulier	lisse / brillante	
145	A	4	3	R	régulière / petite	crème	plate	régulier	lisse / brillante	
146	A	4	3	R	régulière	orange	plate	régulier	lisse / brillante	
147	A	4	3	R	régulière	saumon	légèrement convexe	régulier	lisse / brillante	
148	A	4	3	R	régulière	crème	plate	diffus	lisse / brillante	
149	A	4	3	R	irrégulière	saumon	légèrement convexe	régulier	lisse / brillante	
150	A	4	3	R	régulière	crème	plate	régulier	lisse / brillante	
151	A	5	1	R	régulière	crème	légèrement convexe	régulier	lisse / brillante	jaune
152	A	5	1	R	irrégulière	jaune pâle	plate	diffus	lisse / brillante	
153	A	5	1	R	régulière	crème	légèrement convexe	régulier	lisse / brillante	jaune
154	A	5	1	R	régulière	crème	convexe	régulier	lisse / brillante	jaune
155	A	5	1	R	régulière	blanche / translucide	plate	ondulé / diffus	rugueuse	
156	A	5	1	R	régulière	crème	convexe	régulier	lisse / brillante	jaune
157	A	5	1	R	régulière	jaune pâle / translucide	plate	régulier	lisse / brillante	
158	A	5	1	R	régulière	crème	légèrement convexe	régulier	lisse / brillante	
159	A	5	1	R	irrégulière	jaune pâle	plate	diffus	rugueuse / brillante	
160	A	5	1	R	régulière	crème	convexe	régulier	lisse / brillante	jaune
161	A	5	1	R	irrégulière	jaune pâle / translucide	plate	diffus	rugueuse	
162	A	5	1	R	régulière	crème	convexe	régulier	lisse / brillante	jaune
163	A	5	1	R	régulière / petite	crème / translucide	plate	régulier	lisse / brillante	
164	A	5	1	R	régulière	crème	légèrement convexe	régulier	lisse / brillante	jaune
165	A	5	1	R	régulière	crème	légèrement convexe	régulier	lisse / brillante	jaune
166	A	5	1	R	régulière	crème	convexe	régulier	lisse / brillante	jaune
167	A	5	1	R	régulière	crème	convexe	régulier	lisse / brillante	jaune
168	A	5	1	R	régulière	crème	convexe	régulier	lisse / brillante	jaune
169	A	5	1	R	irrégulière	jaune pale	plate	ondulée	milieu lisse	
170	A	5	1	R	régulière	crème	légèrement convexe	régulier	lisse / brillante	jaune
171	A	5	2	R	régulière	crème	légèrement convexe	régulier	lisse / brillante	
172	A	5	2	R	régulière	crème	légèrement convexe	régulier	lisse / brillante	
173	A	5	2	R	régulière	blanche	plate	régulier	lisse / brillante	
174	A	5	2	R	type bacillus	-	-	-	-	
175	A	5	2	R	régulière	jaune pale	plate	régulier	lisse / brillante	
176	A	5	2	R	régulière	blanche	plate	régulier	lisse / brillante	
177	A	5	2	R	régulière	crème	légèrement convexe	régulier	lisse / brillante	
178	A	5	2	R	régulière / petite	transparente	plate	régulier	lisse / brillante	
179	A	5	2	R	régulière	crème	plate	régulier	rugueuse	
180	A	5	2	R	régulière	blanche	plate	régulier	rugueuse/ mat	
181	A	5	2	R	irrégulière	crème	plate	régulier	lisse / brillante	
182	A	5	2	R	régulière	crème	plate	diffus	lisse	
183	A	5	2	R	régulière	crème	plate	renflé	opaque	
184	A	5	2	R	régulière	blanche	plate	régulier	lisse / brillante	
185	A	5	2	R	régulière	crème	plate	régulier	lisse / brillante	
186	A	5	2	R	irrégulière	jaune	plate	ondulée	rugueuse	
187	A	5	2	R	régulière	crème	convexe	régulier	lisse / brillante	jaune

188	A	5	2	R	irrégulière	blanche	plate	régulier	ombiliquée	
189	A	5	2	R	régulière	crème / translucide	plate	diffus	lisse / brillante	
190	A	5	2	R	régulière	blanche	plate	régulier	mat	
191	A	5	3	R	régulière	blanche	plate	régulier	lisse / brillante	
192	A	5	3	R	régulière	crème	convexe	régulier	lisse / brillante	
193	A	5	3	R	régulière	crème	convexe	régulier	lisse / brillante	
194	A	5	3	R	régulière	crème / translucide	plate	régulier	lisse / brillante	
195	A	5	3	R	régulière	crème	plate	régulier	lisse	
196	A	5	3	R	régulière	jaune pale	plate	régulier	lisse / brillante	
197	A	5	3	R	régulière / petite	crème / translucide	plate	régulier	lisse / brillante	
198	A	5	3	R	régulière	crème	convexe	régulier	lisse / brillante	
199	A	5	3	R	régulière / petite	jaune pâle / translucide	plate	régulier	lisse / brillante	
200	A	5	3	R	régulière / petite	transparente	plate	régulier	lisse / brillante	
201	A	5	3	R	régulière	crème	plate	renflé / diffus	lisse	
202	A	5	3	R	régulière	crème	plate	régulier	lisse / brillante	
203	A	5	3	R	régulière	transparent	plate	régulier	lisse / brillante	
204	A	5	3	R	régulière	crème	plate	régulier	lisse / brillante	
205	A	5	3	R	régulière	blanche	plate	régulier	lisse / brillante	
206	A	5	3	R	régulière	crème	convexe	régulier	lisse / brillante	
207	A	5	3	R	régulière	blanche	plate	régulier	lisse / brillante	
208	A	5	3	R	régulière	crème	convexe	régulier	lisse / brillante	
209	A	5	3	R	régulière	crème	plate	diffus	lisse / brillante	
210	A	5	3	R	régulière	transparente	plate	régulier	lisse / brillante	
211	A	1	1	RS	régulière	blanche / translucide	plate	diffus	lisse	
212	A	1	1	RS	régulière	blanche	plate	régulier	rugueuse	
213	A	1	1	RS	régulière / petite	blanche	plate	régulier	lisse / brillante	
214	A	1	1	RS	irrégulière / petite	blanche	plate	régulier	rugueuse	
215	A	1	1	RS	irrégulière	blanche / translucide	plate	diffus	lisse	
216	A	1	1	RS	régulière	blanche	plate	régulier	lisse / brillante	
217	A	1	1	RS	régulière	jaune	plate	diffus	lisse / brillante	
218	A	1	1	RS	régulière	blanche	plate	régulier	lisse / brillante	
219	A	1	1	RS	régulière	blanche	plate	diffus	lisse	
220	A	1	1	RS	régulière / petite	jaune	plate	régulier	lisse / brillante	
221	A	1	1	RS	régulière	blanche	légèrement convexe	type actino	rugueuse	
222	A	1	1	RS	régulière / petite	blanche	convexe	régulier	lisse / brillante	
223	A	1	1	RS	régulière	blanche / translucide	plate	régulier	rugueuse	
224	A	1	1	RS	régulière / petite	blanche / translucide	plate	régulier	lisse	
225	A	1	1	RS	régulière	crème	légèrement convexe	type actino	duveteuse brun	
226	A	1	1	RS	régulière	crème	légèrement convexe	régulier	lisse / brillante	
227	A	1	1	RS	régulière	crème	légèrement convexe	régulier	lisse / brillante	
228	A	1	1	RS	régulière / petite	jaune pâle	plate	régulier	lisse	
229	A	1	1	RS	irrégulière	blanche / translucide	plate	diffus	lisse / brillante	
230	A	1	1	RS	régulière	ocre	légèrement convexe	régulier	ombiliquée	
231	A	1	1	RS	irrégulière	blanche	plate	régulier	rugueuse	
232	A	1	1	RS	régulière / petite	blanche / translucide	plate	régulier	lisse / brillante	
233	A	1	1	RS	régulière	jaune pâle	plate	régulier	lisse / brillante	
234	A	1	1	RS	régulière	blanche	convexe	régulier	lisse / brillante	
235	A	1	1	RS	régulière	blanche	convexe	régulier	ombiliquée / brillante	

236	A	1	1	RS	régulière	blanche	plate	régulier	lisse / brillante	
237	A	1	1	RS	régulière / petite	jaune pâle / translucide	plate	régulier	lisse / brillante	
238	A	1	1	RS	régulière	crème	plate	régulier	lisse / brillante	
239	A	1	1	RS	régulière	crème	légèrement convexe	régulier	ombiliquée / brillante	
240	A	1	1	RS	régulière / petite	blanche	convexe	régulier	lisse / brillante	
241	A	2	1	RS	régulière	crème	plate	régulier	lisse / brillante	
242	A	2	1	RS	régulière	blanche	légèrement convexe	régulier	cercles concentriques / brillante	
243	A	2	1	RS	régulière	blanche	plate	régulier	lisse / brillante	
244	A	2	1	RS	régulière	jaune	plate	diffus	lisse / brillante	
245	A	2	1	RS	régulière	crème	plate	régulier	lisse / brillante	
246	A	2	1	RS	régulière	blanche	plate	régulier	lisse / brillante	
247	A	2	1	RS	régulière	crème	légèrement convexe	type actino	duveteuse blanche	
248	A	2	1	RS	régulière	blanche	légèrement convexe	régulier	cercles concentriques / brillante	
249	A	2	1	RS	régulière	blanche	plate	régulier	cercles concentriques	
250	A	2	1	RS	irrégulière	blanche	plate	régulier	cercles concentriques	
251	A	2	1	RS	régulière	crème / translucide	plate	régulier	lisse / brillante	
252	A	2	1	RS	régulière	blanche	plate	régulier	cercles concentriques / rugueuse	
253	A	2	1	RS	régulière	blanche	plate	régulier	lisse / brillante	
254	A	2	1	RS	régulière	blanche	convexe	régulier	lisse / brillante	
255	A	2	1	RS	irrégulière	jaune / translucide	plate	diffus	rugueuse	
256	A	2	1	RS	régulière	blanche	légèrement convexe	régulier	cercles concentriques	
257	A	2	1	RS	régulière / petite	jaune / translucide	plate	régulier	lisse / brillante	
258	A	2	1	RS	régulière	blanche	plate	régulier	cercles concentriques / brillante	
259	A	2	1	RS	type bacillus	-	-	-	-	
260	A	2	1	RS	régulière	blanche	légèrement convexe	régulier	cercles concentriques / brillante	
261	A	2	2	RS	type bacillus	-	-	-	-	
262	A	2	2	RS	régulière	crème	plate	régulier	rugueuse	
263	A	2	2	RS	régulière	crème	légèrement convexe	régulier	lisse / brillante	
264	A	2	2	RS	régulière	jaune	plate	régulier	lisse / brillante	
265	A	2	2	RS	irrégulière	blanche	plate	diffus	opaque	
266	A	2	2	RS	régulière	blanche	plate	régulier	opaque	
267	A	2	2	RS	irrégulière	blanche	plate	régulier	cercles concentriques / brillante	
268	A	2	2	RS	régulière	jaune	plate	régulier	lisse / brillante	
269	A	2	2	RS	régulière	blanche	plate	régulier	cercles concentriques	
270	A	2	2	RS	irrégulière	blanche	plate	régulier	rugueuse	
271	A	2	2	RS	régulière	blanche	plate	diffus	rugueuse	
272	A	2	2	RS	irrégulière	blanche	plate	régulier	rugueuse	
273	A	2	2	RS	irrégulière	crème	plate	envahissant	lisse / brillante	
274	A	2	2	RS	régulière	blanche	plate	régulier	cercles concentriques	
275	A	2	2	RS	régulière	ocre	convexe	régulier	rugueuse	
276	A	2	2	RS	régulière	blanche	plate	régulier	lisse / brillante	
277	A	2	2	RS	régulière	crème	plate	régulier	lisse / brillante	
278	A	2	2	RS	régulière	blanche	plate	régulier	cercles concentriques	
279	A	2	2	RS	régulière	crème	plate	régulier	lisse / brillante	
280	A	2	2	RS	irrégulière	crème	plate	régulier	enfoncée au centre / brillante	
281	A	2	3	RS	régulière	blanche	convexe	régulier	lisse / brillante	
282	A	2	3	RS	régulière	blanche	plate	diffus	rugueuse	
283	A	2	3	RS	régulière / petite	blanche / translucide	plate	régulier	lisse / brillante	

284	A	2	3	RS	régulière / petite	blanche / translucide	plate	régulier	lisse / brillante	
285	A	2	3	RS	régulière	blanche	plate	régulier	cercles concentriques	
286	A	2	3	RS	irrégulière	blanche	plate	régulier	lisse / brillante	
287	A	2	3	RS	régulière	jaune / translucide	plate	régulier	lisse / brillante	
288	A	2	3	RS	régulière	blanche	plate	régulier	cercles concentriques	
289	A	2	3	RS	irrégulière	jaune / translucide	plate	diffus	rugueuse / brillante	
290	A	2	3	RS	régulière	blanche	plate	régulier	cercles concentriques	jaune
291	A	2	3	RS	régulière / petite	transparente	plate	régulier	lisse / brillante	
292	A	2	3	RS	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
293	A	2	3	RS	régulière	blanche	plate	régulier	cercles concentriques / rugueuse	
294	A	2	3	RS	régulière	crème	légèrement convexe	type actino	rugueuse	brun
295	A	2	3	RS	irrégulière	jaune / translucide	plate	diffus	rugueuse / brillante	
296	A	2	3	RS	régulière	crème	plate	régulier	cercles concentriques	
297	A	2	3	RS	irrégulière	blanche / translucide	plate	régulier	lisse / brillante	
298	A	2	3	RS	irrégulière	blanche / translucide	plate	régulier	lisse / brillante	
299	A	2	3	RS	régulière	blanche / translucide	plate	régulier	lisse / brillante	
300	A	2	3	RS	régulière	crème	légèrement convexe	régulier	lisse / brillante	
301	A	4	1	RS	régulière	crème	plate	régulier	cercles concentriques / rugueuse	
302	A	4	1	RS	régulière	crème / jaunâtre	légèrement convexe	régulier	lisse / brillante	
303	A	4	1	RS	régulière	crème	légèrement convexe	régulier	cercles concentriques	
304	A	4	1	RS	régulière	crème	plate	régulier	ombiliquée / rugueuse	
305	A	4	1	RS	irrégulière	blanche	plate	légèrement ondulé	rugueuse	
306	A	4	1	RS	régulière / petite	orange pâle	plate	diffus	lisse / brillante	
307	A	4	1	RS	irrégulière	crème	plate	ondulé	rugueuse	
308	A	4	1	RS	régulière / petite	orange pâle	légèrement convexe	régulier	lisse / brillante	
309	A	4	1	RS	régulière / petite	blanche	convexe	régulier	lisse / brillante	
310	A	4	1	RS	régulière	blanche	légèrement convexe	régulier	rugueuse / aplatie au centre	
311	A	4	1	RS	régulière	jaune-orangé	légèrement convexe	diffus	lisse / brillante	
312	A	4	1	RS	régulière	blanche	légèrement convexe	régulier	rugueuse / aplatie au centre	
313	A	4	1	RS	irrégulière	blanche	plate	légèrement ondulé	rugueuse / brillante	
314	A	4	1	RS	irrégulière	blanche	plate	légèrement ondulé	rugueuse	
315	A	4	1	RS	type bacillus	-	-	-	-	
316	A	4	1	RS	irrégulière	crème	plate	légèrement ondulé	lisse / brillante	
317	A	4	1	RS	irrégulière	jaune-orangé	légèrement convexe	diffus	lisse / brillante	
318	A	4	1	RS	régulière	crème	légèrement convexe	régulier	cercles concentriques / rugueuse / brillante	
319	A	4	1	RS	régulière	crème	légèrement convexe	type actino	rugueuse / enfoncée au centre	
320	A	4	1	RS	régulière	crème	légèrement convexe	régulier	ombiliquée / rugueuse	
321	A	4	2	RS	régulière	blanche	plate	type actino	rugueuse	
322	A	4	2	RS	type bacillus	-	-	-	-	
323	A	4	2	RS	irrégulière	transparente	légèrement convexe	régulier	lisse / brillante	
324	A	4	2	RS	irrégulière	crème	légèrement convexe	diffus	lisse / brillante	
325	A	4	2	RS	irrégulière	crème	légèrement convexe	régulier	lisse / brillante	
326	A	4	2	RS	régulière	blanche	plate	type actino	rugueuse	
327	A	4	2	RS	irrégulière	crème	légèrement convexe	régulier	ombiliquée / lisse / brillante	
328	A	4	2	RS	irrégulière	transparente	plate	régulier	rugueuse	
329	A	4	2	RS	irrégulière	crème	plate	ondulé	lisse / brillante	
330	A	4	2	RS	type bacillus	-	-	-	-	
331	A	4	2	RS	régulière	crème	plate	régulier	rugueuse / brillante	

332	A	4	2	RS	régulière	blanche	plate	diffus	rugueuse	
333	A	4	2	RS	irrégulière	blanche	plate	régulier	lisse / brillante	
334	A	4	2	RS	régulière / petite	saumon	plate	régulier	lisse / brillante	
335	A	4	2	RS	irrégulière	transparente	plate	régulier	rugueuse	
336	A	4	2	RS	type bacillus	-	-	-	-	
337	A	4	2	RS	régulière	transparente	plate	diffus	rugueuse	
338	A	4	2	RS	régulière / petite	transparente	plate	régulier	lisse	
339	A	4	2	RS	régulière	jaune pâle	plate	diffus	rugueuse	
340	A	4	2	RS	régulière	crème	plate	régulier	lisse / brillante	
341	A	4	3	RS	régulière / petite	blanche / translucide	plate	régulier	lisse / brillante	
342	A	4	3	RS	régulière	blanche	plate	légèrement ondulé	lisse / brillante	
343	A	4	3	RS	régulière	crème	plate	diffus	rugueuse	
344	A	4	3	RS	régulière	crème	plate	légèrement ondulé	renflée au centre / lisse / brillante	
345	A	4	3	RS	régulière	crème	plate	régulier	lisse / brillante	
346	A	4	3	RS	régulière	jaune	légèrement convexe	régulier	lisse / brillante	
347	A	4	3	RS	régulière	jaune pâle / translucide	plate	ondulé / diffus	rugueuse / brillante	
348	A	4	3	RS	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
349	A	4	3	RS	régulière	blanche	plate	ondulé / diffus	lisse / brillante	
350	A	4	3	RS	régulière	saumon	plate	régulier	cercles concentriques / brillante	
351	A	4	3	RS	régulière	blanche	plate	diffus	rugueuse / brillante	
352	A	4	3	RS	régulière	blanche	plate	régulier	lisse / brillante	
353	A	4	3	RS	régulière	jaune-orangé	convexe	régulier	lisse / brillante	
354	A	4	3	RS	régulière	blanche	légèrement convexe	régulier	ombiliquée / brillante	
355	A	4	3	RS	irrégulière	blanche	légèrement convexe	diffus	ombiliquée	
356	A	4	3	RS	régulière	blanche	légèrement convexe	régulier	ombiliquée / brillante	
357	A	4	3	RS	type bacillus	-	-	-	-	
358	A	4	3	RS	irrégulière	jaune-orangé / translucide	plate	diffus	rugueuse	
359	A	4	3	RS	régulière	blanche	convexe	régulier	ombiliquée / brillante	
360	A	4	3	RS	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
361	A	5	1	RS	régulière	blanche	plate	légèrement ondulé	lisse / brillante	
362	A	5	1	RS	régulière	blanche	plate	ondulé / renflé	lisse / brillante	
363	A	5	1	RS	régulière	blanche	plate	ondulé / renflé	lisse / brillante	
364	A	5	1	RS	régulière	blanche	plate	régulier	lisse / brillante	
365	A	5	1	RS	type bacillus	-	-	-	-	
366	A	5	1	RS	régulière	blanche	plate	ondulé / diffus	rugueuse / brillante	
367	A	5	1	RS	régulière / petite	crème / translucide	plate	régulier	lisse / brillante	
368	A	5	1	RS	type bacillus	-	-	-	-	
369	A	5	1	RS	régulière	blanche	plate	ondulé	lisse / brillante	
370	A	5	1	RS	régulière / petite	transparente	plate	régulier	lisse / brillante	
371	A	5	1	RS	régulière	crème	légèrement convexe	régulier	lisse / brillante	
372	A	5	1	RS	régulière	blanche / translucide	plate	ondulé / diffus	rugueuse	
373	A	5	1	RS	régulière	crème	légèrement convexe	régulier	lisse / brillante	
374	A	5	1	RS	type bacillus	-	-	-	-	
375	A	5	1	RS	type bacillus	-	-	-	-	
376	A	5	1	RS	régulière	crème	légèrement convexe	régulier	lisse / brillante	
377	A	5	1	RS	régulière	blanche	plate	légèrement renflé	lisse / brillante	
378	A	5	1	RS	régulière	crème	plate	ondulé	cratériforme / brillante	
379	A	5	1	RS	régulière / petite	transparente	plate	régulier	lisse / brillante	

380	A	5	1	RS	régulière / petite	blanche	plate	régulier	lisse / brillante	
381	A	5	2	RS	régulière	transparente	plate	régulier	lisse	
382	A	5	2	RS	régulière	blanche	plate	ondulée	rugueuse / mat	
383	A	5	2	RS	régulière	blanche	plate	régulier	lisse / brillante	
384	A	5	2	RS	régulière	blanche	plate	regulier	lisse / brillante	
385	A	5	2	RS	régulière	transparente	plate	régulier	lisse	
386	A	5	2	RS	régulière	blanche	plate	régulier	cercles concentriques	
387	A	5	2	RS	régulière	blanche	légèrement convexe	régulier	ombiliquée / brillante	
388	A	5	2	RS	régulière	blanche	plate	ondulée	rugeuse / mat	
389	A	5	2	RS	irrégulière	transparente	plate	régulier	lisse	
390	A	5	2	RS	régulière	blanche	plate	régulier	lisse / brillante	
391	A	5	2	RS	type bacillus	-	-	-	-	
392	A	5	2	RS	irrégulière	blanche	plate	ondulé	lisse / brillante	
393	A	5	2	RS	régulière	blanche	plate	régulier	rugueuse / brillante	
394	A	5	2	RS	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
395	A	5	2	RS	type bacillus	-	-	-	-	
396	A	5	2	RS	régulière	blanche	convexe	régulier	lisse / brillante	
397	A	5	2	RS	régulière	transparente	plate	régulier	lisse / brillante	
398	A	5	2	RS	régulière	transparente	plate	régulier	lisse	
399	A	5	2	RS	type bacillus	-	-	-	-	
400	A	5	2	RS	régulière	blanche	plate	diffus	rugueuse / brillante	
401	A	5	3	RS	irrégulière	crème	légèrement convexe	régulier	lisse / brillante	
402	A	5	3	RS	régulière	blanche	légèrement convexe	type actino	duveuteux	
403	A	5	3	RS	régulière	blanche	plate	régulier	lisse / brillante	
404	A	5	3	RS	régulière	transparente	plate	régulier	lisse / brillante	
405	A	5	3	RS	régulière	blanche	convexe	régulier	lisse / brillante	
406	A	5	3	RS	type bacillus	jaune	plate	régulier	brillante	
407	A	5	3	RS	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
408	A	5	3	RS	type bacillus	-	-	-	-	
409	A	5	3	RS	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
410	A	5	3	RS	type bacillus	-	-	-	-	
411	A	5	3	RS	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
412	A	5	3	RS	régulière	blanche	plate	régulier	lisse / brillante	
413	A	5	3	RS	irrégulière	blanche	convexe	irrégulière	rugeuse / mat	
414	A	5	3	RS	régulière	saumon	plate	régulier	lisse / brillante	
415	A	5	3	RS	irrégulière	blanche	plate	régulier	rugeuse / mat	
416	A	5	3	RS	régulière	blanche	plate	régulier	lisse / brillante	
417	A	5	3	RS	régulière	blanche	plate	régulier	rugueuse / mat	
418	A	5	3	RS	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
419	A	5	3	RS	régulière	jaune pale	plate	régulier	lisse / brillante	
420	A	5	3	RS	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
421	B	1	1	R	régulière	jaune pâle	plate	régulier	lisse / brillante	
422	B	1	1	R	régulière	crème	légèrement convexe	régulier	ombiliquée / brillante	
423	B	1	1	R	irrégulière	jaune / translucide	plate	régulier	rugueuse	
424	B	1	1	R	irrégulière	jaune / translucide	plate	régulier	rugueuse	
425	B	1	1	R	irrégulière	jaune / translucide	plate	régulier	rugueuse	
426	B	1	1	R	régulière	rouge-rose	légèrement convexe	régulier	lisse / brillante	
427	B	1	1	R	régulière / petite	jaune	plate	régulier	lisse / brillante	

428	B	1	1	R	régulière	jaune	légèrement convexe	régulier	lisse / brillante	
429	B	1	1	R	régulière	crème	convexe	régulier	lisse / brillante	
430	B	1	1	R	régulière	crème	convexe	régulier	lisse / brillante	
431	B	1	1	R	irrégulière	crème	plate	régulier	rugueuse	
432	B	1	1	R	irrégulière	crème	plate	régulier	lisse / brillante	
433	B	1	1	R	irrégulière	crème	convexe	régulier	lisse / brillante	
434	B	1	1	R	irrégulière	crème	convexe	régulier	lisse / brillante	
435	B	1	1	R	régulière	crème	convexe	régulier	lisse / brillante	
436	B	1	1	R	régulière	jaune / translucide	plate	régulier	lisse / brillante	
437	B	1	1	R	régulière / petite	jaune	plate	régulier	lisse / brillante	
438	B	1	1	R	régulière	crème	légèrement convexe	régulier	ombiliquée / opaque	
439	B	1	1	R	régulière / petite	jaune	plate	régulier	lisse / brillante	
440	B	1	1	R	régulière / petite	jaune-orangé	légèrement convexe	régulier	lisse / brillante	
441	B	1	1	R	irrégulière	crème	plate	diffus	lisse / brillante	
442	B	1	1	R	irrégulière	jaune / translucide	plate	régulier	rugueuse / brillante	
443	B	1	1	R	irrégulière	jaunâtre	plate	diffus	rugueuse / brillante	
444	B	1	1	R	irrégulière	crème	plate	régulier	lisse / brillante	
445	B	1	1	R	irrégulière	crème	légèrement convexe	régulier	lisse / brillante	
446	B	1	1	R	régulière / petite	crème	légèrement convexe	régulier	lisse / brillante	
447	B	1	1	R	irrégulière	crème	légèrement convexe	régulier	lisse / brillante	
448	B	1	1	R	régulière	rose	légèrement convexe	régulier	lisse / brillante	
449	B	1	1	R	irrégulière	crème	légèrement convexe	régulier	lisse / brillante	
450	B	1	1	R	irrégulière	jaune / translucide	plate	diffus	rugueuse / brillante	
451	B	2	1	R	irrégulière	jaune / translucide	plate	diffus	rugueuse / brillante	
452	B	2	1	R	régulière / petite	jaune pâle / translucide	plate	régulier	lisse / brillante	
453	B	2	1	R	régulière	crème	plate	régulier	lisse / brillante	
454	B	2	1	R	irrégulière	jaune / translucide	plate	diffus	rugueuse / brillante	
455	B	2	1	R	irrégulière	jaune / translucide	plate	diffus	rugueuse / brillante	
456	B	2	1	R	régulière	jaune pâle	légèrement convexe	régulier	lisse / brillante	
457	B	2	1	R	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
458	B	2	1	R	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
459	B	2	1	R	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
460	B	2	1	R	régulière / petite	jaune pâle / translucide	plate	régulier	lisse / brillante	
461	B	2	1	R	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
462	B	2	1	R	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
463	B	2	1	R	régulière	jaune / translucide	plate	diffus	lisse / brillante	
464	B	2	1	R	régulière	jaune / translucide	plate	régulier	lisse / brillante	
465	B	2	1	R	régulière	jaune / translucide	plate	régulier	lisse / brillante	
466	B	2	1	R	régulière	jaune / translucide	plate	diffus	lisse / brillante	
467	B	2	1	R	régulière	rose	légèrement convexe	régulier	lisse / brillante	
468	B	2	1	R	régulière	orange	légèrement convexe	régulier	lisse / brillante	
469	B	2	1	R	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
470	B	2	1	R	régulière / petite	jaune / translucide	plate	régulier	lisse / brillante	
471	B	2	2	R	régulière	blanche	légèrement convexe	régulier	lisse / brillante	jaune
472	B	2	2	R	régulière / petite	blanche	plate	régulier	lisse / brillante	
473	B	2	2	R	régulière	crème	convexe	type actino	duveteuse blanc et brun	
474	B	2	2	R	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
475	B	2	2	R	régulière	blanche	convexe	régulier	lisse / brillante	

476	B	2	2	R	régulière	crème	convexe	régulier	lisse / brillante	
477	B	2	2	R	régulière	jaune / translucide	plate	régulier	lisse / brillante	
478	B	2	2	R	régulière	blanche	convexe	régulier	lisse / brillante	
479	B	2	2	R	régulière	jaune / translucide	plate	diffus	lisse / brillante	
480	B	2	2	R	régulière	blanche	convexe	régulier	lisse / brillante	
481	B	2	2	R	régulière	crème	légèrement convexe	régulier	lisse / brillante	jaune
482	B	2	2	R	régulière	jaune / translucide	plate	régulier	lisse / brillante	
483	B	2	2	R	régulière	blanche / translucide	plate	régulier	lisse / brillante	jaune
484	B	2	2	R	régulière	blanche	convexe	régulier	lisse / brillante	
485	B	2	2	R	régulière	jaune pâle	convexe	régulier	lisse / brillante	
486	B	2	2	R	régulière	crème	légèrement convexe	régulier	lisse / brillante	
487	B	2	2	R	régulière	crème	légèrement convexe	régulier	lisse / brillante	
488	B	2	2	R	régulière	jaune / translucide	plate	régulier	lisse / brillante	
489	B	2	2	R	régulière	blanche	convexe	régulier	lisse / brillante	
490	B	2	2	R	régulière	crème	convexe	régulier	lisse / brillante	
491	B	2	3	R	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
492	B	2	3	R	type bacillus	-	-	-	-	
493	B	2	3	R	régulière	jaune	plate	diffus	lisse / brillante	
494	B	2	3	R	régulière / petite	blanche	plate	régulier	lisse / brillante	
495	B	2	3	R	régulière	jaune	légèrement convexe	diffus	lisse / brillante	
496	B	2	3	R	régulière	jaune	légèrement convexe	diffus	lisse / brillante	
497	B	2	3	R	régulière	jaune pâle	plate	diffus	lisse / brillante	
498	B	2	3	R	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
499	B	2	3	R	régulière	crème	légèrement convexe	type actino / diffus	rugueuse	
500	B	2	3	R	régulière / petite	blanche / translucide	plate	régulier	lisse / brillante	
501	B	2	3	R	régulière	crème	convexe	régulier	lisse / brillante	
502	B	2	3	R	régulière	jaune	plate	diffus	lisse / brillante	
503	B	2	3	R	régulière	jaune	plate	régulier	lisse / brillante	
504	B	2	3	R	irrégulière	blanche	plate	diffus	rugueuse	
505	B	2	3	R	régulière	jaune	légèrement convexe	diffus	lisse / brillante	
506	B	2	3	R	régulière	crème	légèrement convexe	régulier	lisse / brillante	
507	B	2	3	R	régulière	jaune	plate	diffus	lisse / brillante	
508	B	2	3	R	régulière	jaune pâle	plate	régulier	lisse / brillante	
509	B	2	3	R	régulière	jaune	plate	diffus	lisse / brillante	
510	B	2	3	R	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
511	B	4	1	R	régulière	jaune-orangé	légèrement convexe	régulier	lisse / brillante	
512	B	4	1	R	régulière	crème / translucide	plate	diffus	rugueuse	
513	B	4	1	R	régulière	crème	plate	régulier	lisse / brillante	
514	B	4	1	R	irrégulière	jaune-orangé / translucide	plate	envahissant	rugueuse	
515	B	4	1	R	régulière	jaune pâle / translucide	plate	régulier	lisse / brillante	
516	B	4	1	R	irrégulière	jaune / translucide	plate	ondulé	rugueuse / brillante	
517	B	4	1	R	régulière	saumon	plate	régulier	enfoncée au centre / brillante	
518	B	4	1	R	régulière	jaune-orangé / translucide	plate	envahissant	rugueuse	
519	B	4	1	R	régulière	crème	plate	diffus	lisse / brillante	
520	B	4	1	R	régulière	jaune-orangé / translucide	plate	envahissant	rugueuse	
521	B	4	1	R	irrégulière	crème - jaunâtre	plate	régulier	lisse / brillante	
522	B	4	1	R	irrégulière	crème / translucide	plate	ondulé	rugueuse	
523	B	4	1	R	régulière	jaune-orangé / translucide	plate	envahissant	rugueuse	

524	B	4	1	R	régulière	crème	convexe	régulier	lisse / brillante	jaune
525	B	4	1	R	régulière	saumon	plate	renflé	lisse / brillante	
526	B	4	1	R	régulière	jaune-orangé / translucide	plate	régulier	lisse / brillante	
527	B	4	1	R	régulière	jaune-orangé	plate	régulier	lisse / brillante	
528	B	4	1	R	régulière	jaune-orangé / translucide	plate	envahissant	rugueuse	
529	B	4	1	R	régulière / petite	crème	plate	régulier	lisse / brillante	
530	B	4	1	R	régulière	jaune-orangé	plate	régulier	lisse / brillante	
531	B	4	2	R	irrégulière	transparente jaunâtre	plate	envahissant	rugueuse	
532	B	4	2	R	régulière	jaune-orangé	plate	régulier	lisse / brillante	
533	B	4	2	R	régulière	jaune-orangé	plate	régulier	lisse / brillante	
534	B	4	2	R	régulière	crème	plate	ondulé	lisse	
535	B	4	2	R	régulière	jaune pâle	légèrement convexe	régulier	lisse / brillante	
536	B	4	2	R	régulière	crème	légèrement convexe	régulier	lisse / brillante	
537	B	4	2	R	régulière	crème	plate	régulier	lisse / brillante	
538	B	4	2	R	irrégulière	jaune pâle/ translucide	plate	régulier	lisse / brillante	
539	B	4	2	R	régulière	jaune pâle/ translucide	plate	diffus	rugueuse	
540	B	4	2	R	régulière	crème	plate	ondulé	rugueuse	
541	B	4	2	R	régulière	jaune-orangé	plate	ondulé	rugueuse / brillante	
542	B	4	2	R	irrégulière	jaune pâle/ translucide	plate	envahissant	rugueuse	
543	B	4	2	R	régulière	jaune pâle/ translucide	plate	envahissant	rugueuse	
544	B	4	2	R	régulière	crème	plate	ondulé	lisse	
545	B	4	2	R	régulière	jaune pâle/ translucide	plate	régulier	lisse / brillante	
546	B	4	2	R	régulière	ocre	plate	régulier	enfoncée au centre	
547	B	4	2	R	régulière	jaune-orangé	plate	régulier	lisse / brillante	
548	B	4	2	R	irrégulière	jaune-orangé	plate	ondulé / difus	rugueuse	
549	B	4	2	R	régulière / petite	orange	plate	régulier	lisse / brillante	
550	B	4	2	R	régulière	jaune-orangé / translucide	plate	envahissant	lisse / brillante	
551	B	4	3	R	régulière	crème	plate	diffus	rugueuse	
552	B	4	3	R	régulière	jaune-orangé / translucide	plate	régulier	lisse / brillante	
553	B	4	3	R	régulière	crème	légèrement convexe	régulier	lisse / brillante	
554	B	4	3	R	régulière	orange / translucide	légèrement convexe	régulier	lisse / brillante	
555	B	4	3	R	régulière	crème	plate	ondulé / renflé	läkerol / brillante	
556	B	4	3	R	régulière	crème jaunêtre	plate	régulier	rugueuse	
557	B	4	3	R	régulière	jaune	plate	diffus	lisse / brillante	
558	B	4	3	R	régulière	jaune-orangé / translucide	plate	régulier	lisse / brillante	
559	B	4	3	R	régulière	crème	plate	régulier	lisse / brillante	
560	B	4	3	R	régulière	jaune-orangé / translucide	plate	régulier	lisse / brillante	
561	B	4	3	R	régulière	jaune-orangé	plate	diffus	lisse / brillante	
562	B	4	3	R	régulière / petite	crème	plate	régulier	lisse	
563	B	4	3	R	régulière	crème	légèrement convexe	régulier	lisse / brillante	
564	B	4	3	R	régulière	orange / translucide	plate	régulier	lisse / brillante	
565	B	4	3	R	régulière	orange / translucide	plate	régulier	lisse / brillante	
566	B	4	3	R	régulière	orange / translucide	plate	régulier	lisse / brillante	
567	B	4	3	R	régulière	crème	légèrement convexe	régulier	cercles concentriques / brillante	
568	B	4	3	R	régulière	transparente jaunâtre	plate	régulier	lisse / brillante	
569	B	4	3	R	irrégulière	jaune-orangé / translucide	plate	diffus	rugueuse / brillante	
570	B	4	3	R	irrégulière	jaune-orangé / translucide	plate	diffus	rugueuse / brillante	
571	B	5	1	R	régulière	crème	plate	type actino	rugueuse	brun

572	B	5	1	R	régulière / petite	crème	plate	régulier	rugueuse	
573	B	5	1	R	régulière / petite	crème	plate	régulier	lisse / brillante	
574	B	5	1	R						
575	B	5	1	R	régulière	crème	plate	régulier	cercles concentriques / opaque	
576	B	5	1	R	régulière / petite	crème	plate	régulier	lisse / brillante	
577	B	5	1	R	régulière	crème / translucide	plate	régulier	lisse / brillante	
578	B	5	1	R	régulière	crème	plate	régulier	lisse / brillante	
579	B	5	1	R	irrégulière	blanche	plate	régulier	lisse / brillante	
580	B	5	1	R	régulière	blanche	plate	régulier	lisse / brillante	
581	B	5	1	R	régulière	crème	plate	ondulé	lisse	
582	B	5	1	R	régulière	crème / translucide	plate	ondulé / diffus	lisse	
583	B	5	1	R	régulière	crème	plate	régulier	lisse / brillante	
584	B	5	1	R	régulier	blanche	plate	régulier	lisse / brillante	
585	B	5	1	R	régulière	blanche	plate	régulière	lisse / brillante	
586	B	5	1	R	régulière	jaune pale	plate	régulière	lisse / brillante	
587	B	5	1	R	régulière / petite	crème	plate	type actino	rugueuse	brun
588	B	5	1	R	régulière	jaune pale	plate	régulière	lisse / brillante	
589	B	5	1	R	régulière	crème	plate	diffus	lisse / brillante	
590	B	5	1	R	régulière	jaune pale	plate	régulière	lisse / brillante	
591	B	5	2	R	régulière	crème	plate	régulier	rugueuse	
592	B	5	2	R	régulière / petite	orange / translucide	plate	régulier	lisse / brillante	
593	B	5	2	R	régulière	brun	renflée	convexe aplatie	duveteuse brun	
594	B	5	2	R	régulière / petite	crème / translucide	plate	régulier	lisse / brillante	
595	B	5	2	R	régulière / petite	crème / translucide		régulier	lisse / brillante	
596	B	5	2	R	régulière	crème	plate	régulier	enfoncée au centre	
597	B	5	2	R	régulière / petite	crème / translucide	plate	régulier	lisse / brillante	
598	B	5	2	R	régulière / petite	crème	convexe	régulier	lisse / brillante	
599	B	5	2	R	régulière	crème	plate	régulier	lisse / brillante	
600	B	5	2	R	régulière / petite	crème / translucide	plate	régulier	lisse / brillante	
601	B	5	2	R	régulière / petite	crème / translucide	plate	régulier	lisse / brillante	
602	B	5	2	R	irrégulière	crème	ombiliquée	régulière	?	
603	B	5	2	R	régulière / petite	transparente jaunâtre	plate	régulier	lisse / brillante	
604	B	5	2	R	régulière / petite	crème / translucide	plate	régulier	lisse / brillante	
605	B	5	2	R	irrégulière	jaune pale	plate	légerement ondulé	rugeuse/ mat	
606	B	5	2	R	régulière / petite	crème	convexe	régulier	lisse / brillante	
607	B	5	2	R	régulière	blanche	plate	régulière	lisse / brillante	
608	B	5	2	R	régulière / petite	crème	convexe	régulier	lisse / brillante	
609	B	5	2	R	régulière / petite	crème / translucide	plate	régulier	lisse / brillante	
610	B	5	2	R						
611	B	5	3	R	régulière	crème / translucide	plate	ondulé / diffus	lisse / brillante	
612	B	5	3	R	régulière	saumon	plate	régulier	lisse	
613	B	5	3	R	régulière	crème / translucide	plate	ondulé / diffus	lisse / brillante	
614	B	5	3	R	régulière	crème / translucide	plate	ondulé / diffus	lisse / brillante	
615	B	5	3	R	régulière	crème	convexe	régulier	lisse / brillante	
616	B	5	3	R	régulière / petite	crème / translucide	plate	régulier	lisse / brillante	
617	B	5	3	R	régulière	crème	plate	régulier	lisse	
618	B	5	3	R	régulière	crème	plate	régulier	lisse	
619	B	5	3	R	irrégulière	brun	plate	ondulé	cratériformes	

620	B	5	3	R	régulière	rose clair	plate	ondulé	lisse / brillante	
621	B	5	3	R	régulière	crème	plate	ondulé	rugueuse / brillante	
622	B	5	3	R	régulière / petite	crème	plate	régulier	lisse / brillante	
623	B	5	3	R	régulière	crème	plate	ondulé	rugueuse / brillante	
624	B	5	3	R	régulière	crème	plate	renflé	lisse	
625	B	5	3	R	régulière	crème / translucide	plate	ondulé / diffus	lisse	
626	B	5	3	R	régulière	crème	plate	renflé	lisse	
627	B	5	3	R	régulière	crème	plate	régulier	lisse	
628	B	5	3	R	régulière / petite	transparente jaunâtre	plate	régulier	lisse / brillante	
629	B	5	3	R	régulière / petite	crème / translucide	plate	régulier	lisse / brillante	
630	B	5	3	R	régulière / petite	crème / translucide	plate	régulier	lisse / brillante	
631	B	1	1	RS	régulière / petite	crème / translucide	plate	régulier	lisse	
632	B	1	1	RS	régulière / petite	blanche / translucide	plate	régulier	lisse	
633	B	1	1	RS	irrégulière	crème	légèrement convexe	régulier	lisse / brillante	
634	B	1	1	RS	régulière	crème	légèrement convexe	type actino	rugueuse	jaune
635	B	1	1	RS	irrégulière / petite	blanche / translucide	plate	régulier	rugueuse	
636	B	1	1	RS	régulière	rose	plate	régulier	lisse / brillante	
637	B	1	1	RS	régulière	crème	légèrement convexe	type actino	rugueuse	brun
638	B	1	1	RS	régulière	crème	légèrement convexe	régulier	lisse / brillante	
639	B	1	1	RS	régulière	crème	légèrement convexe	type actino	duveteuse blanche	
640	B	1	1	RS	irrégulière	blanche / translucide	plate	régulier	rugueuse	
641	B	1	1	RS	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
642	B	1	1	RS	irrégulière	blanche	plate	diffus	rugueuse	
643	B	1	1	RS	régulière	crème	plate	régulier	cercles concentriques	
644	B	1	1	RS	régulière	crème	convexe	régulier	lisse / brillante	
645	B	1	1	RS	régulière / petite	blanche	plate	régulier	rugueuse	
646	B	1	1	RS	régulière	jaune	légèrement convexe	régulier	lisse / brillante	
647	B	1	1	RS	régulière	crème	plate	diffus	lisse / brillante	
648	B	1	1	RS	irrégulière / petite	blanche / translucide	plate	régulier	rugueuse	
649	B	1	1	RS	régulière	crème	convexe	régulier	lisse / brillante	
650	B	1	1	RS	régulière / petite	blanche / translucide	plate	régulier	lisse / brillante	
651	B	1	1	RS	régulière	crème	plate	régulier	cercles concentriques	
652	B	1	1	RS	régulière / petite	crème	plate	régulier	rugueuse	
653	B	1	1	RS	régulière	crème	légèrement convexe	régulier	cercles concentriques / brillante	
654	B	1	1	RS	irrégulière	crème	plate	diffus	rugueuse	
655	B	1	1	RS	régulière	crème	convexe	régulier	lisse / brillante	
656	B	1	1	RS	régulière / petite	blanche / translucide	plate	régulier	lisse	
657	B	1	1	RS	régulière / petite	crème	plate	régulier	lisse / brillante	
658	B	1	1	RS	régulière / petite	crème	plate	régulier	lisse / brillante	
659	B	1	1	RS	régulière	crème	légèrement convexe	type actino	duveteuse blanche	
660	B	1	1	RS	régulière	crème	légèrement convexe	régulier	lisse / brillante	
661	B	2	1	RS	régulière / petite	blanche	légèrement convexe	régulier	lisse / brillante	
662	B	2	1	RS	régulière	crème	plate	régulier	cercles concentriques / brillante	
663	B	2	1	RS	régulière	crème	plate	régulier	étoile / brillante	
664	B	2	1	RS	type bacillus	-	-	-	-	
665	B	2	1	RS	régulière	crème	plate	régulier	lisse / brillante	
666	B	2	1	RS	régulière	crème	plate	régulier	lisse / brillante	
667	B	2	1	RS	régulière	crème	plate	régulier	lisse / brillante	

668	B	2	1	RS	régulière / petite	blanche / translucide	plate	régulier	lisse / brillante	
669	B	2	1	RS	régulière	jaune pâle / translucide	plate	diffus	lisse	
670	B	2	1	RS	régulière	blanche	plate	diffus	rugueuse	
671	B	2	1	RS	régulière	crème	convexe	régulier	lisse / brillante	
672	B	2	1	RS	régulière	crème	plate	régulier	lisse / brillante	
673	B	2	1	RS	régulière	crème	plate	régulier	lisse / brillante	
674	B	2	1	RS	régulière	crème	légèrement convexe	type actino	duveteuse blanche et tour brun	
675	B	2	1	RS	régulière	crème	légèrement convexe	régulier	lisse / brillante	
676	B	2	1	RS	régulière	crème	plate	régulier	lisse / brillante	
677	B	2	1	RS	régulière	crème	légèrement convexe	régulier	enfoncée au centre	
678	B	2	1	RS	régulière	crème	légèrement convexe	type actino	duveteuse blanche	brun
679	B	2	1	RS	régulière	blanche / translucide	plate	régulier	lisse / brillante	
680	B	2	1	RS	régulière	jaune pâle	plate	diffus	lisse / brillante	
681	B	2	2	RS	régulière	crème	plate	régulier	rugueuse / enfoncée au centre	
682	B	2	2	RS	régulière	crème	plate	régulier	rugueuse	
683	B	2	2	RS	régulière	crème	plate	régulier	rugueuse / brillante	
684	B	2	2	RS	régulière	crème	plate	régulier	lisse / brillante	
685	B	2	2	RS	régulière	crème	plate	régulier	rugueuse	
686	B	2	2	RS	régulière	blanche / translucide	plate	régulier	lisse / brillante	
687	B	2	2	RS	régulière	crème	légèrement convexe	régulier	lisse / brillante	
688	B	2	2	RS	irrégulière	crème	plate	diffus	lisse	
689	B	2	2	RS	régulière	crème	plate	diffus	lisse / brillante	
690	B	2	2	RS	régulière	crème	plate	diffus	lisse / brillante	
691	B	2	2	RS	type bacillus	-	-	-	-	
692	B	2	2	RS	irrégulière	blanche	plate	diffus	lisse	
693	B	2	2	RS	type bacillus	-	-	-	-	
694	B	2	2	RS	type bacillus	-	-	-	-	
695	B	2	2	RS	régulière	jaune	plate	régulier	lisse / brillante	
696	B	2	2	RS	régulière	crème	légèrement convexe	régulier	enfoncée au centre / brillante	
697	B	2	2	RS	régulière	crème	plate	régulier	rugueuse	
698	B	2	2	RS	régulière	crème	plate	régulier	rugueuse	
699	B	2	2	RS	régulière	blanche	plate	diffus	rugueuse	
700	B	2	2	RS	irrégulière / petite	transparente	plate	régulier	lisse / brillante	
701	B	2	3	RS	régulière / petite	blanche	plate	régulier	lisse / brillante	
702	B	2	3	RS	régulière	crème	plate	type actino / diffus	duveteuse blanche	
703	B	2	3	RS	régulière	crème	plate	type actino / diffus	rugueuse	
704	B	2	3	RS	régulière	crème	plate	type actino / diffus	duveteuse blanche	
705	B	2	3	RS	irrégulière	blanche	plate	diffus	rugueuse	
706	B	2	3	RS	irrégulière	blanche	plate	diffus	rugueuse	
707	B	2	3	RS	régulière	crème	plate	régulier	rugueuse	
708	B	2	3	RS	régulière	crème	plate	diffus	lisse	
709	B	2	3	RS	régulière / petite	blanche	plate	régulier	lisse / brillante	
710	B	2	3	RS	régulière / petite	blanche	plate	régulier	lisse / brillante	
711	B	2	3	RS	régulière	crème	légèrement convexe	type actino	duveteuse blanche	brun
712	B	2	3	RS	régulière	blanche / translucide	plate	régulier	lisse / brillante	
713	B	2	3	RS	régulière	crème	légèrement convexe	type actino	duveteuse blanche	
714	B	2	3	RS	régulière	blanche / translucide	plate	régulier	lisse / brillante	
715	B	2	3	RS	régulière	blanche	plate	régulier	lisse / brillante	

716	B	2	3	RS	régulière / petite	blanche / translucide	plate	régulier	lisse / brillante	
717	B	2	3	RS	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
718	B	2	3	RS	régulière / petite	blanche / translucide	plate	régulier	lisse / brillante	
719	B	2	3	RS	régulière	crème	plate	type actino / diffus	duveteuse blanche	brun
720	B	2	3	RS	régulière	crème	légèrement convexe	type actino	duveteuse blanche	brun
721	B	4	1	RS	régulière	crème	convexe	diffus	lisse / brillante	
722	B	4	1	RS	régulière	crème	plate	légèrement ondulé	lisse / brillante	
723	B	4	1	RS	régulière	crème	plate	diffus	lisse / brillante	
724	B	4	1	RS	régulière	crème	plate	légèrement ondulé	lisse	
725	B	4	1	RS	régulière	crème	plate	diffus	lisse / brillante	
726	B	4	1	RS	régulière	jaune pâle / translucide	légèrement convexe	ondulé	ombiliquée / brillante	
727	B	4	1	RS	irrégulière	jaune	plate	ondulé	lisse	
728	B	4	1	RS	type bacillus	-	-	-	-	
729	B	4	1	RS	régulière	crème	convexe	régulier	lisse / brillante	
730	B	4	1	RS	régulière	crème	légèrement convexe	diffus	lisse / brillante	
731	B	4	1	RS	régulière / petite	crème	légèrement convexe	régulier	lisse / brillante	
732	B	4	1	RS	régulière	crème	plate	diffus	cratériforme	
733	B	4	1	RS	régulière	crème	plate	diffus	cratériforme	
734	B	4	1	RS	régulière	crème	plate	diffus	cratériforme	
735	B	4	1	RS	régulière	crème	plate	diffus	cratériforme	
736	B	4	1	RS	régulière	crème	plate	diffus	cratériforme	
737	B	4	1	RS	régulière	blanche	légèrement convexe	ondulé	lisse / brillante	
738	B	4	1	RS	régulière	crème	plate	diffus	cratériforme	
739	B	4	1	RS	régulière	crème	plate	type actino	duveteuse blanche et tour brun	
740	B	4	1	RS	régulière	crème	plate	diffus	rugueuse	
741	B	4	2	RS	régulière / petite	transparente	plate	régulier	lisse	
742	B	4	2	RS	régulière	crème	légèrement convexe	type actino	duveteuse blanche	
743	B	4	2	RS	régulière	crème	légèrement convexe	régulier	lisse / brillante	
744	B	4	2	RS	régulière / petite	transparente	plate	régulier	lisse	
745	B	4	2	RS	irrégulière	jaune / translucide	plate	envahissant	rugueuse / brillante	
746	B	4	2	RS	régulière	crème	légèrement convexe	diffus	ombiliquée	
747	B	4	2	RS	irrégulière	crème	plate	envahissant / ondulé	lisse / brillante	
748	B	4	2	RS	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
749	B	4	2	RS	régulière	blanche	plate	diffus	lisse	
750	B	4	2	RS	régulière	crème	plate	diffus	lisse / brillante	
751	B	4	2	RS	régulière	crème	convexe	régulier	lisse / brillante	
752	B	4	2	RS	régulière	crème	plate	régulier	lisse / brillante	
753	B	4	2	RS	régulière	crème	légèrement convexe	type actino	duveteuse brune	
754	B	4	2	RS	régulière	orange	plate	régulier	lisse / brillante	
755	B	4	2	RS	régulière	blanche	convexe	régulier	lisse / brillante	
756	B	4	2	RS	régulière	blanche	légèrement convexe	régulier	ombiliquée / brillante	
757	B	4	2	RS	régulière	transparente	plate	régulier	lisse / brillante	
758	B	4	2	RS	régulière	crème	légèrement convexe	type actino	duveteuse brune	
759	B	4	2	RS	type bacillus	-	-	-	-	
760	B	4	2	RS	régulière	jaune	légèrement convexe	régulier	lisse / brillante	
761	B	4	3	RS	régulière	jaune	légèrement convexe	régulier	lisse	
762	B	4	3	RS	régulière	blanche	légèrement convexe	régulier	lisse	
763	B	4	3	RS	régulière	blanche	légèrement convexe	régulier	rugueuse / brillante	

764	B	4	3	RS	régulière	jaune / translucide	légèrement convexe	régulier	lisse / brillante	
765	B	4	3	RS	régulière	blanche	convexe	régulier	lisse / brillante	
766	B	4	3	RS	régulière	saumon	légèrement convexe	ondulé	ombiliquée / brillante	
767	B	4	3	RS	régulière	jaune	légèrement convexe	diffus	lisse / brillante	
768	B	4	3	RS	irrégulière	transparente	plate	régulier	lisse / brillante	
769	B	4	3	RS	régulière	blanche	légèrement convexe	diffus	ombiliquée / brillante	
770	B	4	3	RS	régulière	crème	plate	renflé	cercles concentriques / brillante	
771	B	4	3	RS	régulière	transparente	légèrement convexe	régulier	cratériforme	
772	B	4	3	RS	irrégulière	transparente	plate	diffus	lisse / brillante	
773	B	4	3	RS	régulière	crème	convexe	régulier	lisse / brillante	
774	B	4	3	RS	régulière	blanche	plate	régulier	lisse / brillante	
775	B	4	3	RS	régulière	blanche	plate	régulier	lisse / brillante	
776	B	4	3	RS	régulière	crème	légèrement convexe	régulier	lisse / brillante	
777	B	4	3	RS	irrégulière	transparente	légèrement convexe	régulier	"läkerol" / rugueuse	
778	B	4	3	RS	régulière	jaune	plate	régulier	lisse / brillante	
779	B	4	3	RS	régulière	transparente	plate	diffus	rugueuse	
780	B	4	3	RS	irrégulière	blanche	légèrement convexe	ondulé	ombiliquée / brillante	
781	B	5	1	RS	régulière	blanche	plate	diffus	rugueuse	
782	B	5	1	RS	irrégulière	transparente	plate	diffus	rugueuse/mat	
783	B	5	1	RS	régulière	blanche	plate	régulier	lisse	
784	B	5	1	RS	régulière	blanche	plate	régulier	rugueuse / brillante	
785	B	5	1	RS	irrégulière	blanche	plate	ondulé	rugueuse / brillante	
786	B	5	1	RS	irrégulière	transparente	plate	diffus	rugueuse/mat	
787	B	5	1	RS	régulière	blanche	plate	régulier	lisse / brillante	
788	B	5	1	RS	irrégulière	crème	ombiliqué	régulier	lisse/brillante	
789	B	5	1	RS	régulière	blanche	plate	renflé	lisse	
790	B	5	1	RS	irrégulière	crème	plate	régulier	cercles concentriques/lisse	
791	B	5	1	RS	régulière / petite	transparente	plate	régulier	lisse	
792	B	5	1	RS	régulière	blanche	plate	régulier	lisse/brillante	
793	B	5	1	RS	régulière	blanche	plate	diffus	rugueuse	
794	B	5	1	RS	régulière	brun	légèrement convexe	régulier	mat	
795	B	5	1	RS	régulière	blanche	plate	diffus	lisse / brillante	
796	B	5	1	RS	régulière	transparente	plate	régulier	lisse / brillante	
797	B	5	1	RS	régulière	blanche	plate	régulier	rugueuse/mate	
798	B	5	1	RS	type bacillus	jaune			brillante	
799	B	5	1	RS	type bacillus	-	-	-	-	
800	B	5	1	RS	régulière	blanche	plate	régulier	rugueuse	
801	B	5	2	RS	régulière / petite	transparente	plate	régulier	lisse / brillante	
802	B	5	2	RS	régulière	blanche	plate	diffus	rugueuse/mat	
803	B	5	2	RS	régulière	crème	plate	régulier	rugueuse/mat	
804	B	5	2	RS	régulière	blanche	légèrement convexe	régulier	ombiliquée / brillante	
805	B	5	2	RS	régulière	blanche	convexe	régulier	aplatie au centre / brillante	
806	B	5	2	RS	régulière	blanche	plate	régulier	lisse / brillante	
807	B	5	2	RS	régulière	blanche	plate	régulier	rugueuse/mate	
808	B	5	2	RS	irrégulière	crème	plate	régulier	lisse/brillante	
809	B	5	2	RS	régulière	blanche	plate	régulier	rugeuse/mat	
810	B	5	2	RS	régulière	blanche	convexe	régulier	aplatie au centre / brillante	
811	B	5	2	RS	régulière	jaune	plate	régulier	lisse/brillante	

812	B	5	2	RS	MANQUE					
813	B	5	2	RS	régulière	blanche	convexe	régulier	aplatie au centre / brillante	
814	B	5	2	RS	régulière	blanche	légèrement convexe	légèrement ondulé	rugueuse/mate	
815	B	5	2	RS	régulière	blanche	convexe	régulier	aplatie au centre / brillante	
816	B	5	2	RS	régulière	crème	plate	régulier	brillante/rugueuse	
817	B	5	2	RS	régulière	crème	plate	régulier	rugueuse/mate	
818	B	5	2	RS	régulière	blanche	convexe	régulier	aplatie au centre / brillante	
819	B	5	2	RS	régulière	blanche	convexe	régulier	lisse / brillante	
820	B	5	2	RS	régulière	blanche	plate	régulier	lisse / brillante	
821	B	5	3	RS	régulière	blanche	légèrement convexe	diffus	lisse / brillante	
822	B	5	3	RS	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
823	B	5	3	RS	régulière / petite	transparente	plate	régulier	lisse	
824	B	5	3	RS	régulière	blanche	convexe	régulier	aplatie au centre / brillante	
825	B	5	3	RS	régulière	blanche	convexe	régulier	aplatie au centre / brillante	
826	B	5	3	RS	régulière	crème	légèrement convexe	légèrement ondulé	rugeuse/mat	
827	B	5	3	RS	régulière	crème	plate	régulier	mat/rugueuse	
828	B	5	3	RS	régulière	jaune	plate	régulier	lisse/brillante	
829	B	5	3	RS	régulière	blanche	plate	régulier	lisse / brillante	
830	B	5	3	RS	régulière	transparente	plate	régulier	lisse/brillante	
831	B	5	3	RS	irrégulière	blanche	plate	régulier	rugueuse / brillante	
832	B	5	3	RS	régulière	blanche	convexe	régulier	aplatie au centre / brillante	
833	B	5	3	RS	régulière	blanche	plate	régulier	rugueuse/mate	
834	B	5	3	RS	régulière	blanche	convexe	régulier	aplatie au centre / brillante	
835	B	5	3	RS	régulière	blanche	plate	régulier	rugueuse/mat	
836	B	5	3	RS	régulière	blanche	convexe	régulier	lisse / brillante	
837	B	5	3	RS	irrégulière	blanche	plate	régulier	rugueuse / brillante	
838	B	5	3	RS	régulière	blanche	convexe	régulier	lisse / brillante	
839	B	5	3	RS	régulière	blanche	plate	régulier	rugueuse / brillante	
840	B	5	3	RS	régulière	blanche	convexe	régulier	lisse / brillante	
841	C	1	1	R	régulière	saumon	légèrement convexe	régulier	lisse / brillante	
842	C	1	1	R	régulière / petite	jaune	plate	régulier	lisse / brillante	
843	C	1	1	R	régulière	ocre	légèrement convexe	régulier	lisse / brillante	
844	C	1	1	R	régulière	blanche	plate	régulier	ombiliquée	
845	C	1	1	R	régulière	crème	légèrement convexe	type actino	rugueuse	jaunâtre
846	C	1	1	R	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
847	C	1	1	R	régulière	orange	légèrement convexe	régulier	lisse / brillante	
848	C	1	1	R	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
849	C	1	1	R	irrégulière	crème / translucide	légèrement convexe	régulier	rugueuse	
850	C	1	1	R	régulière	crème	légèrement convexe	type actino	rugueuse	brun
851	C	1	1	R	régulière / petite	jaune	plate	régulier	lisse / brillante	
852	C	1	1	R	régulière / petite	blanche	plate	régulier	lisse / brillante	
853	C	1	1	R	régulière	blanche	légèrement convexe	diffus	rugueuse	
854	C	1	1	R	irrégulière	blanche	plate	diffus	rugueuse	
855	C	1	1	R	régulière	jaune / translucide	plate	régulier	lisse / brillante	
856	C	1	1	R	régulière	crème	légèrement convexe	régulier	lisse / brillante	
857	C	1	1	R	régulière	crème	légèrement convexe	type actino	duveteuse blanche	jaune
858	C	1	1	R	régulière	jaune	légèrement convexe	régulier	lisse / brillante	
859	C	1	1	R	régulière	blanche	légèrement convexe	régulier	lisse / brillante	

860	C	1	1	R	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
861	C	1	1	R	régulière	blanche	légèrement convexe	régulier	lisse	
862	C	1	1	R	type bacillus	-	-	-	-	
863	C	1	1	R	type bacillus	-	-	-	-	
864	C	1	1	R	régulière	blanche	plate	régulier	lisse	
865	C	1	1	R	régulière / petite	jaune / translucide	plate	régulier	lisse / brillante	
866	C	1	1	R	type bacillus	-	-	-	-	
867	C	1	1	R	régulière	crème	légèrement convexe	type actino	rugueuse	
868	C	1	1	R	régulière / petite	blanche / translucide	plate	régulier	lisse / brillante	
869	C	1	1	R	régulière	orange	légèrement convexe	régulier	lisse / brillante	
870	C	1	1	R	type bacillus	-	-	-	-	
871	C	2	1	R	irrégulière	blanche	légèrement convexe	régulier	lisse / brillante	jaune
872	C	2	1	R	régulière / petite	blanche	convexe	régulier	lisse / brillante	
873	C	2	1	R	régulière / petite	blanche	convexe	régulier	lisse / brillante	
874	C	2	1	R	régulière / petite	blanche / translucide	plate	régulier	lisse / brillante	
875	C	2	1	R	régulière / petite	crème	convexe	régulier	lisse / brillante	
876	C	2	1	R	régulière	jaune	légèrement convexe	régulier	lisse / brillante	
877	C	2	1	R	régulière / petite	crème	plate	régulier	lisse / brillante	
878	C	2	1	R	irrégulière	jaune / translucide	plate	diffus	rugueuse	
879	C	2	1	R	régulière / petite	crème	plate	régulier	lisse / brillante	
880	C	2	1	R	régulière / petite	blanche / translucide	plate	régulier	lisse / brillante	
881	C	2	1	R	irrégulière	crème	plate	régulier	lisse / brillante	
882	C	2	1	R	régulière	jaune	légèrement convexe	régulier	lisse / brillante	
883	C	2	1	R	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
884	C	2	1	R	régulière	crème	légèrement convexe	type actino	duveteuse blanc	
885	C	2	1	R	régulière	crème	légèrement convexe	type actino	rugueuse	
886	C	2	1	R	régulière / petite	jaune pâle	légèrement convexe	régulier	lisse / brillante	
887	C	2	1	R	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
888	C	2	1	R	régulière	blanche	convexe	régulier	lisse / brillante	
889	C	2	1	R	régulière / petite	blanche	plate	régulier	lisse / brillante	
890	C	2	1	R	régulière	jaune	légèrement convexe	régulier	lisse / brillante	
891	C	2	2	R	régulière / petite	blanche	légèrement convexe	régulier	lisse / brillante	
892	C	2	2	R	régulière	orange	convexe	régulier	lisse / brillante	
893	C	2	2	R	régulière	orange	légèrement convexe	régulier	lisse / brillante	
894	C	2	2	R	régulière	orange	légèrement convexe	diffus	lisse / brillante	
895	C	2	2	R	irrégulière	jaune / translucide	plate	régulier	rugueuse / brillante	
896	C	2	2	R	régulière	orange	légèrement convexe	diffus	lisse / brillante	
897	C	2	2	R	régulière	crème	convexe	régulier	lisse / brillante	
898	C	2	2	R	régulière	crème	convexe	régulier	lisse / brillante	
899	C	2	2	R	irrégulière	jaune / translucide	plate	régulier	lisse / brillante	
900	C	2	2	R	régulière	orange	légèrement convexe	régulier	lisse / brillante	
901	C	2	2	R	irrégulière	jaune / translucide	plate	régulier	rugueuse / brillante	
902	C	2	2	R	régulière / petite	jaune / translucide	plate	régulier	lisse / brillante	
903	C	2	2	R	régulière	ocre	légèrement convexe	régulier	lisse / brillante	
904	C	2	2	R	régulière / petite	orange	plate	régulier	lisse / brillante	
905	C	2	2	R	irrégulière	jaune / translucide	plate	régulier	rugueuse / brillante	
906	C	2	2	R	régulière	ocre	plate	régulier	lisse / brillante	
907	C	2	2	R	régulière / petite	blanche	plate	régulier	lisse / brillante	

908	C	2	2	R	régulière	crème	légèrement convexe	régulier	lisse / brillante	
909	C	2	2	R	régulière	jaune	convexe	régulier	lisse / brillante	
910	C	2	2	R	irrégulière	jaune / translucide	plate	régulier	rugueuse / brillante	
911	C	2	3	R	régulière	jaune / translucide	plate	diffus	lisse / brillante	
912	C	2	3	R	régulière / petite	transparente	plate	régulier	lisse / brillante	
913	C	2	3	R	irrégulière	jaune / translucide	plate	diffus	rugueuse	
914	C	2	3	R	régulière	blanche / translucide	plate	régulier	lisse / brillante	
915	C	2	3	R	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
916	C	2	3	R	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
917	C	2	3	R	irrégulière	jaune / translucide	plate	diffus	rugueuse / brillante	
918	C	2	3	R	régulière	jaune	légèrement convexe	diffus	lisse / brillante	
919	C	2	3	R	régulière / petite	blanche	plate	régulier	lisse / brillante	
920	C	2	3	R	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
921	C	2	3	R	irrégulière	jaune	plate	diffus	rugueuse / brillante	
922	C	2	3	R	irrégulière	jaune	plate	diffus	rugueuse / brillante	
923	C	2	3	R	régulière	jaune	légèrement convexe	régulier	lisse / brillante	
924	C	2	3	R	régulière	jaune	légèrement convexe	régulier	lisse / brillante	
925	C	2	3	R	irrégulière	jaune	plate	diffus	rugueuse / brillante	
926	C	2	3	R	irrégulière	jaune pâle	plate	diffus	lisse / brillante	
927	C	2	3	R	irrégulière	jaune	plate	diffus	rugueuse / brillante	
928	C	2	3	R	régulière	jaune / translucide	légèrement convexe	diffus	lisse / brillante	
929	C	2	3	R	irrégulière	jaune	plate	diffus	rugueuse / brillante	
930	C	2	3	R	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
931	C	4	1	R	régulière	jaune-orangé / translucide	légèrement convexe	régulier	lisse / brillante	
932	C	4	1	R	régulière	crème / translucide	plate	renflé	rugueuse	
933	C	4	1	R	régulière	jaune-orangé / translucide	légèrement convexe	régulier	lisse / brillante	
934	C	4	1	R	régulière	jaune pâle / translucide	plate	diffus	lisse / brillante	
935	C	4	1	R	régulière	crème	plate	régulier	lisse / brillante	
936	C	4	1	R	régulière	crème	légèrement convexe	régulier	lisse / brillante	
937	C	4	1	R	régulière / petite	orange	plate	régulier	lisse / brillante	
938	C	4	1	R	régulière	rouge rosé	plate	diffus	lisse / brillante	
939	C	4	1	R	régulière	crème jaunâtre	légèrement convexe	régulier	enfoncée au centre / brillante	
940	C	4	1	R	régulière	crème	plate	régulier	lisse / brillante	
941	C	4	1	R	régulière	crème	plate	diffus	lisse / brillante	
942	C	4	1	R	régulière	transparente	plate	régulier	lisse / brillante	
943	C	4	1	R	irrégulière	jaune pâle / translucide	plate	régulier	rugueuse / brillante	
944	C	4	1	R	régulière	jaune-orangé / translucide	légèrement convexe	régulier	lisse / brillante	
945	C	4	1	R	régulière	jaune pâle / translucide	plate	régulier	lisse / brillante	
946	C	4	1	R	régulière	transparente	plate	diffus	lisse / brillante	
947	C	4	1	R	irrégulière	jaune-orangé / translucide	plate	régulier	rugueuse	
948	C	4	1	R	régulière	crème	légèrement convexe	régulier	lisse / brillante	
949	C	4	1	R	régulière	crème	plate	régulier	lisse / brillante	
950	C	4	1	R	régulière	jaune pâle / translucide	plate	diffus	lisse / brillante	
951	C	4	2	R	régulière	jaune	plate	diffus	lisse / brillante	
952	C	4	2	R	régulière	crème	légèrement convexe	diffus	lisse / brillante	
953	C	4	2	R	régulière	jaune	légèrement convexe	diffus	lisse / brillante	
954	C	4	2	R	régulière	rouge rosé	plate	diffus	lisse / brillante	
955	C	4	2	R	régulière	crème / translucide	plate	régulier	lisse / brillante	

956	C	4	2	R	régulière	crème	convexe	régulier	lisse / brillante	
957	C	4	2	R	régulière	crème / translucide	plate	renflé	lisse / brillante	jaune
958	C	4	2	R	régulière	crème / translucide	plate	renflé	lisse / brillante	jaune
959	C	4	2	R	régulière	rose	légèrement convexe	diffus	lisse / brillante	
960	C	4	2	R	régulière	crème	plate	renflé / envahissant	rugueuse / brillante	
961	C	4	2	R	régulière	crème / translucide	plate	renflé	lisse / brillante	jaune
962	C	4	2	R	régulière	jaune	plate	diffus	lisse / brillante	
963	C	4	2	R	régulière	orange / translucide	plate	envahissant /diffus	rugueuse / brillante	
964	C	4	2	R	régulière	jaune	plate	diffus	lisse / brillante	
965	C	4	2	R	régulière	crème / translucide	plate	renflé	lisse / brillante	jaune
966	C	4	2	R	régulière	crème	plate	ondulé / diffus	lisse / brillante	
967	C	4	2	R	régulière	orange / translucide	plate	envahissant /diffus	rugueuse / brillante	
968	C	4	2	R	régulière	jaune	plate	diffus	lisse / brillante	
969	C	4	2	R	régulière	crème	légèrement convexe	diffus	lisse / brillante	
970	C	4	2	R	régulière	jaune-orangé / translucide	plate	envahissant /diffus	rugueuse / brillante	
971	C	4	3	R	régulière	crème / translucide	plate	renflé	lisse / brillante	jaune
972	C	4	3	R	régulière	jaune-orangé / translucide	plate	envahissant /diffus	rugueuse / brillante	
973	C	4	3	R	régulière	crème / translucide	plate	régulier	lisse / brillante	
974	C	4	3	R	régulière	jaune-orangé / translucide	plate	envahissant /diffus	rugueuse / brillante	
975	C	4	3	R	régulière	jaune-orangé / translucide	plate	envahissant /diffus	rugueuse / brillante	
976	C	4	3	R	régulière	jaune-orangé / translucide	plate	envahissant /diffus	rugueuse / brillante	
977	C	4	3	R	régulière	jaune-orangé / translucide	plate	envahissant /diffus	rugueuse / brillante	
978	C	4	3	R	régulière	crème / translucide	plate	régulier	lisse / brillante	
979	C	4	3	R	régulière	jaune-orangé / translucide	plate	envahissant /diffus	rugueuse / brillante	
980	C	4	3	R	régulière	jaune-orangé / translucide	plate	envahissant /diffus	rugueuse / brillante	
981	C	4	3	R	régulière	jaune-orangé / translucide	plate	envahissant /diffus	rugueuse / brillante	
982	C	4	3	R	régulière	jaune	légèrement convexe	régulier	lisse / brillante	
983	C	4	3	R	régulière	jaune / translucide	légèrement convexe	régulier	lisse / brillante	
984	C	4	3	R	régulière	jaune / translucide	légèrement convexe	régulier	lisse / brillante	
985	C	4	3	R	régulière	jaune / translucide	légèrement convexe	régulier	lisse / brillante	
986	C	4	3	R	régulière	crème	plate	ondulé / diffus	lisse / brillante	
987	C	4	3	R	régulière	ocre	convexe	régulier	rugueuse (läkerol)	
988	C	4	3	R	régulière	crème	plate	ondulé / diffus	rugueuse	
989	C	4	3	R	régulière	crème / translucide	plate	renflé	lisse / brillante	jaune
990	C	4	3	R	régulière	crème / translucide	plate	renflé	lisse / brillante	jaune
991	C	5	1	R	régulière	jaune pâle / translucide	plate	diffus	cercles concentriques / brillante	
992	C	5	1	R	régulière / petite	transparente	plate	ondulé	lisse / brillante	
993	C	5	1	R	régulière	crème / translucide	plate	ondulé	lisse / brillante	
994	C	5	1	R	régulière / petite	ocre	légèrement convexe	régulier	lisse / brillante	
995	C	5	1	R	régulière	crème	plate	régulier	lisse / brillante	jaune
996	C	5	1	R	régulière	crème	convexe	régulier	lisse / brillante	
997	C	5	1	R	régulière	crème	convexe	régulier	lisse / brillante	
998	C	5	1	R	régulière	ocre	convexe	régulier	enfoncée au centre / brillante	
999	C	5	1	R	régulière	jaune-orangé / translucide	plate	envahissant / diffus	rugueuse / brillante	
1000	C	5	1	R	régulière	crème	convexe	régulier	lisse / brillante	
1001	C	5	1	R	régulière / petite	transparente	plate	régulier	lisse / brillante	
1002	C	5	1	R	régulière / petite	crème / translucide	plate	renflé	rugueuse	
1003	C	5	1	R	régulière	crème	convexe	ondulé	rugueuse (läkerol)	

1004	C	5	1	R	régulière	crème	légèrement convexe	régulier	rugueuse	
1005	C	5	1	R	régulière / petite	crème	plate	régulier	lisse / brillante	
1006	C	5	1	R	régulière / petite	transparente	plate	régulier	lisse / brillante	
1007	C	5	1	R	régulière	jaune-orangé / translucide	plate	envahissant / diffus	rugueuse / brillante	
1008	C	5	1	R	régulière	jaune-orangé / translucide	plate	envahissant / diffus	rugueuse / brillante	
1009	C	5	1	R	régulière	jaune-orangé / translucide	plate	envahissant / diffus	rugueuse / brillante	
1010	C	5	1	R	régulière / petite	crème / translucide	plate	renflé	rugueuse	
1011	C	5	2	R	régulière / petite	transparente	plate	ondulé	lisse / brillante	
1012	C	5	2	R	régulière	crème (bords) brun (centre)	plate ondulée	type actino / renflé	enfoncée au centre / rugueuse	
1013	C	5	2	R	régulière / petite	crème / translucide	plate	ondulé	lisse / brillante	
1014	C	5	2	R	régulière / petite	transparente	plate	régulier	lisse / brillante	
1015	C	5	2	R	régulière	crème	plate	régulier	cercles concentriques / opaque	
1016	C	5	2	R	régulière	jaune / translucide	plate	régulier	lisse / brillante	
1017	C	5	2	R	régulière	jaune / translucide	plate	envahissant	lisse / brillante	
1018	C	5	2	R	régulière	crème	plate	régulier	cercles concentriques / opaque	
1019	C	5	2	R	régulière	transparente	plate	régulier	lisse / brillante	
1020	C	5	2	R	régulière	transparente	plate	régulier	cercles concentriques / opaque	
1021	C	5	2	R	régulière	crème	plate	ondulé	lisse / brillante	
1022	C	5	2	R	régulière	transparente	plate	régulier	cercles concentriques / opaque	
1023	C	5	2	R	régulière	crème	plate	ondulé	lisse / brillante	
1024	C	5	2	R	régulière	transparente	plate	régulier	lisse / brillante	
1025	C	5	2	R	régulière / petite	jaune	légèrement convexe	régulier	lisse / brillante	
1026	C	5	2	R	irrégulière	jaune-orangé / translucide	plate	régulier	rugueuse	
1027	C	5	2	R	régulière / petite	transparente	plate	régulier	lisse / brillante	
1028	C	5	2	R	régulière	crème	convexe	régulier	lisse / brillante	
1029	C	5	2	R	régulière	transparente	plate	renflé	lisse	
1030	C	5	2	R	régulière	transparente	plate	renflé	lisse	
1031	C	5	3	R	régulière	crème / translucide	plate	ondulé	rugueuse	
1032	C	5	3	R	régulière / petite	crème / translucide	plate	régulier	lisse / brillante	
1033	C	5	3	R	régulière	ocre	plate	envahissant / diffus	lisse / brillante	
1034	C	5	3	R	irrégulière	transparente	plate	diffus	rugueuse	
1035	C	5	3	R	régulière / petite	transparente	plate	régulier	lisse / brillante	
1036	C	5	3	R	irrégulière	transparente	plate	diffus	rugueuse	
1037	C	5	3	R	régulière	ocre	plate	renflé / ondulé	rugueuse	
1038	C	5	3	R	régulière / petite	crème / translucide	plate	régulier	lisse / brillante	
1039	C	5	3	R	irrégulière	transparente	plate	diffus	rugueuse	
1040	C	5	3	R	régulière	orange	légèrement convexe	régulier	lisse / brillante	
1041	C	5	3	R	irrégulière	transparente	plate	diffus	rugueuse	
1042	C	5	3	R	irrégulière	transparente	plate	diffus	rugueuse	
1043	C	5	3	R	régulière	jaune pâle / translucide	plate	régulier	lisse / brillante	
1044	C	5	3	R	régulière	jaune -orangé	légèrement convexe	régulier	lisse / brillante	
1045	C	5	3	R	irrégulière	transparente	plate	diffus	rugueuse	
1046	C	5	3	R	irrégulière	transparente	plate	diffus	rugueuse	
1047	C	5	3	R	régulière	crème	légèrement convexe	diffus	lisse	
1048	C	5	3	R	régulière	crème	plate	ondulé	rugueuse	
1049	C	5	3	R	irrégulière	transparente	plate	diffus	rugueuse	
1050	C	5	3	R	régulière	crème	convexe	ondulé	lisse	
1051	C	1	1	RS	régulière	blanche	légèrement convexe	régulier	lisse / brillante	

1052	C	1	1	RS	régulière	blanche	plate	régulier	cercles concentriques	
1053	C	1	1	RS	régulière	ocre	plate	diffus	lisse / brillante	
1054	C	1	1	RS	régulière	orange	plate	régulier	lisse / brillante	
1055	C	1	1	RS	régulière	crème	légèrement convexe	type actino	rugueuse	jaune
1056	C	1	1	RS	régulière	jaune	plate	régulier	lisse / brillante	
1057	C	1	1	RS	régulière	jaune	plate	régulier	lisse / brillante	
1058	C	1	1	RS	régulière	orange	légèrement convexe	régulier	lisse / brillante	
1059	C	1	1	RS	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
1060	C	1	1	RS	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
1061	C	1	1	RS	régulière / petite	blanche	plate	régulier	lisse / brillante	
1062	C	1	1	RS	régulière / petite	blanche	plate	régulier	lisse / brillante	
1063	C	1	1	RS	régulière	crème	légèrement convexe	régulier	ombiliquée	
1064	C	1	1	RS	régulière / petite	blanche	plate	régulier	lisse / brillante	
1065	C	1	1	RS	régulière	orange	légèrement convexe	régulier	lisse / brillante	
1066	C	1	1	RS	régulière	blanche	plate	régulier	rugueuse	
1067	C	1	1	RS	régulière	jaune / translucide	plate	régulier	lisse / brillante	
1068	C	1	1	RS	régulière	blanche	plate	régulier	lisse / brillante	
1069	C	1	1	RS	régulière	blanche	plate	régulier	rugueuse / brillante	
1070	C	1	1	RS	régulière	saumon	légèrement convexe	régulier	lisse / brillante	
1071	C	1	1	RS	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
1072	C	1	1	RS	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
1073	C	1	1	RS	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
1074	C	1	1	RS	régulière	blanche	plate	régulier	lisse / brillante	
1075	C	1	1	RS	régulière	orange	légèrement convexe	régulier	lisse / brillante	
1076	C	1	1	RS	régulière	blanche	plate	régulier	lisse / brillante	
1077	C	1	1	RS	régulière	crème	légèrement convexe	type actino	rugueuse	
1078	C	1	1	RS	irrégulière	jaune / translucide	plate	régulier	rugueuse / brillante	
1079	C	1	1	RS	type bacillus	-	-	-	-	
1080	C	1	1	RS	régulière	blanche	plate	régulier	lisse / brillante	
1081	C	2	1	RS	régulière	crème	légèrement convexe	type actino	duveteuse brune	
1082	C	2	1	RS	irrégulière	blanche	plate	envahissant / diffus	lisse / brillante	
1083	C	2	1	RS	régulière	crème	plate	régulier	lisse / brillante	
1084	C	2	1	RS	régulière	crème	légèrement convexe	régulier	lisse / brillante	
1085	C	2	1	RS	régulière	blanche	plate	régulier	lisse / brillante	
1086	C	2	1	RS	régulière / petite	jaune pâle	plate	régulier	lisse / brillante	
1087	C	2	1	RS	régulière / petite	blanche	plate	régulier	lisse / brillante	
1088	C	2	1	RS	régulière	crème	plate	régulier	lisse / brillante	
1089	C	2	1	RS	régulière / petite	jaune pâle	plate	régulier	lisse / brillante	
1090	C	2	1	RS	régulière	crème	convexe	régulier	lisse / brillante	
1091	C	2	1	RS	type bacillus	-	-	-	-	
1092	C	2	1	RS	irrégulière	crème	plate	régulier	lisse / brillante	
1093	C	2	1	RS	régulière	crème	légèrement convexe	type actino	rugueuse	brun
1094	C	2	1	RS	régulière	crème	convexe	régulier	lisse / brillante	
1095	C	2	1	RS	irrégulière	jaune / translucide	plate	régulier	lisse / brillante	
1096	C	2	1	RS	régulière	blanche	convexe	régulier	lisse / brillante	
1097	C	2	1	RS	régulière	crème	plate	diffus	lisse / brillante	
1098	C	2	1	RS	régulière	crème	légèrement convexe	diffus	lisse / brillante	
1099	C	2	1	RS	régulière	crème	plate	régulier	rugueuse	

1100	C	2	1	RS	type bacillus	-	-	-	-	
1101	C	2	2	RS	régulière / petite	blanche / translucide	plate	régulier	lisse / brillante	
1102	C	2	2	RS	régulière	jaune pâle	plate	régulier	rugueuse / brillante	
1103	C	2	2	RS	irrégulière	jaune / translucide	plate	diffus	lisse / brillante	
1104	C	2	2	RS	irrégulière	jaune / translucide	plate	diffus	lisse / brillante	
1105	C	2	2	RS	régulière	blanche	plate	régulier	lisse / brillante	
1106	C	2	2	RS	irrégulière	jaune / translucide	plate	envahissant	rugueuse / brillante	
1107	C	2	2	RS	irrégulière	jaune / translucide	plate	diffus	lisse / brillante	
1108	C	2	2	RS	irrégulière	orange-rosé / translucide	plate	diffus	lisse / brillante	
1109	C	2	2	RS	irrégulière	jaune / translucide	plate	diffus	lisse / brillante	
1110	C	2	2	RS	régulière	jaune	plate	régulier	lisse / brillante	
1111	C	2	2	RS	régulière	blanche	plate	régulier	lisse / brillante	
1112	C	2	2	RS	irrégulière	jaune / translucide	plate	diffus	lisse / brillante	
1113	C	2	2	RS	irrégulière	jaune / translucide	plate	diffus	lisse / brillante	
1114	C	2	2	RS	régulière	crème	plate	régulier	lisse	
1115	C	2	2	RS	régulière	crème	plate	régulier	lisse / brillante	
1116	C	2	2	RS	régulière	crème	plate	régulier	cercles concentriques / rugueuse	
1117	C	2	2	RS	régulière	ocre	plate	régulier	lisse / brillante	
1118	C	2	2	RS	régulière	jaune	légèrement convexe	régulier	lisse / brillante	jaune
1119	C	2	2	RS	régulière	blanche / translucide	plate	régulier	lisse / brillante	
1120	C	2	2	RS	irrégulière	crème	plate	diffus	rugueuse	
1121	C	2	3	RS	régulière	blanche	plate	régulier	cercles concentriques	
1122	C	2	3	RS	type bacillus	-	-	-	-	
1123	C	2	3	RS	irrégulière	blanche	plate	diffus	lisse	
1124	C	2	3	RS	régulière / petite	blanche	plate	régulier	lisse	
1125	C	2	3	RS	régulière	jaune	légèrement convexe	régulier	lisse / brillante	
1126	C	2	3	RS	régulière	blanche	convexe		lisse / brillante	
1127	C	2	3	RS	type bacillus	-	-	-	-	
1128	C	2	3	RS	régulière	blanche	plate	régulier	lisse	
1129	C	2	3	RS	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
1130	C	2	3	RS	régulière	jaune / translucide	plate	régulier	lisse / brillante	
1131	C	2	3	RS	régulière	crème	plate	régulier	lisse / brillante	
1132	C	2	3	RS	régulière	crème	légèrement convexe	régulier	lisse / brillante	
1133	C	2	3	RS	régulière	crème	plate	régulier	cercles concentriques	
1134	C	2	3	RS	régulière	jaune / translucide	plate	régulier	lisse / brillante	
1135	C	2	3	RS	régulière / petite	blanche / translucide	plate	régulier	lisse / brillante	
1136	C	2	3	RS	irrégulière	jaune	plate	diffus	lisse / brillante	
1137	C	2	3	RS	irrégulière	jaune	plate	diffus	lisse / brillante	
1138	C	2	3	RS	irrégulière	blanche	légèrement convexe	régulier	lisse / brillante	
1139	C	2	3	RS	irrégulière	jaune	plate	diffus	lisse / brillante	
1140	C	2	3	RS	régulière	blanche	plate	régulier	lisse / brillante	
1141	C	4	1	RS	régulière / petite	transparente	plate	régulier	lisse / brillante	
1142	C	4	1	RS	régulière	crème	légèrement convexe	diffus	lisse / brillante	
1143	C	4	1	RS	régulière	crème	légèrement convexe	régulier	lisse / brillante	
1144	C	4	1	RS	régulière	crème	plate	régulier	rugueuse	
1145	C	4	1	RS	régulière	transparente	plate	régulier	rugueuse / brillante	
1146	C	4	1	RS	régulière	transparente	plate	régulier	rugueuse / brillante	
1147	C	4	1	RS	régulière	crème	légèrement convexe	régulier	lisse / brillante	

1148	C	4	1	RS	régulière	blanche	plate	régulier	lisse / brillante	
1149	C	4	1	RS	irrégulière	crème	légèrement convexe	régulier	lisse / brillante	
1150	C	4	1	RS	régulière	jaune / translucide	convexe	régulier	lisse / brillante	
1151	C	4	1	RS	régulière	jaune / translucide	légèrement convexe	diffus	lisse / brillante	
1152	C	4	1	RS	régulière	crème	plate	diffus	lisse / brillante	
1153	C	4	1	RS	type bacillus	-	-	-	-	
1154	C	4	1	RS	régulière	transparente	plate	régulier	lisse / brillante	
1155	C	4	1	RS	régulière	blanche	convexe	régulier	aplatie au centre / brillante	
1156	C	4	1	RS	irrégulière	blanche	légèrement convexe	ondulé	ombiliquée / brillante	
1157	C	4	1	RS	irrégulière	blanche	plate	diffus	rugueuse / brillante	
1158	C	4	1	RS	régulière	saumon / translucide	convexe	régulier	lisse / brillante	
1159	C	4	1	RS	régulière	blanche	plate	régulier	lisse / brillante	
1160	C	4	1	RS	irrégulière	transparente	plate	régulier	rugueuse	
1161	C	4	2	RS	régulière	crème	convexe	régulier	lisse / brillante	
1162	C	4	2	RS	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
1163	C	4	2	RS	régulière	blanche	plate	diffus	rugueuse	
1164	C	4	2	RS	régulière	transparente	plate	diffus	bord brillant / centre rugueux	
1165	C	4	2	RS	régulière	orange	convexe	régulier	lisse / brillante	
1166	C	4	2	RS	irrégulière	blanche	convexe	régulier	lisse / brillante	
1167	C	4	2	RS	irrégulière	blanche	plate	régulier	rugueuse	
1168	C	4	2	RS	irrégulière	jaune / translucide	plate	régulier	lisse / brillante	
1169	C	4	2	RS	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
1170	C	4	2	RS	irrégulière	jaune-orangé	plate	ondulé	lisse / brillante	
1171	C	4	2	RS	type bacillus	-	-	-	-	
1172	C	4	2	RS	irrégulière	jaune / translucide	plate	diffus	rugueuse	
1173	C	4	2	RS	régulière	transparente	plate	régulier	rugueuse	
1174	C	4	2	RS	régulière	transparente	plate	régulier	bord brillant / centre rugueux	
1175	C	4	2	RS	irrégulière	blanche	plate	régulier	rugueuse	
1176	C	4	2	RS	régulière	blanche	plate	régulier	läkerol	
1177	C	4	2	RS	régulière	transparente	plate	régulier	bord brillant / centre rugueux	jaune
1178	C	4	2	RS	régulière	blanche	convexe	régulier	lisse / brillante	
1179	C	4	2	RS	régulière	blanche	plate	diffus	rugueuse	
1180	C	4	2	RS	régulière	jaune / translucide	légèrement convexe	régulier	lisse / brillante	
1181	C	4	3	RS	irrégulière	transparente	plate	régulier	rugueuse	
1182	C	4	3	RS	irrégulière	transparente	plate	régulier	rugueuse	
1183	C	4	3	RS	régulière	blanche	légèrement convexe	régulier	enfoncée au centre / brillante	
1184	C	4	3	RS	régulière	jaune-orangé / translucide	convexe	régulier	lisse / brillante	
1185	C	4	3	RS	irrégulière	blanche	légèrement convexe	régulier	läkerol	
1186	C	4	3	RS	régulière	crème	convexe	diffus	lisse / brillante	
1187	C	4	3	RS	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
1188	C	4	3	RS	régulière	blanche	plate	régulier	rugueuse	
1189	C	4	3	RS	régulière	blanche	convexe	diffus	aplatie au centre / brillante	
1190	C	4	3	RS	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
1191	C	4	3	RS	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
1192	C	4	3	RS	régulière	blanche	plate	régulier	rugueuse / brillante	
1193	C	4	3	RS	type bacillus	-	-	-	-	
1194	C	4	3	RS	régulière	transparente	plate	régulier	bord brillant / centre rugueux	
1195	C	4	3	RS	irrégulière	transparente	plate	régulier	rugueuse	

1196	C	4	3	RS	régulière	blanche	légèrement convexe	diffus	lisse / brillante	
1197	C	4	3	RS	régulière	blanche	plate	diffus	ombiliquée / brillante	
1198	C	4	3	RS	régulière	blanche	plate	ondulé	ombiliquée / brillante	
1199	C	4	3	RS	régulière	blanche	plate	renflé / diffus	lisse / brillante	
1200	C	4	3	RS	régulière	blanche	plate	diffus	lisse / brillante	
1201	C	5	1	RS	régulière	blanche	convexe	régulier	ombiliquée / brillante	
1202	C	5	1	RS	régulière	blanche	légèrement convexe	régulier	rugueuse	
1203	C	5	1	RS	régulière	blanche	légèrement convexe	régulier	rugueuse / brillante	
1204	C	5	1	RS	régulière	blanche	plate	régulier	lisse / brillante	
1205	C	5	1	RS	régulière	transparente	plate	régulier	lisse / brillante	
1206	C	5	1	RS	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
1207	C	5	1	RS	régulière	transparente	légèrement convexe	régulier	lisse / brillante	
1208	C	5	1	RS	régulière	blanche	plate	diffus	rugueuse	
1209	C	5	1	RS	régulière	jaune	légèrement convexe	régulier	lisse / brillante	
1210	C	5	1	RS	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
1211	C	5	1	RS	régulière	blanche	légèrement convexe	régulier	ombiliquée	
1212	C	5	1	RS	régulière	blanche	convexe	régulier	lisse / brillante	
1213	C	5	1	RS	irrégulière	blanche	plate	régulier	lisse / brillante	
1214	C	5	1	RS	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
1215	C	5	1	RS	régulière	blanche	plate	régulier	lisse / brillante	
1216	C	5	1	RS	régulière	blanche	plate	régulier	lisse / brillante	
1217	C	5	1	RS	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
1218	C	5	1	RS	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
1219	C	5	1	RS	régulière	transparente	plate	régulier	lisse / brillante	
1220	C	5	1	RS	régulière	blanche	légèrement convexe	régulier	ombiliquée	
1221	C	5	2	RS	Régulière	blanche	plate	r	lisse/brillante	
1222	C	5	2	RS	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
1223	C	5	2	RS	régulière	saumon	légèrement convexe	régulier	lisse / brillante	
1224	C	5	2	RS	régulière	blanche	plate	régulier	rugueuse / brillante	
1225	C	5	2	RS	régulière / petite	transparente	plate	régulier	lisse	
1226	C	5	2	RS	irrégulière	blanche	plate	régulier	lisse / brillante	
1227	C	5	2	RS	régulière	blanche	plate	régulier	lisse / brillante	
1228	C	5	2	RS	régulière	crème	légèrement convexe	type actino	duveteuse blanche et brune	
1229	C	5	2	RS	régulière / petite	transparente	plate	régulier	lisse	
1230	C	5	2	RS	type bacillus	-	-	-	-	
1231	C	5	2	RS	régulière	blanche	élévation	régulier	ombiliquée	
1232	C	5	2	RS	type bacillus	-	-	-	-	
1233	C	5	2	RS	irrégulière	blanche	plate	ondulé	ombiliquée	
1234	C	5	2	RS	type bacillus	-	-	-	-	
1235	C	5	2	RS	régulière	blanche	plate	régulier	lisse	
1236	C	5	2	RS	irrégulière	blanche	plate	type actino / ondulé	rugueuse	
1237	C	5	2	RS	régulière	blanche	plate	régulier	lisse	
1238	C	5	2	RS	régulière	blanche	légèrement convexe	régulier	rugueuse	
1239	C	5	2	RS	régulière	blanche	plate	régulier	lisse	
1240	C	5	2	RS	régulière / petite	blanche	plate	régulier	lisse / brillante	
1241	C	5	3	RS	régulière / petite	transparente	plate	régulier	lisse	
1242	C	5	3	RS	régulière	blanche	plate	régulier	rugueuse	
1243	C	5	3	RS	régulière	blanche	plate	régulier	rugueuse / brillante	

1244	C	5	3	RS	régulière	saumon	plate	régulier	rugueuse / brillante	
1245	C	5	3	RS	régulière	saumon	plate	régulier	lisse / brillante	
1246	C	5	3	RS	régulière / petite	transparente	plate	régulier	lisse	
1247	C	5	3	RS	régulière	blanche	plate	régulier	lisse / brillante	
1248	C	5	3	RS	régulière	blanche	plate	régulier	rugueuse	
1249	C	5	3	RS	régulière	jaune	plate	régulier	rugueuse	
1250	C	5	3	RS	régulière / petite	blanche	plate	régulier	lisse / brillante	
1251	C	5	3	RS	régulière	crème	légèrement convexe	type actino	duveteuse brune	
1252	C	5	3	RS	régulière	rose	légèrement convexe	régulier	lisse / brillante	
1253	C	5	3	RS	régulière	blanche	plate	ondulé	rugueuse	
1254	C	5	3	RS	régulière	blanche	plate	diffus	rugueuse	
1255	C	5	3	RS	régulière	rouge-orangé	plate	régulier	lisse / brillante	
1256	C	5	3	RS	régulière	blanche	plate	régulier	lisse / brillante	
1257	C	5	3	RS	régulière	transparente	plate	régulier	lisse	
1258	C	5	3	RS	régulière	crème	légèrement convexe	type actino	duveteuse blanche	
1259	C	5	3	RS	régulière	blanche	plate	régulier	lisse / brillante	
1260	C	5	3	RS	régulière	saumon	légèrement convexe	régulier	ombiliquée / brillante	
1261	L	1	1	R	régulière	jaune pâle	plate	régulier	lisse / brillante	
1262	L	1	1	R	régulière	saumon	légèrement convexe	régulier	lisse / brillante	
1263	L	1	1	R	régulière	jaune	convexe	régulier	lisse / brillante	
1264	L	1	1	R	régulière	blanche	convexe	régulier	lisse / brillante	
1265	L	1	1	R	irrégulière	crème	plate	régulier	lisse / brillante	
1266	L	1	1	R	régulière	orange	légèrement convexe	régulier	lisse / brillante	
1267	L	1	1	R	régulière	jaune	légèrement convexe	régulier	lisse / brillante	
1268	L	1	1	R	régulière	blanche	légèrement convexe	régulier	ombiliquée / brillante	
1269	L	1	1	R	irrégulière	jaune / translucide	plate	diffus	rugueuse	
1270	L	1	1	R	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
1271	L	1	1	R	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
1272	L	1	1	R	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
1273	L	1	1	R	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
1274	L	1	1	R	régulière	crème	légèrement convexe	type actino	rugueuse	
1275	L	1	1	R	régulière	jaune	légèrement convexe	régulier	ombiliquée / brillante	
1276	L	1	1	R	régulière / petite	blanche	plate	régulier	lisse / brillante	
1277	L	1	1	R	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
1278	L	1	1	R	régulière	jaune	convexe	régulier	ombiliquée / brillante	
1279	L	1	1	R	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
1280	L	1	1	R	régulière / petite	rose	légèrement convexe	régulier	lisse / brillante	
1281	L	1	1	R	régulière	jaune pâle / translucide	plate	régulier	lisse / brillante	
1282	L	1	1	R	irrégulière	blanche	plate	diffus	lisse / brillante	
1283	L	1	1	R	régulière	blanche	plate	régulier	rugueuse	
1284	L	1	1	R	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
1285	L	1	1	R	régulière / petite	blanche	plate	régulier	lisse / brillante	
1286	L	1	1	R	régulière	blanche	convexe	régulier	lisse / brillante	
1287	L	1	1	R	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
1288	L	1	1	R	régulière	jaune / translucide	plate	régulier	lisse / brillante	
1289	L	1	1	R	irrégulière	blanche	plate	régulier	lisse / brillante	
1290	L	1	1	R	régulière	blanche	convexe	régulier	lisse / brillante	
1291	L	2	1	R	régulière	jaune	légèrement convexe	diffus	lisse / brillante	

1292	L	2	1	R	irrégulière	crème	plate	régulier	lisse / brillante	jaune
1293	L	2	1	R	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
1294	L	2	1	R	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
1295	L	2	1	R	régulière	jaune / translucide	légèrement convexe	diffus	lisse / brillante	
1296	L	2	1	R	régulière / petite	jaune pâle	convexe	régulier	lisse / brillante	
1297	L	2	1	R	irrégulière	jaune	plate	diffus	rugueuse / brillante	
1298	L	2	1	R	irrégulière	crème	plate	envahissant / diffus	lisse / brillante	jaune
1299	L	2	1	R	régulière	orange	légèrement convexe	diffus	lisse / brillante	
1300	L	2	1	R	régulière	jaune / translucide	légèrement convexe	diffus	lisse	
1301	L	2	1	R	régulière	jaune / translucide	légèrement convexe	régulier	lisse / brillante	
1302	L	2	1	R	régulière	jaune / translucide	légèrement convexe	diffus	lisse / brillante	
1303	L	2	1	R	régulière	jaune	légèrement convexe	diffus	lisse / brillante	
1304	L	2	1	R	régulière	crème	légèrement convexe	type actino	rugueuse	
1305	L	2	1	R	régulière	blanche	plate	régulier	lisse / brillante	
1306	L	2	1	R	régulière / petite	jaune	plate	régulier	lisse / brillante	
1307	L	2	1	R	régulière	jaune / translucide	plate	diffus	rugueuse	
1308	L	2	1	R	régulière	jaune	légèrement convexe	diffus	lisse / brillante	
1309	L	2	1	R	régulière	jaune	légèrement convexe	diffus	lisse / brillante	
1310	L	2	1	R	irrégulière	blanche	plate	régulier	lisse / brillante	jaune
1311	L	2	2	R	régulière	ocre	plate	régulier	lisse / brillante	
1312	L	2	2	R	régulière	jaune	légèrement convexe	régulier	lisse / brillante	
1313	L	2	2	R	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
1314	L	2	2	R	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
1315	L	2	2	R	régulière / petite	jaune pâle / translucide	plate	régulier	lisse / brillante	
1316	L	2	2	R	régulière	crème	légèrement convexe	type actino	rugueuse	brun
1317	L	2	2	R	régulière / petite	jaune pâle	plate	régulier	lisse / brillante	
1318	L	2	2	R	irrégulière	jaune	plate	diffus	rugueuse / brillante	
1319	L	2	2	R	régulière	jaune pâle / translucide	plate	régulier	lisse / brillante	
1320	L	2	2	R	régulière / petite	transparente	plate	régulier	lisse / brillante	
1321	L	2	2	R	régulière	jaune	légèrement convexe	diffus	lisse / brillante	
1322	L	2	2	R	régulière	jaune	légèrement convexe	diffus	lisse / brillante	
1323	L	2	2	R	irrégulière	jaune / translucide	plate	diffus	rugueuse	
1324	L	2	2	R	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
1325	L	2	2	R	régulière	blanche / translucide	plate	régulier	lisse / brillante	
1326	L	2	2	R	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
1327	L	2	2	R	régulière	jaune	légèrement convexe	diffus	lisse / brillante	
1328	L	2	2	R	régulière	blanche / translucide	légèrement convexe	régulier	lisse / brillante	
1329	L	2	2	R	régulière	jaune	légèrement convexe	diffus	lisse / brillante	
1330	L	2	2	R	régulière	blanche	plate	diffus	rugueuse	
1331	L	2	3	R	régulière	jaune / translucide	plate	diffus	rugueuse	
1332	L	2	3	R	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
1333	L	2	3	R	régulière	blanche / translucide	plate	régulier	lisse	
1334	L	2	3	R	régulière	jaune	légèrement convexe	régulier	lisse / brillante	
1335	L	2	3	R	régulière	blanche / translucide	plate	régulier	lisse / brillante	
1336	L	2	3	R	régulière	crème	légèrement convexe	type actino	duveteuse blanc	brun
1337	L	2	3	R	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
1338	L	2	3	R	régulière / petite	jaune / translucide	plate	régulier	lisse / brillante	
1339	L	2	3	R	régulière	jaune / translucide	plate	diffus	rugueuse	

1340	L	2	3	R	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
1341	L	2	3	R	irrégulière	jaune / translucide	plate	diffus	rugueuse	
1342	L	2	3	R	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
1343	L	2	3	R	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
1344	L	2	3	R	régulière	blanche / translucide	plate	régulier	lisse / brillante	
1345	L	2	3	R	régulière	jaune pâle / translucide	plate	régulier	lisse / brillante	
1346	L	2	3	R	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
1347	L	2	3	R	régulière	blanche / translucide	plate	régulier	lisse / brillante	
1348	L	2	3	R	régulière	jaune / translucide	plate	diffus	rugueuse	
1349	L	2	3	R	régulière	jaune	légèrement convexe	régulier	lisse / brillante	
1350	L	2	3	R	irrégulière	jaune / translucide	plate	diffus	rugueuse	
1351	L	4	1	R	régulière	crème	plate	régulier	lisse / brillante	
1352	L	4	1	R	régulière	transparente	plate	diffus	lisse / brillante	
1353	L	4	1	R	irrégulière	jaune pâle / translucide	plate	régulier	rugueuse / brillante	
1354	L	4	1	R	régulière	orange / translucide	légèrement convexe	régulier	lisse / brillante	
1355	L	4	1	R	régulière	crème / translucide	plate	régulier	lisse / brillante	
1356	L	4	1	R	régulière	crème	plate	régulier	lisse / brillante	
1357	L	4	1	R	régulière	jaune / translucide	plate	régulier	lisse / brillante	
1358	L	4	1	R	régulière	jaune-orangé / translucide	plate	régulier	lisse / brillante	
1359	L	4	1	R	régulière	orange	légèrement convexe	régulier	lisse / brillante	
1360	L	4	1	R	régulière	crème	légèrement convexe	régulier	lisse / brillante	
1361	L	4	1	R	régulière	orange / translucide	plate	régulier	lisse / brillante	
1362	L	4	1	R	irrégulière	jaune pâle / translucide	plate	régulier	rugueuse / brillante	
1363	L	4	1	R	régulière	crème	convexe	régulier	lisse / aplati au centre	
1364	L	4	1	R	régulière	jaune-orangé / translucide	plate	diffus	rugueuse / brillante	
1365	L	4	1	R	régulière	crème	plate	régulier	lisse / brillante	
1366	L	4	1	R	régulière / petite	orange / translucide	plate	régulier	lisse / brillante	
1367	L	4	1	R	régulière	orange / translucide	plate	régulier	lisse / brillante	
1368	L	4	1	R	régulière	orange / translucide	plate	régulier	lisse / brillante	
1369	L	4	1	R	régulière	crème	plate	renflé	lisse	
1370	L	4	1	R	régulière	jaune / translucide	plate	régulier	lisse / brillante	
1371	L	4	2	R	régulière	jaune-orangé / translucide	plate	régulier	rugueuse / brillante	
1372	L	4	2	R	régulière	jaune-orangé / translucide	plate	régulier	rugueuse / brillante	
1373	L	4	2	R	régulière	crème	plate	renflé	rugueuse	
1374	L	4	2	R	régulière	jaune	légèrement convexe	régulier	lisse / brillante	
1375	L	4	2	R	irrégulière	jaune pâle / translucide	plate	diffus	lisse / brillante	
1376	L	4	2	R	régulière	crème	plate	envahissante	rugueuse	
1377	L	4	2	R	irrégulière	jaune-orangé	plate	envahissante	rugueuse	
1378	L	4	2	R	irrégulière	jaune-orangé	plate	envahissante	rugueuse	
1379	L	4	2	R	irrégulière	jaune-orangé	plate	envahissante	rugueuse	
1380	L	4	2	R	régulière	crème	plate	ondulé / diffus	lisse / brillante	
1381	L	4	2	R	régulière	crème	plate	diffus	rugueuse	
1382	L	4	2	R	régulière	jaune-orangé / translucide	plate	régulier	rugueuse / brillante	
1383	L	4	2	R	régulière	jaune-orangé / translucide	plate	diffus	rugueuse / brillante	
1384	L	4	2	R	régulière	crème	légèrement convexe	régulier	lisse / brillante	
1385	L	4	2	R	régulière	jaune	plate	diffus	lisse / brillante	
1386	L	4	2	R	régulière	jaune-orangé / translucide	plate	régulier	rugueuse / brillante	
1387	L	4	2	R	régulière	jaune-orangé / translucide	plate	régulier	rugueuse / brillante	

1388	L	4	2	R	régulière	jaune-orangé / translucide	plate	régulier	rugueuse / brillante	
1389	L	4	2	R	régulière	crème / translucide	plate	régulier	lisse / brillante	
1390	L	4	2	R	irrégulière	jaune-orangé / translucide	plate	régulier	rugueuse	
1391	L	4	3	R	irrégulière	jaune pâle / translucide	plate	régulier	lisse / brillante	
1392	L	4	3	R	irrégulière	jaune pâle / translucide	plate	envahissant	lisse / brillante	
1393	L	4	3	R	régulière	jaune-orangé / translucide	plate	régulier	rugueuse / brillante	
1394	L	4	3	R	régulière	rose / translucide	plate	régulier	lisse / brillante	
1395	L	4	3	R	régulière	crème	plate	régulier	lisse / brillante	
1396	L	4	3	R	régulière	jaune-orangé / translucide	plate	régulier	rugueuse / brillante	
1397	L	4	3	R	régulière	jaune / translucide	plate	régulier	lisse / brillante	
1398	L	4	3	R	régulière	crème	plate	ondulé	lisse / brillante	
1399	L	4	3	R	régulière	crème	plate	régulier	lisse / brillante	
1400	L	4	3	R	régulière	rose / translucide	plate	régulier	lisse / brillante	
1401	L	4	3	R	régulière / petite	jaune pâle / translucide	plate	régulier	lisse / brillante	
1402	L	4	3	R	régulière	crème	plate	régulier	lisse / brillante	
1403	L	4	3	R	régulière	jaune pâle	plate	régulier	lisse / brillante	
1404	L	4	3	R	régulière	jaune / translucide	plate	régulier	lisse / brillante	
1405	L	4	3	R	régulière	saumon / translucide	plate	régulier	lisse / brillante	
1406	L	4	3	R	régulière	crème	plate	régulier	lisse / brillante	
1407	L	4	3	R	régulière	jaune / translucide	plate	régulier	lisse / brillante	
1408	L	4	3	R	régulière	crème	plate	régulier	lisse / brillante	
1409	L	4	3	R	régulière	transparente	plate	régulier	lisse / brillante	
1410	L	4	3	R	régulière	crème	plate	régulier	lisse / brillante	
1411	L	5	1	R	régulière	crème (bord) brun (centre)	plate	type actino / renflé / ondulé	rugueuse	brun
1412	L	5	1	R	régulière	crème (bord) brun (centre)	plate	type actino / renflé / ondulé	rugueuse	
1413	L	5	1	R	régulière	crème	plate	régulier	lisse / brillante	
1414	L	5	1	R	régulière	crème / translucide	plate	régulier	lisse / brillante	
1415	L	5	1	R	régulière	ocre	plate	ondulé / diffus	lisse / brillante	
1416	L	5	1	R	régulière	crème (bord) ocre (centre)	plate	type actino / renflé / ondulé	rugueuse	
1417	L	5	1	R	régulière	orange	plate	diffus	lisse / brillante	
1418	L	5	1	R	régulière	crème	plate	diffus	lisse / brillante	
1419	L	5	1	R	régulière	crème	plate	envahissant / ondulé	rugueuse / brillante	
1420	L	5	1	R	régulière / petite	transparent	plate	régulier	lisse / brillante	
1421	L	5	1	R	régulière	jaune	plate	diffus	lisse / brillante	
1422	L	5	1	R	régulière	crème	légèrement convexe	type actino	rugueuse	
1423	L	5	1	R	régulière	saumon / translucide	plate	ondulé	lisse / brillante	
1424	L	5	1	R	régulière	transparent	plate	régulier	lisse / brillante	
1425	L	5	1	R	irrégulière	jaune pâle	plate	régulier	lisse / brillante	
1426	L	5	1	R	régulière	crème	légèrement convexe	régulier	lisse / brillante	
1427	L	5	1	R	irrégulière	jaune-orangé	plate	envahissant	rugueuse	
1428	L	5	1	R	régulière	crème	convexe	régulier	rugueuse	
1429	L	5	1	R	régulière	orange	plate	diffus	lisse / brillante	
1430	L	5	1	R	régulière	crème	convexe	régulier	enfoncée au centre / brillante	
1431	L	5	2	R	régulière	crème	plate	régulier	lisse / brillante	
1432	L	5	2	R	régulière / petite	jaune pâle / translucide	plate	régulier	lisse / brillante	
1433	L	5	2	R	régulière	crème	convexe	régulier	lisse / brillante	
1434	L	5	2	R	régulière	jaune -orangé / translucide	plate	diffus	lisse / brillante	
1435	L	5	2	R	régulière	crème	plate	régulier	rugueuse	

1436	L	5	2	R	régulière	crème	plate	envahissant / diffus	lisse	
1437	L	5	2	R	régulière / petite	jaune / translucide	plate	régulier	lisse / brillante	
1438	L	5	2	R	régulière	crème (bords) brun (centre)	plate	type actino	rugueuse	
1439	L	5	2	R	régulière / petite	transparente	plate	régulier	lisse / brillante	
1440	L	5	2	R	régulière	crème / translucide	plate	diffus	lisse / brillante	
1441	L	5	2	R	régulière	jaune / translucide	plate	régulier	lisse / brillante	
1442	L	5	2	R	régulière	crème	légèrement convexe	régulier	lisse / brillante	
1443	L	5	2	R	régulière	crème (bords) brun (centre)	plate	type actino	rugueuse	
1444	L	5	2	R	régulière	crème	plate	ondulé	rugueuse / brillante	
1445	L	5	2	R	régulière	crème	plate	régulier	lisse / brillante	
1446	L	5	2	R	régulière	jaune / translucide	plate	régulier	lisse / brillante	
1447	L	5	2	R	régulière	crème	plate	type actino / diffus	rugueuse	
1448	L	5	2	R	régulière	jaune / translucide	plate	régulier	lisse / brillante	
1449	L	5	2	R	régulière	crème	plate	type actino / diffus	rugueuse	
1450	L	5	2	R	régulière	crème	plate	type actino / diffus	rugueuse	
1451	L	5	3	R	régulière / petite	transparente	plate	régulier	lisse	
1452	L	5	3	R	régulière	crème	plate	régulier	enfoncée au centre / opaque	
1453	L	5	3	R	régulière	crème	cratériforme	type actino	lisse	
1454	L	5	3	R	régulière	crème	légèrement convexe	régulier	enfoncée au centre / brillante	
1455	L	5	3	R	régulière / petite	crème / translucide	plate	régulier	lisse / brillante	
1456	L	5	3	R	régulière / petite	rose / translucide	plate	régulier	lisse / brillante	
1457	L	5	3	R	régulière	crème	convexe	régulier	lisse / brillante	
1458	L	5	3	R	régulière / petite	rose / translucide	plate	régulier	lisse / brillante	
1459	L	5	3	R	régulière	crème	plate	régulier	lisse / brillante	
1460	L	5	3	R	régulière / petite	crème / translucide	plate	régulier	lisse / brillante	
1461	L	5	3	R	régulière	saumon / translucide	plate	régulier	lisse / brillante	
1462	L	5	3	R	régulière	transparente	plate	ondulé	rugueuse	
1463	L	5	3	R	régulière	orange / translucide	légèrement convexe	régulier	lisse / brillante	
1464	L	5	3	R	régulière	crème	plate	ondulé / diffus	rugueuse / brillante	
1465	L	5	3	R	irrégulière	transparente	plate	diffus	rugueuse	
1466	L	5	3	R	type bacillus	-	-	-	-	
1467	L	5	3	R	régulière	jaune-orangé	plate	diffus	lisse / brillante	
1468	L	5	3	R	régulière / petite	transparente	plate	régulier	lisse	
1469	L	5	3	R	régulière	crème / translucide	plate	régulier	lisse / brillante	
1470	L	5	3	R	régulière	crème / translucide	plate	régulier	lisse / brillante	
1471	L	1	1	RS	régulière	jaune	convexe	régulier	lisse / brillante	
1472	L	1	1	RS	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
1473	L	1	1	RS	régulière	transparente	plate	envahissant	rugueuse	
1474	L	1	1	RS	régulière	jaune / translucide	légèrement convexe	régulier	lisse	
1475	L	1	1	RS	irrégulière	blanche	plate	régulier	lisse	
1476	L	1	1	RS	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
1477	L	1	1	RS	irrégulière	blanche	plate	régulier	lisse / brillante	
1478	L	1	1	RS	type bacillus	-	-	-	-	
1479	L	1	1	RS	irrégulière	blanche	plate	diffus	rugueuse	
1480	L	1	1	RS	irrégulière	blanche	plate	régulier	rugueuse	
1481	L	1	1	RS	régulière	blanche	légèrement convexe	régulier	ombiliquée	
1482	L	1	1	RS	irrégulière	blanche	plate	diffus	lisse	
1483	L	1	1	RS	régulière	jaune	plate	régulier	lisse	

1484	L	1	1	RS	régulière	blanche / translucide	plate	régulier	lisse	
1485	L	1	1	RS	régulière	crème	légèrement convexe	type actino	rugueuse	brun
1486	L	1	1	RS	type bacillus	-	-	-	-	
1487	L	1	1	RS	régulière	crème	plate	diffus	lisse	
1488	L	1	1	RS	régulière	jaune / translucide	plate	régulier	lisse	
1489	L	1	1	RS	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
1490	L	1	1	RS	régulière	blanche	légèrement convexe	régulier	ombiliquée	
1491	L	1	1	RS	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
1492	L	1	1	RS	régulière	saumon	plate	régulier	lisse / brillante	
1493	L	1	1	RS	régulière	blanche	légèrement convexe	régulier	ombiliquée / brillante	
1494	L	1	1	RS	régulière	blanche	plate	régulier	lisse / brillante	
1495	L	1	1	RS	irrégulière	blanche	plate	régulier	rugueuse	
1496	L	1	1	RS	régulière	blanche	plate	régulier	rugueuse	
1497	L	1	1	RS	régulière	blanche	plate	régulier	rugueuse	
1498	L	1	1	RS	régulière / petite	blanche / translucide	plate	régulier	lisse	
1499	L	1	1	RS	régulière	blanche	plate	régulier	rugueuse	
1500	L	1	1	RS	régulière	jaune	convexe	régulier	lisse / brillante	
1501	L	2	1	RS	régulière	jaune	plate	diffus	lisse / brillante	
1502	L	2	1	RS	régulière	jaune / translucide	plate	diffus	lisse / brillante	
1503	L	2	1	RS	régulière	blanche	plate	régulier	lisse	
1504	L	2	1	RS	régulière	blanche	plate	régulier	lisse / brillante	
1505	L	2	1	RS	régulière / petite	crème	plate	régulier	lisse / brillante	
1506	L	2	1	RS	régulière	blanche	convexe	régulier	lisse / brillante	
1507	L	2	1	RS	régulière / petite	crème	plate	régulier	lisse / brillante	
1508	L	2	1	RS	régulière	jaune / translucide	plate	régulier	lisse / brillante	
1509	L	2	1	RS	régulière	blanche	plate	diffus	lisse	
1510	L	2	1	RS	régulière	blanche	plate	régulier	cercles concentriques / brillante	
1511	L	2	1	RS	régulière	crème	plate	régulier	lisse / brillante	
1512	L	2	1	RS	régulière	blanche	plate	régulier	lisse / brillante	
1513	L	2	1	RS	irrégulière	jaune / translucide	plate	diffus	lisse / brillante	
1514	L	2	1	RS	type bacillus	-	-	-	-	
1515	L	2	1	RS	régulière	blanche	plate	régulier	lisse / brillante	
1516	L	2	1	RS	irrégulière	blanche	plate	diffus	lisse / brillante	jaune
1517	L	2	1	RS	régulière	blanche	plate	régulier	lisse / brillante	
1518	L	2	1	RS	régulière	blanche	plate	régulier	lisse / brillante	
1519	L	2	1	RS	régulière	blanche	plate	régulier	lisse / brillante	
1520	L	2	1	RS	régulière	blanche / translucide	plate	diffus	lisse	
1521	L	2	2	RS	type bacillus	-	-	-	-	
1522	L	2	2	RS	régulière	jaune / translucide	plate	diffus	lisse / brillante	
1523	L	2	2	RS	régulière	blanche	plate	régulier	cercles concentriques / brillante	
1524	L	2	2	RS	régulière	crème	légèrement convexe	type actino	duveteuse blanche	
1525	L	2	2	RS	régulière	blanche	plate	ondulé	lisse / brillante	
1526	L	2	2	RS	régulière	blanche / translucide	plate	régulier	lisse / brillante	
1527	L	2	2	RS	régulière	blanche	plate	régulier	cercles concentriques / brillante	
1528	L	2	2	RS	régulière	crème	plate	régulier	lisse / brillante	
1529	L	2	2	RS	type bacillus	-	-	-	-	
1530	L	2	2	RS	régulière	crème	plate	régulier	cercles concentriques	
1531	L	2	2	RS	régulière	crème	plate	régulier	lisse / brillante	

1532	L	2	2	RS	régulière	blanche	plate	diffus	rugueuse	
1533	L	2	2	RS	irrégulière	blanche	plate	régulier	lisse	
1534	L	2	2	RS	régulière	blanche	plate	régulier	cercles concentriques	
1535	L	2	2	RS	régulière	blanche	plate	régulier	cercles concentriques / brillante	
1536	L	2	2	RS	irrégulière	crème	plate	ondulé / diffus	lisse / brillante	
1537	L	2	2	RS	irrégulière	crème	plate	ondulé / diffus	lisse / brillante	
1538	L	2	2	RS	type bacillus	-	-	-	-	
1539	L	2	2	RS	régulière	crème	plate	régulier	lisse / brillante	
1540	L	2	2	RS	régulière	jaune / translucide	plate	diffus	lisse / brillante	
1541	L	2	3	RS	irrégulière	blanche	plate	régulier	lisse / brillante	
1542	L	2	3	RS	régulière	blanche	plate	régulier	cercles concentriques	
1543	L	2	3	RS	régulière	blanche	plate	régulier	cercles concentriques	
1544	L	2	3	RS	régulière	blanche	plate	diffus	rugueuse	
1545	L	2	3	RS	régulière	ocre	convexe	régulier	étoile / brillante	
1546	L	2	3	RS	régulière	crème	plate	régulier	cercles concentriques	
1547	L	2	3	RS	irrégulière	blanche	plate	diffus	rugueuse	
1548	L	2	3	RS	régulière	ocre	plate	régulier	lisse / brillante	
1549	L	2	3	RS	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
1550	L	2	3	RS	irrégulière	blanche	plate	diffus	rugueuse	
1551	L	2	3	RS	régulière	crème	convexe	régulier	enfoncée au centre / rugueuse	
1552	L	2	3	RS	irrégulière	jaune / translucide	plate	diffus	rugueuse	
1553	L	2	3	RS	type bacillus	-	-	-	-	
1554	L	2	3	RS	type bacillus	-	-	-	-	
1555	L	2	3	RS	régulière	blanche	plate	régulier	lisse / brillante	
1556	L	2	3	RS	irrégulière	blanche	plate	envahissant	lisse / brillante	
1557	L	2	3	RS	irrégulière	crème	plate	régulier	lisse / brillante	
1558	L	2	3	RS	irrégulière	crème	plate	envahissant / diffus	lisse / brillante	
1559	L	2	3	RS	régulière	blanche	plate	diffus	rugueuse	
1560	L	2	3	RS	régulière	blanche	plate	lobé / diffus	rugueuse	
1561	L	4	1	RS	régulière	blanche / translucide	plate	diffus	rugueuse	
1562	L	4	1	RS	type bacillus	-	-	-		
1563	L	4	1	RS	régulière	blanche / translucide	plate	diffus	rugueuse	
1564	L	4	1	RS	irrégulière	jaune-orangé / translucide	plate	régulier	lisse / brillante	
1565	L	4	1	RS	régulière	blanche / translucide	plate	diffus	rugueuse	
1566	L	4	1	RS	régulière	blanche / translucide	plate	diffus	lisse / brillante	
1567	L	4	1	RS	régulière	blanche / translucide	plate	diffus	rugueuse	
1568	L	4	1	RS	régulière	blanche / translucide	plate	diffus	rugueuse	
1569	L	4	1	RS	type bacillus	-	-	-	-	
1570	L	4	1	RS	régulière / petite	blanche	plate	régulier	rugueuse	
1571	L	4	1	RS	régulière	jaune-orangé / translucide	plate	diffus	lisse / brillante	
1572	L	4	1	RS	régulière	crème	légèrement convexe	régulier	lisse / brillante	
1573	L	4	1	RS	régulière	crème	plate	ondulé	ombiliquée / rugueuse	
1574	L	4	1	RS	type bacillus	-	-	-	-	
1575	L	4	1	RS	régulière	blanche / translucide	plate	diffus	rugueuse	
1576	L	4	1	RS	type bacillus	-	-	-	-	
1577	L	4	1	RS	régulière	jaune-orangé / translucide	plate	régulier	lisse / brillante	
1578	L	4	1	RS	régulière	jaune	plate	diffus	lisse / brillante	
1579	L	4	1	RS	régulière	crème	légèrement convexe	régulier	lisse / brillante	

1580	L	4	1	RS	régulière	blanche	plate	régulier	lisse / brillante	
1581	L	4	2	RS	type bacillus	-	-	-	-	
1582	L	4	2	RS	régulière	crème	plate	diffus	rugueuse	
1583	L	4	2	RS	régulière	crème	plate	ondulé / diffus	rugueuse / brillante	
1584	L	4	2	RS	régulière	crème	plate	diffus	rugueuse	
1585	L	4	2	RS	régulière	crème	plate	diffus	cercles concentriques / rugueuse	
1586	L	4	2	RS	régulière / petite	crème	plate	régulier	lisse / brillante	
1587	L	4	2	RS	régulière	crème	plate	diffus	cercles concentriques / rugueuse	
1588	L	4	2	RS	régulière / petite	blanche / translucide	plate	ondulé / diffus	lisse / brillante	
1589	L	4	2	RS	régulière	crème	plate	diffus	ombiliquée / brillante	
1590	L	4	2	RS	type bacillus	-	-	-	-	
1591	L	4	2	RS	régulière	brune	plate	type actino / diffus	duveteuse blanche	
1592	L	4	2	RS	régulière	blanche	plate	régulier	cercles concentriques / rugueuse	
1593	L	4	2	RS	régulière	crème	plate	régulier	lisse	
1594	L	4	2	RS	type bacillus	-	-	-	-	
1595	L	4	2	RS	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
1596	L	4	2	RS	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
1597	L	4	2	RS	régulière / petite	crème	plate	régulier	lisse / brillante	
1598	L	4	2	RS	régulière	blanche	plate	ondulé / diffus	rugueuse / brillante	
1599	L	4	2	RS	régulière	blanche	légèrement convexe	régulier	lisse / brillante	
1600	L	4	2	RS	type bacillus	-	-	-	-	
1601	L	4	3	RS	régulière / petite	transparente	plate	diffus	rugueuse	
1602	L	4	3	RS	irrégulière	jaune / translucide	plate	régulier	lisse / brillante	
1603	L	4	3	RS	régulière / petite	crème	plate	régulier	lisse / brillante	
1604	L	4	3	RS	type bacillus	-	-	-	-	
1605	L	4	3	RS	régulière	blanche	plate	renflé / diffus	rugueuse	
1606	L	4	3	RS	régulière	jaune / translucide	légèrement convexe	régulier	lisse / brillante	
1607	L	4	3	RS	type bacillus	-	-	-	-	
1608	L	4	3	RS	régulière	crème	légèrement convexe	régulier	lisse / brillante	
1609	L	4	3	RS	régulière / petite	crème	plate	régulier	rugueuse / brillante	
1610	L	4	3	RS	irrégulière	transparente	plate	régulier	rugueuse	jaune
1611	L	4	3	RS	régulière	blanche	plate	diffus	cercles concentriques / rugueuse	
1612	L	4	3	RS	régulière	blanche	plate	ondulé	lisse / brillante	
1613	L	4	3	RS	type bacillus	-	-	-	-	
1614	L	4	3	RS	régulière	jaune-orangé / translucide	plate	diffus	lisse / brillante	
1615	L	4	3	RS	irrégulière	transparente	plate	régulier	rugueuse	jaune
1616	L	4	3	RS	type bacillus	-	-	-	-	
1617	L	4	3	RS	régulière	blanche	plate	renflé / diffus	rugueuse / brillante	
1618	L	4	3	RS	irrégulière	transparente	plate	régulier	rugueuse	jaune
1619	L	4	3	RS	irrégulière	transparente	plate	régulier	lisse / brillante	
1620	L	4	3	RS	régulière / petite	blanche	plate	régulier	lisse / brillante	
1621	L	5	1	RS	régulière	blanche / translucide	plate	renflé	rugueuse	
1622	L	5	1	RS	régulière	crème	plate	diffus	anneau blanc / rugueuse	
1623	L	5	1	RS	régulière	crème	légèrement convexe	régulier	lisse / brillante	
1624	L	5	1	RS	régulière / petite	crème	plate	régulier	lisse / brillante	
1625	L	5	1	RS	régulière	crème	convexe	ondulé / diffus	lisse / brillante	
1626	L	5	1	RS	régulière / petite	jaune-orangé	plate	régulier	lisse / brillante	
1627	L	5	1	RS	régulière / petite	blanche	légèrement convexe	régulier	lisse / brillante	

1628	L	5	1	RS	régulière	brune (centre) / crème (bord)	plate	type actino / ondulé	rugueuse	brun
1629	L	5	1	RS	régulière / petite	transparente	plate	régulier	lisse / brillante	
1630	L	5	1	RS	régulière	crème	plate	diffus	anneau blanc / rugueuse	
1631	L	5	1	RS	régulière	crème	légèrement convexe	régulier	lisse / brillante	
1632	L	5	1	RS	régulière	ocre	légèrement convexe	régulier	lisse / brillante	
1633	L	5	1	RS	régulière / petite	blanche	légèrement convexe	régulier	lisse / brillante	
1634	L	5	1	RS	régulière	blanche	plate	régulier	rugueuse/mate	
1635	L	5	1	RS	régulière	crème	plate	type actino / ondulé	duveteuse blanche	
1636	L	5	1	RS	régulière	crème	plate	type actino / ondulé	duveteuse blanche / cratériforme	
1637	L	5	1	RS	régulière	crème	plate	ondulé / diffus	rugueuse / brillante	
1638	L	5	1	RS	type bacillus	-	-	-	-	
1639	L	5	1	RS						
1640	L	5	1	RS	régulière	crème	légèrement convexe	régulier	lisse / brillante	
1641	L	5	2	RS	régulière	crème	légèrement convexe	diffus	enfoncée au centre / lisse / brillante	
1642	L	5	2	RS	régulière	crème	légèrement convexe	diffus	enfoncée au centre / rugueuse / brillante	
1643	L	5	2	RS	régulière	crème	plate	diffus	cercles concentriques / rugueuse	
1644	L	5	2	RS	régulière	crème	convexe	régulier	lisse / brillante	
1645	L	5	2	RS	régulière / petite	transparente	plate	régulier	rugueuse	
1646	L	5	2	RS	régulière / petite	transparente	plate	ondulé	rugueuse	
1647	L	5	2	RS	régulière / petite	transparente	plate	régulier	lisse / brillante	
1648	L	5	2	RS	régulière	crème	plate	ondulé	rugueuse	
1649	L	5	2	RS	régulière / petite	transparente	plate	régulier	lisse / brillante	
1650	L	5	2	RS	régulière	crème	plate	diffus	anneau blanc / rugueuse	
1651	L	5	2	RS	régulière	transparente	plate	ondulé	rugueuse	
1652	L	5	2	RS	régulière	blanche / translucide	plate	régulier	lisse / brillante	
1653	L	5	2	RS	régulière	crème	convexe	ondulé / diffus	enfoncée au centre / rugueuse	
1654	L	5	2	RS	régulière / petite	transparente	plate	régulier	lisse / brillante	
1655	L	5	2	RS	régulière / petite	blanche / translucide	plate	régulier	lisse / brillante	
1656	L	5	2	RS	régulière / petite	transparente	plate	diffus	rugueuse	
1657	L	5	2	RS	régulière / petite	blanche / translucide	plate	régulier	lisse / brillante	
1658	L	5	2	RS	régulière / petite	blanche / translucide	plate	ondulé	lisse / brillante	
1659	L	5	2	RS	régulière	ocre	plate	ondulé / diffus	lisse / brillante	
1660	L	5	2	RS						
1661	L	5	3	RS	irrégulière	jaune-orangé / translucide	plate	régulier	lisse / brillante	
1662	L	5	3	RS	irrégulière	crème	convexe	régulier	lisse / brillante	jaune
1663	L	5	3	RS	irrégulière	jaune-orangé / translucide	plate	diffus	rugueuse / brillante	
1664	L	5	3	RS	régulière	blanche / translucide	plate	régulier	cercles concentriques / lisse	
1665	L	5	3	RS	régulière / petite	transparente	légèrement convexe	régulier	lisse / brillante	
1666	L	5	3	RS	régulière	crème	légèrement convexe	régulier	lisse / brillante	
1667	L	5	3	RS	régulière	crème	plate	ondulé / diffus	cercles concentriques / rugueuse	
1668	L	5	3	RS	régulière	crème	légèrement convexe	ondulé	boursouflé / brillant	
1669	L	5	3	RS						
1670	L	5	3	RS	régulière	crème	plate	diffus	anneau blanc / rugueuse	
1671	L	5	3	RS	régulière	blanche	plate	ondulé / diffus	rugueuse	
1672	L	5	3	RS	régulière	crème	plate	diffus	lisse / brillante	
1673	L	5	3	RS	régulière	brun	plate	type actino / diffus	rugueuse	
1674	L	5	3	RS	régulière	ocre	plate	diffus	rugueuse	
1675	L	5	3	RS	régulière / petite	orange / translucide	légèrement convexe	régulier	lisse / brillante	

1676	L	5	3	RS	régulière	crème	plate	ondulé / diffus	lisse / brillante	
1677	L	5	3	RS	régulière	transparente	plate	régulier	lisse / brillante	
1678	L	5	3	RS	régulière	crème	plate	diffus	anneau blanc / rugueuse	
1679	L	5	3	RS	régulière	orange	plate	régulier	lisse / brillante	
1680	L	5	3	RS	régulière	blanche	plate	diffus	lisse / brillante	
1681	T	1	1	S	régulière	jaune / translucide	plate	régulier	lisse	
1682	T	1	1	S	régulière	jaunâtre	plate	diffus	lisse	
1683	T	1	1	S	régulière / petite	blanche	plate	régulier	lisse / brillante	
1684	T	1	1	S	régulière / petite	blanche	plate	régulier	lisse / brillante	
1685	T	1	1	S	régulière / petite	crème	plate	régulier	lisse / brillante	
1686	T	1	1	S	irrégulière	blanche	plate	régulier	lisse / brillante	
1687	T	1	1	S	régulière	jaune pâle	plate	diffus	lisse	
1688	T	1	1	S	régulière	jaune / translucide	plate	régulier	rugueuse	
1689	T	1	1	S	régulière	jaune	convexe	régulier	lisse	
1690	T	1	1	S	type bacillus	-	-	-	-	
1691	T	1	1	S	régulière / petite	blanche	plate	régulier	lisse / brillante	
1692	T	1	1	S	irrégulière	blanche	légèrement convexe	diffus	lisse / brillante	
1693	T	1	1	S	régulière	jaune / translucide	plate	diffus	lisse / brillante	
1694	T	1	1	S	régulière	crème	légèrement convexe	régulier	lisse / brillante	
1695	T	1	1	S	régulière	jaune	plate	régulier	lisse / brillante	
1696	T	1	1	S	régulière	crème	plate	régulier	lisse / brillante	
1697	T	1	1	S	irrégulière	jaune / translucide	plate	régulier	rugueuse	
1698	T	1	1	S	régulière	ocre	légèrement convexe	régulier	lisse / brillante	
1699	T	1	1	S	irrégulière	jaune / translucide	plate	régulier	rugueuse	
1700	T	1	1	S	régulière	blanche	plate	régulier	lisse / brillante	
1701	T	1	1	S	type bacillus	-	-	-	-	
1702	T	1	1	S	régulière / petite	crème / translucide	plate	régulier	lisse	
1703	T	1	1	S	régulière / petite	blanche	plate	régulier	lisse / brillante	
1704	T	1	1	S	irrégulière	jaune / translucide	plate	régulier	lisse / brillante	
1705	T	1	1	S	régulière / petite	blanche	plate	régulier	lisse / brillante	
1706	T	1	1	S	régulière	jaune-orangé	légèrement convexe	régulier	lisse / brillante	
1707	T	1	1	S	régulière / petite	crème	plate	régulier	lisse / brillante	
1708	T	1	1	S	irrégulière	crème	légèrement convexe	diffus	lisse / brillante	
1709	T	1	1	S	irrégulière	jaune / translucide	plate	régulier	rugueuse	
1710	T	1	1	S	irrégulière	jaune / translucide	plate	régulier	rugueuse / brillante	
1711	T	1	2	S	régulière	crème	légèrement convexe	type actino	duveteuse brune	
1712	T	1	2	S	régulière	crème	plate	régulier	lisse / brillante	
1713	T	1	2	S	régulière	blanche	plate	diffus	lisse / brillante	
1714	T	1	2	S	irrégulière	blanche / translucide	plate	diffus	lisse / brillante	
1715	T	1	2	S	régulière / petite	blanche / translucide	plate	régulier	lisse	
1716	T	1	2	S	régulière	jaune pâle	légèrement convexe	régulier	lisse / brillante	
1717	T	1	2	S	régulière	jaune	légèrement convexe	régulier	lisse / brillante	
1718	T	1	2	S	régulière	crème	légèrement convexe	régulier	lisse / brillante	
1719	T	1	2	S	régulière	crème	plate	régulier	lisse / brillante	
1720	T	1	2	S	régulière	crème	plate	régulier	lisse / brillante	
1721	T	1	2	S	régulière	crème	plate	régulier	ombiliquée	
1722	T	1	2	S	régulière	crème	plate	régulier	lisse / brillante	
1723	T	1	2	S	régulière / petite	jaune / translucide	plate	diffus	lisse	

1724	T	1	2	S	régulière	crème	plate	diffus	lisse / brillante	
1725	T	1	2	S	régulière	crème	légèrement convexe	régulier	lisse / brillante	
1726	T	1	2	S	régulière	jaune	légèrement convexe	régulier	lisse / brillante	
1727	T	1	2	S	régulière	rose	plate	régulier	lisse / brillante	
1728	T	1	2	S	régulière	crème	convexe	régulier	lisse / brillante	
1729	T	1	2	S	régulière	jaune	plate	régulier	lisse / brillante	
1730	T	1	2	S	régulière	crème	plate	régulier	ombiliquée	
1731	T	1	2	S	régulière / petite	transparente	plate	régulier	lisse	
1732	T	1	2	S	régulière	crème	légèrement convexe	type actino	rugueuse	brun
1733	T	1	2	S	régulière	crème	légèrement convexe	type actino	rugueuse	brun
1734	T	1	2	S	régulière	jaune	légèrement convexe	régulier	lisse / brillante	
1735	T	1	2	S	irrégulière	blanche	plate	régulier	lisse / brillante	
1736	T	1	2	S	régulière	crème	plate	régulier	cercles concentriques / brillante	
1737	T	1	2	S	régulière	crème	légèrement convexe	régulier	ombiliquée / brillante	
1738	T	1	2	S	régulière	jaune	convexe	régulier	lisse / brillante	
1739	T	1	2	S	régulière	orange	plate	régulier	lisse / brillante	
1740	T	1	2	S	régulière	crème	plate	régulier	lisse / brillante	
1741	T	1	3	S	irrégulière	blanche	plate	régulier	rugueuse	
1742	T	1	3	S	régulière	jaune pâle	plate	régulier	lisse / brillante	
1743	T	1	3	S	régulière / petite	crème	plate	régulier	lisse / brillante	
1744	T	1	3	S	régulière	blanche	plate	régulier	lisse / brillante	
1745	T	1	3	S	régulière	crème	plate	régulier	lisse / brillante	
1746	T	1	3	S	régulière	blanche	convexe	régulier	lisse / brillante	
1747	T	1	3	S	régulière	blanche	plate	régulier	lisse / brillante	
1748	T	1	3	S	régulière	crème	plate	régulier	cercles concentriques / brillante	
1749	T	1	3	S	régulière	crème	convexe	régulier	cercles concentriques	
1750	T	1	3	S	régulière	crème	légèrement convexe	régulier	étoile / brillante	
1751	T	1	3	S	irrégulière	jaune / translucide	plate	régulier	rugueuse / brillante	
1752	T	1	3	S	régulière	crème	légèrement convexe	type actino	duveteuse blanche	
1753	T	1	3	S	régulière	orange	légèrement convexe	régulier	enfoncée au centre / brillante	
1754	T	1	3	S	régulière / petite	blanche	plate	régulier	rugueuse	
1755	T	1	3	S	régulière	crème	plate	régulier	cercles concentriques / brillante	
1756	T	1	3	S	régulière	crème	plate	régulier	cercles concentriques / brillante	
1757	T	1	3	S	régulière	rose	légèrement convexe	régulier	lisse / brillante	
1758	T	1	3	S	régulière	crème	plate	régulier	cercles concentriques / brillante	
1759	T	1	3	S	régulière	jaune / translucide	plate	régulier	lisse / brillante	
1760	T	1	3	S	régulière	jaune	plate	régulier	lisse / brillante	
1761	T	1	3	S	irrégulière	jaune / translucide	plate	régulier	rugueuse	
1762	T	1	3	S	régulière	crème	plate	régulier	cercles concentriques / brillante	
1763	T	1	3	S	régulière	crème	légèrement convexe	régulier	lisse / brillante	
1764	T	1	3	S	régulière	crème / translucide	plate	régulier	lisse / brillante	
1765	T	1	3	S	type bacillus	-	-	-	-	
1766	T	1	3	S	régulière	crème	légèrement convexe	type actino	duveteuse blanche	
1767	T	1	3	S	régulière	crème	plate	régulier	lisse / brillante	
1768	T	1	3	S	régulière	crème	légèrement convexe	régulier	cercles concentriques / brillante	
1769	T	1	3	S	régulière	crème	plate	régulier	lisse / brillante	
1770	T	1	3	S	irrégulière	crème / translucide	plate	régulier	lisse / brillante	
1771	T	2	1	S	type bacillus	-	-	-	-	

1772	T	2	1	S	régulière / petite	blanche / translucide	plate	régulier	lisse	
1773	T	2	1	S	régulière / petite	blanche / translucide	plate	régulier	lisse	
1774	T	2	1	S	régulière	jaune / translucide	plate	régulier	lisse	
1775	T	2	1	S	irrégulière	blanche / translucide	plate	régulier	lisse / brillante	
1776	T	2	1	S	régulière	crème	plate	régulier	lisse / brillante	
1777	T	2	1	S	régulière / petite	blanche / translucide	plate	régulier	lisse / brillante	
1778	T	2	1	S	régulière / petite	crème	plate	régulier	lisse / brillante	
1779	T	2	1	S	régulière / petite	crème	plate	régulier	lisse / brillante	
1780	T	2	1	S	régulière	jaune / translucide	plate	régulier	lisse	
1781	T	2	1	S	régulière	crème	plate	régulier	lisse / brillante	
1782	T	2	1	S	régulière	jaune pâle	plate	régulier	lisse / brillante	
1783	T	2	1	S	régulière	crème	légèrement convexe	régulier	ombiliquée	
1784	T	2	1	S	régulière	crème	légèrement convexe	type actino	lisse	
1785	T	2	1	S	régulière	crème	plate	régulier	lisse / brillante	
1786	T	2	1	S	type bacillus	-	-	-	-	
1787	T	2	1	S	régulière	crème	plate	régulier	lisse / brillante	
1788	T	2	1	S	régulière / petite	crème / translucide	plate	régulier	lisse / brillante	
1789	T	2	1	S	régulière	crème	légèrement convexe	type actino	lisse	brun
1790	T	2	1	S	régulière	crème	plate	régulier	cercles concentriques	
1791	T	2	2	S	régulière	crème	plate	régulier	lisse	
1792	T	2	2	S	régulière	crème	plate	régulier	cercles concentriques	
1793	T	2	2	S	régulière / petite	crème / translucide	plate	régulier	lisse / brillante	
1794	T	2	2	S	irrégulière	crème	plate	envahissant / diffus	lisse	
1795	T	2	2	S	régulière	crème	légèrement convexe	régulier	lisse / brillante	
1796	T	2	2	S	régulière / petite	crème	plate	régulier	lisse / brillante	
1797	T	2	2	S	régulière	crème	plate	régulier	ombiliquées	
1798	T	2	2	S	régulière	crème	plate	régulier	lisse / brillante	
1799	T	2	2	S	irrégulière	transparente	plate	régulier	rugueuse	
1800	T	2	2	S	régulière	crème	plate	régulier	lisse / brillante	
1801	T	2	2	S	régulière	crème	légèrement convexe	type actino	duveteuse blanche	
1802	T	2	2	S	régulière	jaune	plate	régulier	lisse / brillante	
1803	T	2	2	S	régulière	crème	légèrement convexe	régulier	enfoncée au centre / brillante	
1804	T	2	2	S	régulière	crème	légèrement convexe	type actino	rugueuse	jaune
1805	T	2	2	S	régulière	crème	plate	régulier	lisse / brillante	
1806	T	2	2	S	régulière / petite	crème / translucide	plate	régulier	lisse / brillante	
1807	T	2	2	S	régulière	crème	plate	régulier	rugueuse	
1808	T	2	2	S	régulière	crème	légèrement convexe	type actino	rugueuse	brun
1809	T	2	2	S	régulière	crème	plate	régulier	rugueuse / brillante	
1810	T	2	2	S	régulière	crème	légèrement convexe	régulier	ombiliquée / brillante	
1811	T	2	3	S	irrégulière	crème	plate	diffus	lisse	
1812	T	2	3	S	régulière	crème	plate	régulier	cercles concentriques / brillante	
1813	T	2	3	S	régulière	crème	légèrement convexe	type actino	rugueuse	
1814	T	2	3	S	régulière / petite	crème / translucide	plate	régulier	lisse / brillante	
1815	T	2	3	S	irrégulière	crème	plate	diffus	rugueuse	
1816	T	2	3	S	irrégulière	crème	plate	diffus	cercles concentriques / rugueuse	
1817	T	2	3	S	type bacillus	-	-	-	-	
1818	T	2	3	S	régulière	crème	plate	régulier	cercles concentriques / rugueuse	
1819	T	2	3	S	régulière	crème	plate	régulier	lisse	

1820	T	2	3	S	type bacillus	-	-	-	-	
1821	T	2	3	S	régulière	crème	plate	régulier	cercles concentriques / rugueuse	
1822	T	2	3	S	irrégulière	crème	plate	régulier	lisse / brillante	
1823	T	2	3	S	type bacillus	-	-	-	-	
1824	T	2	3	S	régulière	crème	plate	régulier	cercles concentriques / rugueuse	
1825	T	2	3	S	régulière / petite	crème / translucide	plate	régulier	lisse / brillante	
1826	T	2	3	S	régulière	crème	légèrement convexe	type actino	duveteuse blanche	
1827	T	2	3	S	régulière	crème	plate	régulier	cercles concentriques / brillante	
1828	T	2	3	S	type bacillus	-	-	-	-	
1829	T	2	3	S	régulière	crème	plate	régulier	cercles concentriques / rugueuse	
1830	T	2	3	S	irrégulière	jaune pâle	plate	régulier	lisse / brillante	
1831	T	4	1	S	régulière	brune	plate	type actino / diffus	duveteuse blanche	brun
1832	T	4	1	S	régulière	crème	plate	ondulé / diffus	cratériforme / rugueuse	
1833	T	4	1	S	régulière	crème	plate	régulier	cercles concentriques / lisse	
1834	T	4	1	S	régulière	crème	plate	régulier	cercles concentriques / lisse	
1835	T	4	1	S	régulière	transparente	plate	régulier	lisse / brillante	
1836	T	4	1	S	type bacillus	-	-	-	-	
1837	T	4	1	S	régulière	crème	plate	renflé / diffus	cercles concentriques / lisse	
1838	T	4	1	S	régulière	crème	plate	régulier	lisse / brillante	
1839	T	4	1	S	régulière	brune	plate	type actino	duveteuse blanche	
1840	T	4	1	S	régulière	transparente	plate	renflé	rugueuse	
1841	T	4	1	S	régulière	crème	plate	régulier	lisse / brillante	
1842	T	4	1	S	régulière	crème	plate	régulier	cercles concentriques / lisse	
1843	T	4	1	S	régulière	crème / translucide	plate	régulier	lisse / brillante	
1844	T	4	1	S	régulière	ocre	plate	ondulé	rugueuse	
1845	T	4	1	S	régulière	crème	légèrement convexe	type actino / diffus	duveteuse brune	
1846	T	4	1	S	régulière	crème	plate	type actino / diffus	anneau blanc / rugueuse	
1847	T	4	1	S	régulière	crème	plate	renflé / diffus	cercles concentriques / lisse	
1848	T	4	1	S	régulière	transparente	plate	régulier	lisse / brillante	
1849	T	4	1	S	type bacillus	-	-	-	-	
1850	T	4	1	S	régulière	crème / translucide	légèrement convexe	régulier	rugueuse / brillante	
1851	T	4	2	S	régulière	crème	légèrement convexe	diffus	cercles concentriques / lisse	
1852	T	4	2	S	régulière	crème / translucide	plate	régulier	lisse / brillante	
1853	T	4	2	S	régulière	crème	légèrement convexe	diffus	enfoncée au centre / lisse	
1854	T	4	2	S	régulière	crème / translucide	plate	renflé	lisse	
1855	T	4	2	S	régulière	crème	plate	ondulé	rugueuse	
1856	T	4	2	S	régulière	crème	légèrement convexe	diffus	cercles concentriques / lisse	
1857	T	4	2	S	régulière	crème	légèrement convexe	diffus	cercles concentriques / lisse	
1858	T	4	2	S	régulière / petite	transparente	plate	diffus	lisse	
1859	T	4	2	S	régulière	crème / translucide	plate	régulier	enfoncée au centre / lisse	
1860	T	4	2	S	régulière	crème	légèrement convexe	diffus	cercles concentriques / lisse	
1861	T	4	2	S	régulière / petite	crème	plate	régulier	cercles concentriques / brillante	
1862	T	4	2	S	type bacillus	-	-	-	-	
1863	T	4	2	S	régulière / petite	crème	plate	régulier	cercles concentriques / lisse	
1864	T	4	2	S	type bacillus	-	-	-	-	
1865	T	4	2	S	régulière	crème	légèrement convexe	régulier	cercles concentriques / lisse	
1866	T	4	2	S	régulière	crème / translucide	plate	diffus	lisse / brillante	
1867	T	4	2	S	régulière	crème	légèrement convexe	diffus	cercles concentriques / lisse	

1868	T	4	2	S	type bacillus	-	-	-	-	
1869	T	4	2	S	régulière	crème / translucide	plate	ondulé	lisse / brillante	
1870	T	4	2	S	régulière	crème	plate	régulier	cercles concentriques / rugueuse	
1871	T	4	3	S	type bacillus	-	-	-	-	
1872	T	4	3	S	régulière	crème / translucide	plate	régulier	lisse / brillante	
1873	T	4	3	S	régulière	crème	convexe	ondulé / diffus	cercles concentriques / lisse	
1874	T	4	3	S	régulière	crème	légèrement convexe	régulier	cercles concentriques / brillante	
1875	T	4	3	S	type bacillus	-	-	-	-	
1876	T	4	3	S	régulière	crème / translucide	plate	diffus	ombiliquée / rugueuse	
1877	T	4	3	S	régulière	crème	plate	régulier	cercles concentriques / brillante	
1878	T	4	3	S	régulière	crème / translucide	plate	régulier	lisse / brillante	
1879	T	4	3	S	régulière	crème	plate	diffus	cercles concentriques / rugueuse	
1880	T	4	3	S	régulière / petite	crème	plate	régulier	lisse	
1881	T	4	3	S	régulière	crème	plate	ondulé / diffus	cercles concentriques / rugueuse	
1882	T	4	3	S	type bacillus	-	-	-	-	
1883	T	4	3	S	régulière	crème	plate	diffus	cercles concentriques / lisse	
1884	T	4	3	S	régulière	crème / translucide	plate	régulier	cercles concentriques / lisse	
1885	T	4	3	S	régulière	crème / translucide	plate	diffus	lisse	
1886	T	4	3	S	régulière	crème	plate	diffus	ombiliquée / brillante	
1887	T	4	3	S	régulière	crème	convexe	régulier	enfoncée au centre / lisse	
1888	T	4	3	S	régulière	crème	légèrement convexe	régulier	lisse / brillante	
1889	T	4	3	S	régulière	crème	plate	diffus	cercles concentriques / lisse	
1890	T	4	3	S	régulière	ocre / translucide	convexe	régulier	cercles concentriques / lisse	
1891	T	5	1	S	régulière	crème	plate	régulier	cercles concentriques / lisse	
1892	T	5	1	S	régulière / petite	transparente	plate	régulier	lisse / brillante	
1893	T	5	1	S	régulière / petite	blanche / translucide	plate	régulier	lisse / brillante	
1894	T	5	1	S	régulière	crème	plate	ondulé	rugueuse	
1895	T	5	1	S	type bacillus	-	-	-	-	
1896	T	5	1	S	régulière	crème	plate	ondulé / diffus	lisse	jaune
1897	T	5	1	S	régulière / petite	blanche / translucide	plate	régulier	lisse	
1898	T	5	1	S	régulière	crème	convexe	régulier	lisse / brillante	
1899	T	5	1	S	régulière	blanche / translucide	plate	régulier	lisse / brillante	
1900	T	5	1	S	régulière	crème	plate	type actino	enfoncée au centre / rugueuse	brun
1901	T	5	1	S	régulière	blanche	plate	régulier	rugueuse/mate	
1902	T	5	1	S	type bacillus	-	-	-	-	
1903	T	5	1	S	régulière	blanche / translucide	plate	diffus	rugueuse	
1904	T	5	1	S	régulière	transparente	légèrement convexe	régulier	lisse / brillante	
1905	T	5	1	S	régulière / petite	saumon	légèrement convexe	régulier	lisse / brillante	
1906	T	5	1	S	régulière	crème	légèrement convexe	ondulé	ombiliquée / lisse	
1907	T	5	1	S	régulière	crème	convexe	régulier	lisse / brillante	jaune
1908	T	5	1	S	régulière	crème	plate	régulier	cercles concentriques / rugueuse	
1909	T	5	1	S	régulière	transparente	plate	ondulé	lisse	
1910	T	5	1	S	régulière	crème / translucide	plate	renflé	lisse	
1911	T	5	2	S	régulière	crème	plate	diffus	rugueuse	
1912	T	5	2	S	régulière / petite	crème	plate	régulier	ombiliquée / lisse	
1913	T	5	2	S	régulière	crème	légèrement convexe	régulier	cercles concentriques / lisse	
1914	T	5	2	S	régulière	blanche	plate	régulier	rugueuse/mate	
1915	T	5	2	S	régulière	blanche	légèrement convexe	diffus	brillante	

1916	T	5	2	S	régulière	crème	plate	régulier	lisse / brillante	
1917	T	5	2	S	type bacillus	-	-	-	-	
1918	T	5	2	S	régulière	crème	plate	renflé / ondulé	rugueuse	
1919	T	5	2	S	régulière	crème / translucide	plate	ondulé	lisse / brillante	
1920	T	5	2	S	régulière	crème	légèrement convexe	régulier	enfoncée au centre / lisse	
1921	T	5	2	S	régulière / petite	crème	légèrement convexe	régulier	enfoncée au centre / lisse	
1922	T	5	2	S	type bacillus	-	-	-	-	
1923	T	5	2	S	régulière	crème	plate	ondulé	lisse / brillante	
1924	T	5	2	S	type bacillus	-	-	-	-	
1925	T	5	2	S	régulière	transparente	plate	diffus	?	
1926	T	5	2	S	régulière	transparente	plate	régulier	rugueuse /mate	
1927	T	5	2	S	régulière	blanche	plate	diffus	rugueuse/mate	
1928	T	5	2	S	type bacillus	-	-	-	-	
1929	T	5	2	S	régulière	crème	légèrement convexe	régulier	lisse / brillante	
1930	T	5	2	S	régulière	crème	plate	ondulé / diffus	rugueuse / brillante	
1931	T	5	3	S	régulière	crème	plate	régulier	lisse / brillante	
1932	T	5	3	S	régulière	blanche / translucide	plate	diffus	rugueuse	
1933	T	5	3	S	type bacillus	-	-	-	-	
1934	T	5	3	S	régulière	crème	plate	régulier	lisse	
1935	T	5	3	S	régulière	blanche	plate	diffus	rugueuse/mate	
1936	T	5	3	S	régulière	blanche / translucide	plate	diffus	rugueuse / brillante	
1937	T	5	3	S	régulière / petite	crème	plate	régulier	lisse / brillante	
1938	T	5	3	S	régulière	crème	légèrement convexe	régulier	enfoncée au centre / lisse	
1939	T	5	3	S	régulière	crème	légèrement convexe	régulier	cercles concentriques / brillante	
1940	T	5	3	S	régulière	blanche	plate	régulier	lisse / brillante	
1941	T	5	3	S	type bacillus	-	-	-	-	
1942	T	5	3	S	type bacillus	-	-	-	-	
1943	T	5	3	S	régulière	crème	plate	régulier	lisse / brillante	
1944	T	5	3	S	régulière	crème	plate	régulier	cercles concentriques / lisse	
1945	T	5	3	S	régulière	crème	plate	régulier	cercles concentriques / lisse	
1946	T	5	3	S	régulière	blanche	plate	diffus	rugueuse	
1947	T	5	3	S	régulière	blanche	plate	diffus	rugueuse	
1948	T	5	3	S	régulière	crème / translucide	plate	ondulé	rugueuse	
1949	T	5	3	S	régulière	crème / translucide	plate	ondulé	rugueuse	
1950	T	5	3	S	régulière	blanche	plate	diffus	rugueuse	

Isolates description : Functional abilities

souche	cv	stade	repl.	fract.	gram+	oxyd+	eps+	sid+	psb+	phyG	phyCEm	rednit+	denit+	nitram+	case+	gelat+	nahl+ c4-c8	nahl+ c6-c12	aia+	hcn+	Sulf+	Sulf +Smin
1	A	1	1	R	0	0	1	1	0	0	0	0	0	0	1	1	1	1	0	0	0	0
2	A	1	1	R	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
3	A	1	1	R	0	0	1	0	1	1	0	0	0	0	0	0	1	0	1	0	0	0
4	A	1	1	R	0	0	1	1	0	0	0	1	0	0	0	1	1	1	1	0	0	0
5	A	1	1	R	1	1	0	0	0	NA	NA	0	0	0	0	0	1	0	0	0	NA	NA
6	A	1	1	R	0	0	1	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0
7	A	1	1	R	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
8	A	1	1	R	0	0	1	1	0	0	0	1	0	0	1	1	1	1	1	0	1	1
9	A	1	1	R	0	1	1	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0
10	A	1	1	R	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
11	A	1	1	R	0	0	1	0	1	1	0	1	0	0	1	0	0	0	0	0	0	0
12	A	1	1	R	0	1	0	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0
13	A	1	1	R	1	0	0	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0
14	A	1	1	R	1	1	0	0	0	NA	NA	1	0	0	1	1	0	0	0	0	0	0
15	A	1	1	R	0	0	0	0	1	1	0	1	0	0	0	0	0	0	1	0	0	0
16	A	1	1	R	0	0	0	1	1	0	0	1	0	0	0	0	1	1	1	0	0	0
17	A	1	1	R	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0
18	A	1	1	R	0	0	0	1	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
19	A	1	1	R	0	0	0	0	1	1	0	0	0	0	1	1	0	1	0	0	0	0
20	A	1	1	R	0	1	0	0	0	NA	NA	0	0	0	1	1	0	0	0	0	NA	NA
21	A	1	1	R	0	0	0	1	1	1	0	0	0	0	1	0	0	0	0	0	0	0
22	A	1	1	R	1	1	1	0	0	NA	NA	0	0	0	1	1	0	0	0	0	NA	NA
23	A	1	1	R	1	0	1	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0
24	A	1	1	R	0	0	0	1	0	0	0	1	0	0	1	0	0	0	0	0	0	0
25	A	1	1	R	0	0	1	1	0	0	0	1	0	0	NA	NA	NA	NA	NA	NA	0	0
26	A	1	1	R	1	0	0	1	0	0	0	1	0	0	1	1	0	0	1	0	0	0
27	A	1	1	R	1	0	0	0	0	NA	NA	1	1	0	1	1	0	0	0	0	0	0
28	A	1	1	R	0	0	0	1	0	0	0	0	0	0	1	1	0	1	1	0	0	0
29	A	1	1	R	1	1	0	1	0	0	0	1	1	0	1	1	0	0	0	0	0	0
30	A	1	1	R	0	1	0	1	0	0	0	1	0	0	0	0	0	0	1	0	NA	NA
31	A	2	1	R	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
32	A	2	1	R	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
33	A	2	1	R	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
34	A	2	1	R	0	0	1	1	0	NA	NA	0	0	0	0	1	0	1	0	0	0	0
35	A	2	1	R	0	0	0	1	0	0	0	1	0	0	0	1	0	1	0	0	0	0
36	A	2	1	R	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
37	A	2	1	R	0	0	0	1	1	0	0	1	0	0	0	0	0	1	0	0	0	0
38	A	2	1	R	0	0	0	0	1	1	0	0	0	0	0	0	1	1	1	0	0	0
39	A	2	1	R	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
40	A	2	1	R	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
41	A	2	1	R	0	1	0	0	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
42	A	2	1	R	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
43	A	2	1	R	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

44	A	2	1	R	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0
45	A	2	1	R	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0
46	A	2	1	R	0	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0
47	A	2	1	R	0	1	1	1	0	NA	NA	1	0	0	0	0	0	1	0	0	1	1
48	A	2	1	R	0	1	1	1	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
49	A	2	1	R	1	1	0	0	0	NA	NA	0	0	1	1	1	0	0	0	0	0	0
50	A	2	1	R	0	1	0	1	0	0	0	1	0	0	0	0	0	1	0	0	0	0
51	A	2	2	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
52	A	2	2	R	0	0	0	1	0	0	0	1	0	0	1	1	0	0	1	0	0	0
53	A	2	2	R	0	0	0	1	0	0	0	0	0	0	1	1	1	1	1	0	0	0
54	A	2	2	R	0	1	0	1	0	1	0	0	0	0	0	0	1	0	1	0	0	0
55	A	2	2	R	0	0	0	0	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
56	A	2	2	R	0	0	0	1	0	NA	NA	0	0	0	1	1	1	1	1	0	0	0
57	A	2	2	R	0	1	0	0	0	1	0	1	1	0	0	0	1	0	1	0	0	0
58	A	2	2	R	1	0	0	0	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
59	A	2	2	R	0	0	0	0	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
60	A	2	2	R	0	1	0	1	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
61	A	2	2	R	0	1	0	1	0	1	0	0	0	0	0	0	1	0	1	0	0	0
62	A	2	2	R	0	1	0	0	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
63	A	2	2	R	0	1	0	0	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
64	A	2	2	R	0	0	0	1	0	NA	NA	0	0	0	1	1	0	1	0	0	0	0
65	A	2	2	R	0	1	0	1	0	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
66	A	2	2	R	0	0	0	0	0	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
67	A	2	2	R	0	1	0	0	0	NA	NA	0	0	0	0	1	0	0	0	0	NA	NA
68	A	2	2	R	0	0	0	0	0	1	0	0	0	1	1	1	0	1	0	0	0	0
69	A	2	2	R	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
70	A	2	2	R	0	1	0	0	0	0	0	1	0	0	0	0	0	1	1	0	1	1
71	A	2	3	R	0	1	1	1	0	0	0	1	1	0	0	0	0	0	1	0	0	0
72	A	2	3	R	0	0	0	1	0	1	1	0	0	0	0	1	1	1	0	0	1	1
73	A	2	3	R	0	1	0	1	1	0	0	0	0	0	1	1	1	0	0	1	0	0
74	A	2	3	R	0	0	0	0	1	0	0	0	0	0	0	0	1	1	1	0	0	0
75	A	2	3	R	0	0	0	1	0	1	0	0	0	0	0	1	1	1	0	0	1	1
76	A	2	3	R	0	1	1	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0
77	A	2	3	R	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
78	A	2	3	R	0	NA	0	NA	NA	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
79	A	2	3	R	0	0	0	0	0	NA	NA	0	0	0	0	0	1	0	0	0	0	0
80	A	2	3	R	0	1	0	0	0	1	1	0	0	0	0	0	0	0	1	0	0	0
81	A	2	3	R	0	0	0	1	1	0	0	1	1	0	0	0	0	1	0	0	0	0
82	A	2	3	R	0	0	0	0	0	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
83	A	2	3	R	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
84	A	2	3	R	0	0	1	0	0	0	0	1	1	0	0	0	1	0	0	0	1	1
85	A	2	3	R	0	0	0	1	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
86	A	2	3	R	0	0	1	1	0	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
87	A	2	3	R	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0	0	1	1
88	A	2	3	R	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0	0	1	1
89	A	2	3	R	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
90	A	2	3	R	0	1	1	0	1	1	1	0	0	0	0	0	1	1	1	0	0	0
91	A	4	1	R	0	0	1	0	1	0	0	0	0	0	1	1	0	0	0	1	0	0

92	A	4	1	R	NA	NA	0	NA	NA	NA	0	NA	NA	NA	0	0	0	0	0	0	0	0
93	A	4	1	R	0	0	1	1	0	1	0	1	0	0	1	0	0	1	1	0	0	0
94	A	4	1	R	0	1	0	0	0	NA	NA	1	0	0	1	1	0	0	1	0	0	0
95	A	4	1	R	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0
96	A	4	1	R	0	1	0	0	0	NA	NA	1	1	0	1	1	0	1	0	0	0	0
97	A	4	1	R	0	1	1	1	0	0	0	0	0	0	0	1	0	1	0	0	1	1
98	A	4	1	R	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0
99	A	4	1	R	0	1	1	1	0	1	1	1	0	0	1	1	0	1	1	0	1	1
100	A	4	1	R	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
101	A	4	1	R	0	1	0	1	0	0	0	1	0	0	1	1	0	1	1	0	0	1
102	A	4	1	R	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
103	A	4	1	R	1	1	0	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0
104	A	4	1	R	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
105	A	4	1	R	1	0	0	1	0	0	0	1	0	0	1	1	0	0	1	0	0	0
106	A	4	1	R	0	0	1	1	0	0	0	1	0	0	0	0	1	0	0	0	0	0
107	A	4	1	R	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0
108	A	4	1	R	1	1	0	1	0	1	1	0	0	0	0	0	0	1	1	0	0	0
109	A	4	1	R	1	0	1	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
110	A	4	1	R	0	1	1	1	0	1	0	0	0	0	1	1	0	1	0	0	0	0
111	A	4	2	R	0	1	0	0	0	NA	NA	0	0	0	0	0	0	1	0	0	0	0
112	A	4	2	R	1	1	0	0	1	1	0	0	0	0	1	1	0	0	0	0	0	0
113	A	4	2	R	NA	NA	0	NA	NA	NA	0	NA	NA	NA	0	0	0	0	0	0	0	0
114	A	4	2	R	0	0	0	1	0	0	0	1	0	0	0	0	1	0	0	0	0	0
115	A	4	2	R	1	1	0	0	1	1	1	0	0	0	1	1	0	0	0	0	0	0
116	A	4	2	R	1	1	0	0	0	NA	NA	0	0	0	1	1	0	0	1	0	0	0
117	A	4	2	R	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0
118	A	4	2	R	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
119	A	4	2	R	1	0	0	1	0	1	0	1	1	0	1	1	0	0	0	0	0	0
120	A	4	2	R	1	1	0	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0
121	A	4	2	R	0	0	0	1	1	0	0	0	0	0	1	1	0	0	0	0	0	0
122	A	4	2	R	1	1	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
123	A	4	2	R	0	0	0	1	0	0	0	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
124	A	4	2	R	0	1	0	1	0	0	0	1	1	0	1	1	0	0	1	0	0	0
125	A	4	2	R	0	1	1	0	0	0	0	1	0	0	0	0	0	1	1	0	1	1
126	A	4	2	R	1	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0
127	A	4	2	R	1	1	0	0	0	1	0	1	1	0	1	1	0	0	1	0	0	0
128	A	4	2	R	1	1	0	0	1	1	1	0	0	0	1	1	0	0	0	0	0	0
129	A	4	2	R	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
130	A	4	2	R	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	NA	NA
131	A	4	3	R	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
132	A	4	3	R	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
133	A	4	3	R	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
134	A	4	3	R	1	0	0	0	1	1	1	0	0	0	1	0	0	1	0	0	0	0
135	A	4	3	R	0	0	0	1	0	0	0	1	0	0	1	1	0	1	0	0	0	0
136	A	4	3	R	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0
137	A	4	3	R	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
138	A	4	3	R	0	0	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0
139	A	4	3	R	0	1	1	1	0	1	0	1	0	0	1	1	0	1	0	0	0	0

140	A	4	3	R	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0
141	A	4	3	R	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0
142	A	4	3	R	1	0	0	1	0	0	0	1	0	0	1	1	0	0	0	0	0
143	A	4	3	R	0	1	0	1	0	NA	NA	0	0	0	1	1	0	1	0	0	0
144	A	4	3	R	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
145	A	4	3	R	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
146	A	4	3	R	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0
147	A	4	3	R	0	0	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
148	A	4	3	R	1	0	0	0	1	1	0	0	0	0	NA	NA	NA	NA	NA	NA	NA
149	A	4	3	R	0	0	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
150	A	4	3	R	1	0	0	0	1	1	0	0	0	0	1	0	0	1	0	0	0
151	A	5	1	R	0	1	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0
152	A	5	1	R	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
153	A	5	1	R	0	1	0	1	1	0	0	0	0	0	0	0	0	0	1	0	0
154	A	5	1	R	0	1	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0
155	A	5	1	R	1	1	0	1	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA
156	A	5	1	R	0	1	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0
157	A	5	1	R	0	0	1	1	0	0	0	1	0	0	1	1	1	0	0	0	0
158	A	5	1	R	0	1	0	1	1	0	0	1	1	0	0	0	0	1	0	0	0
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160	A	5	1	R	0	1	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0
161	A	5	1	R	0	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	1	1
162	A	5	1	R	0	1	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0
163	A	5	1	R	0	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0
164	A	5	1	R	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
165	A	5	1	R	0	1	1	1	0	0	0	1	0	0	1	1	1	0	0	1	0
166	A	5	1	R	0	1	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0
167	A	5	1	R	0	1	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0
168	A	5	1	R	0	1	1	0	0	0	0	1	1	0	1	1	0	0	0	1	0
169	A	5	1	R	0	1	0	1	1	0	0	1	0	0	0	1	0	1	1	0	1
170	A	5	1	R	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
171	A	5	2	R	1	0	0	0	0	NA	NA	1	0	0	1	1	0	0	0	0	0
172	A	5	2	R	1	0	0	1	0	1	0	1	0	0	1	1	0	0	1	0	NA
173	A	5	2	R	0	1	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0
174	A	5	2	R	0	1	0	1	0	0	0	1	0	0	1	1	0	0	0	0	0
175	A	5	2	R	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0
176	A	5	2	R	0	1	0	1	0	0	0	0	0	0	0	1	1	1	1	0	0
177	A	5	2	R	0	1	0	0	1	0	0	0	0	0	0	0	1	1	1	0	0
178	A	5	2	R	1	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0
179	A	5	2	R	1	0	0	0	0	1	0	1	0	0	1	1	0	0	1	0	0
180	A	5	2	R	1	1	0	0	0	NA	NA	1	0	0	0	0	0	1	1	0	0
181	A	5	2	R	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
182	A	5	2	R	1	0	0	1	0	0	0	1	0	0	1	1	0	0	1	0	0
183	A	5	2	R	1	0	0	0	0	1	0	1	0	0	0	1	0	0	1	0	0
184	A	5	2	R	0	1	0	0	0	NA	NA	0	0	0	1	0	0	0	0	0	0
185	A	5	2	R	0	1	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0
186	A	5	2	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
187	A	5	2	R	0	1	1	0	1	1	1	1	1	0	0	0	0	1	0	0	1	1

188	A	5	2	R	1	0	0	1	0	1	0	0	0	0	1	1	0	0	1	0	0	0
189	A	5	2	R	0	1	0	0	0	0	0	1	1	0	1	1	0	0	0	0	0	0
190	A	5	2	R	1	0	0	0	0	NA	NA	1	0	0	1	1	0	0	0	0	0	0
191	A	5	3	R	1	0	0	0	0	NA	NA	1	0	0	0	0	0	0	0	0	0	0
192	A	5	3	R	0	1	1	0	0	1	0	1	1	0	0	0	1	1	0	0	1	1
193	A	5	3	R	0	1	1	1	0	0	0	1	1	0	0	0	1	1	0	0	1	1
194	A	5	3	R	1	1	0	0	1	1	0	0	0	0	1	1	0	0	0	0	0	0
195	A	5	3	R	1	0	0	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0
196	A	5	3	R	0	0	0	0	1	1	0	1	0	0	0	0	0	1	0	0	0	0
197	A	5	3	R	1	0	0	1	0	0	0	1	0	0	1	0	0	1	0	0	0	0
198	A	5	3	R	0	1	1	0	0	1	1	1	1	0	0	0	1	1	0	0	1	1
199	A	5	3	R	0	0	0	0	0	0	0	1	0	0	1	1	0	0	1	0	0	0
200	A	5	3	R	1	1	0	0	0	NA	NA	0	0	0	1	1	0	0	0	0	0	0
201	A	5	3	R	1	0	0	0	0	0	0	1	0	0	0	1	0	0	1	0	0	0
202	A	5	3	R	1	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
203	A	5	3	R	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
204	A	5	3	R	0	0	0	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0
205	A	5	3	R	0	1	0	1	0	0	0	1	1	0	0	0	0	1	0	0	1	1
206	A	5	3	R	0	1	1	1	0	1	0	1	1	0	0	0	0	1	0	0	NA	NA
207	A	5	3	R	0	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	NA	NA
208	A	5	3	R	0	1	1	1	0	1	0	1	1	0	0	0	0	1	0	0	NA	NA
209	A	5	3	R	0	1	0	1	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
210	A	5	3	R	1	1	0	0	0	NA	NA	1	1	0	0	0	0	0	0	0	NA	NA
211	A	1	1	RS	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	NA	NA
212	A	1	1	RS	1	0	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
213	A	1	1	RS	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
214	A	1	1	RS	1	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	0	0
215	A	1	1	RS	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0	NA	NA
216	A	1	1	RS	1	1	0	1	0	NA	NA	1	0	0	0	0	0	0	1	0	NA	NA
217	A	1	1	RS	0	0	0	1	0	0	0	1	0	0	1	1	0	0	1	0	NA	NA
218	A	1	1	RS	0	0	0	1	1	1	0	1	0	0	0	0	0	1	0	0	0	0
219	A	1	1	RS	0	0	0	1	0	0	0	1	0	0	1	1	0	0	1	0	0	0
220	A	1	1	RS	0	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0
221	A	1	1	RS	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1	0	NA	NA
222	A	1	1	RS	0	0	0	0	0	1	0	1	0	0	1	1	0	1	1	0	0	0
223	A	1	1	RS	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	NA	NA
224	A	1	1	RS	1	0	0	1	0	0	0	1	0	0	1	1	0	1	0	0	0	0
225	A	1	1	RS	1	0	0	1	0	1	1	1	0	0	1	1	0	0	0	0	0	0
226	A	1	1	RS	0	0	0	1	1	1	0	1	0	0	0	0	0	1	1	0	0	0
227	A	1	1	RS	0	0	0	1	1	1	0	1	1	0	0	0	0	1	1	0	0	0
228	A	1	1	RS	0	0	0	1	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
229	A	1	1	RS	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
230	A	1	1	RS	1	0	1	0	1	1	0	1	1	0	0	0	0	1	0	0	0	0
231	A	1	1	RS	1	0	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
232	A	1	1	RS	1	0	0	0	0	NA	NA	0	0	0	1	1	0	0	0	0	NA	NA
233	A	1	1	RS	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
234	A	1	1	RS	1	1	1	1	0	0	0	1	0	0	1	1	0	0	1	0	0	0
235	A	1	1	RS	1	0	0	0	0	NA	NA	1	0	0	0	0	0	0	0	0	NA	NA

236	A	1	1	RS	0	0	0	1	1	0	0	1	1	0	0	0	1	1	1	0	0	0
237	A	1	1	RS	0	0	0	1	0	0	0	1	0	0	1	1	0	0	1	0	NA	NA
238	A	1	1	RS	1	0	0	0	0	1	0	1	1	0	1	1	0	0	1	0	0	0
239	A	1	1	RS	1	0	1	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0
240	A	1	1	RS	0	0	0	0	0	NA	NA	1	1	0	NA	NA	NA	NA	NA	NA	NA	NA
241	A	2	1	RS	1	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	0	0
242	A	2	1	RS	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
243	A	2	1	RS	1	0	0	1	1	1	0	1	0	0	0	0	0	1	1	0	0	0
244	A	2	1	RS	0	0	0	1	0	NA	NA	0	0	0	1	1	0	1	0	0	0	0
245	A	2	1	RS	1	1	0	1	0	NA	NA	1	0	0	1	1	0	0	0	0	0	0
246	A	2	1	RS	1	1	0	1	0	1	0	1	0	0	0	0	0	0	1	0	0	0
247	A	2	1	RS	1	0	0	1	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
248	A	2	1	RS	1	1	0	0	0	0	0	1	0	0	1	1	0	0	1	0	0	0
249	A	2	1	RS	1	0	0	0	0	NA	NA	1	0	0	1	1	0	0	0	0	0	0
250	A	2	1	RS	1	0	0	0	0	NA	NA	1	0	0	1	1	0	0	0	0	0	0
251	A	2	1	RS	1	1	0	0	0	NA	NA	0	0	0	1	0	0	0	0	0	0	0
252	A	2	1	RS	1	0	0	1	0	1	0	1	0	0	0	0	0	0	1	0	0	0
253	A	2	1	RS	1	1	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0
254	A	2	1	RS	1	1	1	1	0	0	0	1	0	0	1	1	0	0	1	0	0	0
255	A	2	1	RS	0	1	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0
256	A	2	1	RS	1	0	0	0	0	1	0	1	0	0	0	0	0	1	1	0	0	0
257	A	2	1	RS	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
258	A	2	1	RS	1	0	0	1	0	0	0	1	0	0	1	1	0	0	1	0	0	0
259	A	2	1	RS	1	0	0	NA	NA	NA	0	1	1	0	1	1	0	0	0	0	0	0
260	A	2	1	RS	1	0	0	1	0	1	0	1	0	0	1	0	0	0	1	0	0	0
261	A	2	2	RS	1	1	0	1	0	1	0	1	1	0	1	1	0	0	0	0	0	0
262	A	2	2	RS	1	1	0	0	0	1	0	1	0	0	1	1	0	1	0	0	0	0
263	A	2	2	RS	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
264	A	2	2	RS	0	1	0	1	0	1	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
265	A	2	2	RS	1	1	0	1	0	1	0	1	1	0	1	1	0	0	0	0	0	0
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267	A	2	2	RS	0	1	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
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269	A	2	2	RS	1	0	0	0	0	1	0	1	0	0	1	0	0	0	1	0	0	0
270	A	2	2	RS	1	1	0	0	0	1	0	1	1	0	1	1	0	0	0	0	0	0
271	A	2	2	RS	1	1	0	1	0	0	0	1	0	0	1	1	0	0	1	0	0	0
272	A	2	2	RS	1	0	0	1	0	1	0	1	0	0	0	0	0	0	1	0	0	0
273	A	2	2	RS	1	1	0	1	1	1	0	0	0	0	1	1	0	0	0	0	0	0
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276	A	2	2	RS	0	0	0	0	1	1	0	1	1	0	0	0	1	0	1	0	0	0
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279	A	2	2	RS	1	1	0	1	0	0	0	1	0	0	1	1	0	0	1	0	0	0
280	A	2	2	RS	1	1	1	1	0	0	0	1	0	0	1	1	0	0	0	0	0	0
281	A	2	3	RS	1	0	0	0	0	1	0	0	0	0	0	0	0	1	1	0	0	0
282	A	2	3	RS	1	0	0	1	0	1	0	1	0	0	0	0	0	0	1	0	0	0
283	A	2	3	RS	0	0	0	0	1	1	0	0	0	0	0	0	0	1	1	0	0	0

284	A	2	3	RS	0	0	0	0	0	NA	NA	0	0	0	1	1	0	0	1	0	0	0
285	A	2	3	RS	0	1	0	0	0	0	0	1	0	0	1	1	0	0	1	0	0	0
286	A	2	3	RS	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0
287	A	2	3	RS	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
288	A	2	3	RS	0	1	0	0	0	0	0	1	0	0	1	0	0	0	1	0	0	0
289	A	2	3	RS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
290	A	2	3	RS	1	0	0	1	1	1	0	0	0	0	1	1	0	0	0	0	0	0
291	A	2	3	RS	1	1	0	0	0	NA	NA	1	1	0	0	0	0	1	1	0	0	0
292	A	2	3	RS	0	0	1	1	1	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
293	A	2	3	RS	1	0	0	1	0	1	0	1	0	0	1	0	0	0	1	0	0	0
294	A	2	3	RS	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
295	A	2	3	RS	0	0	0	1	0	0	0	0	0	0	1	1	0	1	0	0	0	0
296	A	2	3	RS	1	0	0	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0
297	A	2	3	RS	0	0	0	0	1	1	0	0	0	0	0	0	0	1	1	0	0	0
298	A	2	3	RS	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
299	A	2	3	RS	0	1	0	0	0	NA	NA	0	0	0	0	0	0	0	0	0	0	0
300	A	2	3	RS	1	1	1	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
301	A	4	1	RS	1	0	0	1	0	1	0	1	0	0	1	0	0	0	1	0	0	0
302	A	4	1	RS	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	NA	NA
303	A	4	1	RS	1	0	0	1	0	1	0	1	0	0	1	0	0	0	1	0	NA	NA
304	A	4	1	RS	1	0	0	1	0	1	0	1	0	0	1	0	0	0	1	0	NA	NA
305	A	4	1	RS	1	1	0	1	0	1	0	1	1	0	1	1	0	0	0	0	NA	NA
306	A	4	1	RS	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
307	A	4	1	RS	1	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	NA	NA
308	A	4	1	RS	1	1	0	1	0	NA	NA	0	0	0	1	1	0	0	0	0	0	0
309	A	4	1	RS	0	0	0	0	0	NA	NA	1	1	0	NA	NA	NA	NA	NA	NA	NA	NA
310	A	4	1	RS	1	1	0	1	0	1	0	1	0	0	1	1	0	0	1	0	NA	NA
311	A	4	1	RS	0	1	1	1	0	0	0	1	0	0	1	1	0	1	1	0	0	0
312	A	4	1	RS	1	1	0	1	0	0	0	1	0	0	1	1	0	0	1	0	0	0
313	A	4	1	RS	1	0	0	1	0	NA	NA	1	0	0	0	0	0	1	1	0	NA	NA
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315	A	4	1	RS	1	1	0	1	0	NA	NA	1	1	0	NA	NA	NA	NA	NA	NA	NA	NA
316	A	4	1	RS	1	1	0	0	0	1	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
317	A	4	1	RS	0	1	1	1	0	1	0	1	0	0	1	0	0	0	1	0	0	0
318	A	4	1	RS	1	1	0	0	0	0	0	1	0	0	1	0	0	0	1	0	0	0
319	A	4	1	RS	1	0	0	1	0	1	0	1	0	0	1	0	0	0	1	0	0	0
320	A	4	1	RS	1	0	0	1	0	1	0	1	0	0	1	0	0	0	1	0	0	0
321	A	4	2	RS	0	0	0	1	0	0	0	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
322	A	4	2	RS	1	1	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0
323	A	4	2	RS	1	1	1	1	0	1	0	1	1	0	1	1	0	0	1	0	0	0
324	A	4	2	RS	1	1	1	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0
325	A	4	2	RS	0	1	1	0	0	1	0	1	1	0	0	0	1	1	1	0	1	1
326	A	4	2	RS	0	1	0	1	0	0	0	1	0	0	0	0	0	1	1	0	0	0
327	A	4	2	RS	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
328	A	4	2	RS	1	0	0	1	0	1	0	1	1	0	1	1	0	0	0	0	0	0
329	A	4	2	RS	1	1	0	0	1	1	0	0	0	0	1	1	0	0	0	0	0	0
330	A	4	2	RS	0	1	0	1	0	1	0	1	1	0	0	0	1	0	0	0	1	1
331	A	4	2	RS	1	0	0	1	0	0	0	1	0	0	1	1	0	0	1	0	0	0

332	A	4	2	RS	1	1	0	0	0	NA	NA	0	0	0	0	0	0	0	0	0
333	A	4	2	RS	1	1	0	0	1	1	0	0	0	0	1	1	0	0	0	0
334	A	4	2	RS	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0
335	A	4	2	RS	1	1	0	0	1	1	0	0	0	0	1	1	0	0	0	0
336	A	4	2	RS	1	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0
337	A	4	2	RS	1	0	0	1	0	1	0	1	0	0	1	1	0	0	0	0
338	A	4	2	RS	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
339	A	4	2	RS	0	1	0	0	0	NA	NA	1	1	0	0	0	0	0	0	0
340	A	4	2	RS	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
341	A	4	3	RS	0	0	0	1	0	1	0	1	1	0	0	0	1	1	0	0
342	A	4	3	RS	1	0	0	1	0	1	0	1	0	0	0	1	0	0	1	0
343	A	4	3	RS	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0
344	A	4	3	RS	0	0	0	0	0	1	0	1	0	0	1	0	0	0	1	0
345	A	4	3	RS	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0
346	A	4	3	RS	0	0	0	0	0	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA
347	A	4	3	RS	NA	NA	0	NA	NA	NA	0	NA	NA	NA	0	0	0	0	1	0
348	A	4	3	RS	0	1	1	0	0	0	0	1	1	0	0	0	1	1	0	0
349	A	4	3	RS	1	0	0	0	0	0	0	1	1	0	0	0	0	1	1	0
350	A	4	3	RS	1	0	0	0	1	1	0	0	0	0	1	0	0	1	0	0
351	A	4	3	RS	0	0	0	0	0	1	0	1	1	0	1	1	0	0	1	0
352	A	4	3	RS	1	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0
353	A	4	3	RS	1	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0
354	A	4	3	RS	1	0	0	0	0	NA	NA	0	0	0	0	0	0	0	0	0
355	A	4	3	RS	1	0	0	0	0	1	0	0	0	0	1	0	0	1	0	0
356	A	4	3	RS	1	1	0	0	0	NA	NA	0	0	0	0	0	0	0	0	0
357	A	4	3	RS	1	1	0	1	0	NA	NA	1	0	0	1	1	0	0	0	0
358	A	4	3	RS	0	0	0	1	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA
359	A	4	3	RS	1	1	0	1	0	0	0	0	0	0	1	1	0	0	0	0
360	A	4	3	RS	1	0	0	1	0	1	0	1	0	0	1	1	0	0	0	0
361	A	5	1	RS	1	1	0	0	1	1	0	0	0	0	1	1	0	0	0	0
362	A	5	1	RS	1	1	0	1	0	1	0	1	1	0	1	1	0	0	0	0
363	A	5	1	RS	1	1	0	1	0	1	0	1	1	0	1	1	0	0	0	0
364	A	5	1	RS	1	0	0	0	0	1	0	1	0	0	1	1	0	0	0	0
365	A	5	1	RS	1	1	0	1	0	0	0	1	1	0	1	1	0	0	0	0
366	A	5	1	RS	1	0	0	0	0	1	0	1	0	0	1	1	0	0	0	0
367	A	5	1	RS	1	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0
368	A	5	1	RS	1	0	0	1	0	NA	NA	1	1	0	1	1	0	0	0	0
369	A	5	1	RS	1	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0
370	A	5	1	RS	1	0	0	1	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA
371	A	5	1	RS	1	0	1	1	0	1	0	1	0	0	1	1	0	0	1	0
372	A	5	1	RS	1	1	0	1	0	1	0	1	0	0	1	1	0	0	1	0
373	A	5	1	RS	1	0	0	0	0	1	0	1	0	0	1	1	0	0	0	0
374	A	5	1	RS	1	0	0	0	0	NA	NA	1	0	0	1	1	0	0	0	0
375	A	5	1	RS	1	0	0	1	0	1	0	1	0	0	1	1	0	0	0	1
376	A	5	1	RS	1	0	0	0	0	1	0	1	0	0	1	1	0	0	1	0
377	A	5	1	RS	1	0	0	1	0	1	0	1	0	0	1	1	0	0	0	0
378	A	5	1	RS	1	0	0	1	0	1	0	1	0	0	1	1	0	0	1	0
379	A	5	1	RS	1	0	0	1	0	NA	NA	0	0	0	0	1	0	0	1	0

380	A	5	1	RS	1	0	0	0	0	NA	NA	1	0	0	0	0	0	1	1	0	0	0
381	A	5	2	RS	0	0	0	0	0	NA	NA	0	0	0	1	1	0	0	1	0	0	0
382	A	5	2	RS	0	0	0	1	0	0	0	1	0	0	1	1	0	0	1	0	0	0
383	A	5	2	RS	1	1	0	1	0	NA	NA	0	0	0	1	1	0	0	0	0	0	0
384	A	5	2	RS	0	1	1	0	0	0	0	1	1	0	0	0	1	1	0	0	1	1
385	A	5	2	RS	1	0	0	0	0	NA	NA	1	0	0	1	0	0	1	1	0	0	0
386	A	5	2	RS	1	0	0	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0
387	A	5	2	RS	1	1	0	1	0	0	0	1	0	0	1	1	0	0	1	0	0	0
388	A	5	2	RS	1	1	0	0	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
389	A	5	2	RS	1	1	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0
390	A	5	2	RS	1	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0
391	A	5	2	RS	1	1	0	1	0	0	0	1	1	0	1	1	0	0	0	0	0	0
392	A	5	2	RS	1	1	1	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
393	A	5	2	RS	1	0	1	0	0	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
394	A	5	2	RS	1	0	1	1	0	1	0	1	0	0	0	1	0	0	1	0	0	0
395	A	5	2	RS	1	1	0	1	0	0	0	1	1	0	1	1	0	0	0	0	0	0
396	A	5	2	RS	0	1	0	0	0	1	0	1	1	0	0	0	1	1	0	0	1	1
397	A	5	2	RS	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
398	A	5	2	RS	1	1	0	0	0	NA	NA	0	0	0	0	0	0	0	0	0	0	0
399	A	5	2	RS	1	0	0	1	0	1	0	1	1	0	1	1	0	0	0	0	0	0
400	A	5	2	RS	1	1	1	0	0	1	0	1	0	0	1	1	0	0	0	0	0	0
401	A	5	3	RS	1	1	1	1	0	0	0	1	0	0	1	1	0	0	0	0	0	0
402	A	5	3	RS	1	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0
403	A	5	3	RS	1	0	1	0	0	NA	NA	1	0	0	0	1	0	0	1	0	0	0
404	A	5	3	RS	1	1	0	1	0	0	0	1	0	0	1	1	0	0	0	0	0	0
405	A	5	3	RS	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0
406	A	5	3	RS	0	1	0	0	0	NA	NA	1	1	0	1	1	0	1	0	0	0	0
407	A	5	3	RS	1	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
408	A	5	3	RS	1	1	0	0	0	NA	NA	1	1	0	1	1	0	0	0	0	0	0
409	A	5	3	RS	1	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
410	A	5	3	RS	1	1	0	1	0	0	0	1	0	0	1	1	0	0	0	0	0	0
411	A	5	3	RS	1	0	0	0	1	1	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
412	A	5	3	RS	1	0	1	1	0	1	0	1	0	0	0	1	0	0	1	0	0	0
413	A	5	3	RS	1	1	0	1	0	1	0	1	0	0	0	0	0	0	1	0	0	0
414	A	5	3	RS	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0
415	A	5	3	RS	1	0	0	1	0	1	0	1	0	0	0	0	0	0	1	0	0	0
416	A	5	3	RS	1	0	1	1	0	0	0	1	0	0	0	0	0	0	1	0	0	0
417	A	5	3	RS	0	0	0	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0
418	A	5	3	RS	1	0	0	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0
419	A	5	3	RS	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0
420	A	5	3	RS	0	1	0	0	0	0	0	1	1	0	0	0	0	1	0	0	1	1
421	B	1	1	R	0	0	0	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0
422	B	1	1	R	0	1	1	1	0	0	0	1	0	0	1	1	0	1	1	0	0	0
423	B	1	1	R	0	0	0	0	0	NA	NA	1	0	0	1	1	0	1	0	0	0	0
424	B	1	1	R	0	0	0	0	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
425	B	1	1	R	0	0	0	0	1	1	0	1	1	0	1	1	0	1	0	0	1	1
426	B	1	1	R	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
427	B	1	1	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

428	B	1	1	R	0	0	1	0	0	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
429	B	1	1	R	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
430	B	1	1	R	0	0	1	0	1	1	0	0	0	0	0	0	0	1	0	0	1	1
431	B	1	1	R	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
432	B	1	1	R	0	1	0	0	1	0	0	1	1	0	1	0	0	0	0	0	0	0
433	B	1	1	R	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
434	B	1	1	R	0	0	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0
435	B	1	1	R	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
436	B	1	1	R	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
437	B	1	1	R	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0
438	B	1	1	R	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0
439	B	1	1	R	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0
440	B	1	1	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
441	B	1	1	R	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
442	B	1	1	R	0	0	0	0	1	1	0	1	0	0	1	1	0	1	0	0	0	0
443	B	1	1	R	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
444	B	1	1	R	1	0	0	1	1	1	0	1	0	0	0	0	0	1	0	0	0	0
445	B	1	1	R	0	1	1	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0
446	B	1	1	R	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
447	B	1	1	R	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
448	B	1	1	R	0	1	0	1	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
449	B	1	1	R	0	0	1	1	1	0	0	1	0	0	0	0	0	0	0	0	1	1
450	B	1	1	R	0	0	0	1	0	1	0	1	1	0	1	1	0	1	0	0	1	1
451	B	2	1	R	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
452	B	2	1	R	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
453	B	2	1	R	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
454	B	2	1	R	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
455	B	2	1	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
456	B	2	1	R	0	0	1	1	1	0	0	1	0	0	0	0	0	0	0	0	1	1
457	B	2	1	R	0	0	1	0	1	0	0	0	0	0	0	0	1	1	0	0	0	0
458	B	2	1	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
459	B	2	1	R	0	1	1	1	0	0	0	0	0	0	0	0	1	1	0	0	1	1
460	B	2	1	R	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0
461	B	2	1	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
462	B	2	1	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
463	B	2	1	R	0	0	0	1	0	0	0	1	0	0	0	0	0	0	1	0	0	0
464	B	2	1	R	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0
465	B	2	1	R	0	0	0	1	1	0	0	1	0	0	0	0	0	1	1	0	0	0
466	B	2	1	R	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
467	B	2	1	R	0	1	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0
468	B	2	1	R	0	1	0	0	1	1	1	0	0	0	1	1	0	1	0	0	0	0
469	B	2	1	R	1	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
470	B	2	1	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
471	B	2	2	R	0	1	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0
472	B	2	2	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
473	B	2	2	R	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	NA	NA
474	B	2	2	R	0	0	0	0	0	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
475	B	2	2	R	0	0	1	0	0	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA

476	B	2	2	R	0	0	0	1	1	0	0	1	0	0	0	0	0	1	0	0	0	0
477	B	2	2	R	0	0	0	1	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
478	B	2	2	R	0	0	1	1	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
479	B	2	2	R	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
480	B	2	2	R	1	0	1	0	0	1	0	1	1	0	0	0	1	1	0	0	1	1
481	B	2	2	R	0	1	0	0	1	0	0	0	0	0	1	1	1	0	0	1	0	0
482	B	2	2	R	0	0	0	1	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
483	B	2	2	R	0	1	0	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0
484	B	2	2	R	0	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
485	B	2	2	R	0	0	1	1	0	1	0	1	0	0	0	0	0	1	0	0	0	0
486	B	2	2	R	0	0	0	0	1	0	0	0	0	0	0	0	1	1	1	0	0	0
487	B	2	2	R	0	1	0	1	0	NA	NA	1	1	0	NA	NA	NA	NA	NA	NA	NA	NA
488	B	2	2	R	0	0	0	1	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
489	B	2	2	R	0	0	1	0	0	1	0	1	1	0	0	0	1	1	0	0	1	1
490	B	2	2	R	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
491	B	2	3	R	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
492	B	2	3	R	1	0	0	1	0	1	0	1	1	0	1	1	0	0	0	0	0	0
493	B	2	3	R	0	0	0	0	0	NA	NA	1	0	0	1	1	0	1	1	0	0	0
494	B	2	3	R	0	0	0	1	1	0	0	1	0	0	0	0	0	1	0	0	0	0
495	B	2	3	R	0	0	0	0	0	NA	NA	0	0	0	1	1	0	1	0	0	NA	NA
496	B	2	3	R	0	0	1	0	0	1	0	0	0	0	1	1	0	1	0	0	0	0
497	B	2	3	R	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
498	B	2	3	R	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
499	B	2	3	R	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
500	B	2	3	R	1	1	0	0	0	NA	NA	0	0	0	1	1	0	0	0	0	0	0
501	B	2	3	R	1	1	0	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0
502	B	2	3	R	0	0	0	0	0	NA	NA	1	0	0	1	1	0	1	1	0	0	0
503	B	2	3	R	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	1	1
504	B	2	3	R	1	0	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
505	B	2	3	R	0	0	1	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0
506	B	2	3	R	0	1	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0
507	B	2	3	R	0	0	0	0	0	NA	NA	0	0	0	1	1	0	1	0	0	0	0
508	B	2	3	R	0	0	0	0	0	NA	NA	1	0	0	0	0	0	1	0	0	0	0
509	B	2	3	R	1	0	0	0	0	1	0	0	0	0	1	1	0	1	0	0	0	0
510	B	2	3	R	0	1	1	0	0	0	0	1	1	0	1	1	0	0	0	1	0	0
511	B	4	1	R	0	1	1	1	0	1	0	0	0	0	0	1	0	1	0	0	1	1
512	B	4	1	R	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	NA	NA
513	B	4	1	R	0	0	0	1	0	1	0	1	0	0	0	0	0	1	0	0	0	0
514	B	4	1	R	0	1	0	0	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	0	0
515	B	4	1	R	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
516	B	4	1	R	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0
517	B	4	1	R	1	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
518	B	4	1	R	0	0	0	1	0	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
519	B	4	1	R	1	1	0	0	1	1	1	0	0	0	1	1	0	0	0	0	0	0
520	B	4	1	R	0	0	0	1	0	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
521	B	4	1	R	0	1	1	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0
522	B	4	1	R	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0
523	B	4	1	R	0	0	0	1	0	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA

524	B	4	1	R	0	1	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0
525	B	4	1	R	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
526	B	4	1	R	0	1	1	1	0	1	0	0	0	0	1	1	0	1	0	0	0	0
527	B	4	1	R	0	1	1	1	0	NA	NA	0	0	0	0	1	0	1	0	0	1	1
528	B	4	1	R	0	0	0	0	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
529	B	4	1	R	0	0	0	1	0	0	0	1	0	0	0	0	0	1	0	0	0	0
530	B	4	1	R	0	1	1	1	0	NA	NA	1	0	0	0	1	0	0	0	0	1	1
531	B	4	2	R	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	NA	NA
532	B	4	2	R	0	1	1	1	0	1	0	0	0	0	0	1	0	1	0	0	1	1
533	B	4	2	R	0	1	1	1	0	1	0	0	0	0	1	1	0	0	0	0	0	0
534	B	4	2	R	0	1	0	1	0	0	0	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
535	B	4	2	R	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
536	B	4	2	R	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1
537	B	4	2	R	0	0	0	0	1	0	0	0	0	0	0	0	1	1	1	0	0	0
538	B	4	2	R	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
539	B	4	2	R	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
540	B	4	2	R	1	1	0	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0
541	B	4	2	R	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1
542	B	4	2	R	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
543	B	4	2	R	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
544	B	4	2	R	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
545	B	4	2	R	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
546	B	4	2	R	0	1	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0
547	B	4	2	R	0	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
548	B	4	2	R	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
549	B	4	2	R	0	1	0	0	0	NA	NA	0	0	0	0	0	0	0	0	0	NA	NA
550	B	4	2	R	0	1	1	1	0	1	0	0	0	0	1	1	0	1	0	0	0	0
551	B	4	3	R	1	0	0	1	0	1	1	1	0	0	1	1	0	0	1	0	0	0
552	B	4	3	R	1	0	1	0	0	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
553	B	4	3	R	1	1	1	0	0	NA	NA	0	0	0	0	0	0	0	0	0	NA	NA
554	B	4	3	R	0	1	0	0	0	1	0	0	0	0	0	1	0	1	0	0	0	0
555	B	4	3	R	1	0	0	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0
556	B	4	3	R	1	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0
557	B	4	3	R	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
558	B	4	3	R	0	0	1	0	0	1	0	0	0	0	0	1	0	1	0	0	0	0
559	B	4	3	R	1	1	1	0	0	1	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
560	B	4	3	R	1	0	1	0	0	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
561	B	4	3	R	0	0	1	1	0	1	0	0	0	0	1	1	0	1	0	1	0	0
562	B	4	3	R	0	0	0	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0
563	B	4	3	R	1	1	1	0	0	NA	NA	0	0	0	0	0	0	1	0	0	NA	NA
564	B	4	3	R	0	1	1	1	0	NA	NA	0	0	0	0	1	0	1	0	0	1	1
565	B	4	3	R	0	1	1	1	0	1	0	0	0	0	1	1	0	0	0	0	0	0
566	B	4	3	R	1	0	1	0	0	1	0	1	0	0	0	1	0	1	0	0	0	0
567	B	4	3	R	1	1	0	0	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
568	B	4	3	R	1	1	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0
569	B	4	3	R	0	0	1	0	0	1	0	0	0	0	1	1	0	1	0	0	0	0
570	B	4	3	R	0	1	1	1	0	NA	NA	0	0	0	1	1	0	1	0	0	0	0
571	B	5	1	R	0	0	0	1	1	0	0	0	0	0	0	1	0	1	0	0	0	0

572	B	5	1	R	1	1	0	0	1	1	0	0	0	0	1	1	0	0	0	0	0	0
573	B	5	1	R	0	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0
574	B	5	1	R	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0
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576	B	5	1	R	1	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0
577	B	5	1	R	1	1	0	0	0	1	0	1	1	0	0	0	0	1	0	0	0	0
578	B	5	1	R	1	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
579	B	5	1	R	1	0	0	0	0	1	0	1	0	0	0	1	0	1	0	0	0	0
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581	B	5	1	R	1	0	0	0	0	NA	NA	0	0	0	0	0	0	0	0	0	0	0
582	B	5	1	R	1	1	0	0	1	1	0	0	0	0	1	1	0	0	0	0	0	0
583	B	5	1	R	1	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
584	B	5	1	R	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0
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587	B	5	1	R	0	1	0	1	1	NA	NA	1	0	0	0	1	0	0	1	0	NA	NA
588	B	5	1	R	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
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591	B	5	2	R	0	0	0	1	1	1	0	0	0	0	0	1	0	1	0	0	0	0
592	B	5	2	R	0	1	0	1	0	1	0	0	0	0	0	0	0	1	0	0	0	0
593	B	5	2	R	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0
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599	B	5	2	R	1	0	0	1	0	0	0	1	0	0	1	1	0	0	0	0	0	0
600	B	5	2	R	0	1	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0
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602	B	5	2	R	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
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605	B	5	2	R	0	0	0	1	0	0	0	1	0	0	0	0	1	0	0	0	0	0
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607	B	5	2	R	0	1	0	0	1	1	0	0	0	0	1	0	0	0	0	0	0	0
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611	B	5	3	R	1	1	0	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0
612	B	5	3	R	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1
613	B	5	3	R	1	1	0	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0
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615	B	5	3	R	0	1	1	0	0	1	0	1	1	0	0	0	1	0	0	0	1	1
616	B	5	3	R	1	1	0	0	1	1	0	0	0	0	1	1	0	0	0	0	0	0
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618	B	5	3	R	1	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
619	B	5	3	R	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0

620	B	5	3	R	0	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
621	B	5	3	R	1	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0
622	B	5	3	R	1	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
623	B	5	3	R	1	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	0	0
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625	B	5	3	R	1	1	0	0	1	1	0	0	0	0	1	1	0	0	0	0	0	0
626	B	5	3	R	1	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
627	B	5	3	R	1	0	0	0	0	1	0	1	0	0	1	1	0	0	0	0	0	0
628	B	5	3	R	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0
629	B	5	3	R	1	0	0	1	0	0	0	1	0	0	1	1	0	0	0	0	0	0
630	B	5	3	R	1	1	0	0	0	NA	NA	0	0	0	1	1	0	0	0	0	0	0
631	B	1	1	RS	1	1	0	0	0	NA	NA	0	0	0	1	0	0	0	0	0	0	0
632	B	1	1	RS	0	1	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0
633	B	1	1	RS	0	1	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0
634	B	1	1	RS	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
635	B	1	1	RS	1	0	0	0	0	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
636	B	1	1	RS	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0
637	B	1	1	RS	1	0	0	1	1	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
638	B	1	1	RS	0	0	1	1	0	1	0	1	0	0	1	0	0	0	1	0	0	0
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640	B	1	1	RS	1	0	0	0	1	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
641	B	1	1	RS	0	1	1	1	0	1	0	1	1	0	0	0	0	1	0	0	1	1
642	B	1	1	RS	1	1	0	1	0	0	0	1	0	0	1	1	0	0	0	0	0	0
643	B	1	1	RS	1	1	0	1	0	0	0	1	0	0	1	1	0	0	1	0	0	0
644	B	1	1	RS	0	1	1	0	0	0	0	1	1	0	0	0	1	1	0	0	1	1
645	B	1	1	RS	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
646	B	1	1	RS	0	0	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0
647	B	1	1	RS	1	1	0	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0
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649	B	1	1	RS	0	1	1	0	0	1	0	1	1	0	0	0	1	1	0	0	1	1
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651	B	1	1	RS	1	1	0	1	0	0	0	1	0	0	1	1	0	0	1	0	0	0
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653	B	1	1	RS	1	0	1	1	0	1	0	0	0	0	1	1	0	0	0	0	0	0
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657	B	1	1	RS	0	1	0	0	1	0	0	1	1	0	1	0	0	0	0	0	0	0
658	B	1	1	RS	1	1	0	0	0	NA	NA	1	0	0	0	0	0	0	1	0	0	0
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660	B	1	1	RS	0	1	1	1	1	NA	NA	1	1	0	NA	NA	NA	NA	NA	NA	NA	NA
661	B	2	1	RS	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0
662	B	2	1	RS	0	1	0	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0
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664	B	2	1	RS	1	0	0	0	0	NA	NA	1	0	0	1	1	0	0	0	0	0	0
665	B	2	1	RS	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
666	B	2	1	RS	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0
667	B	2	1	RS	1	1	0	1	0	NA	NA	1	0	0	1	1	0	0	1	0	0	0

668	B	2	1	RS	1	0	0	0	0	NA	NA	1	0	0	1	1	0	0	0	0	0	0
669	B	2	1	RS	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
670	B	2	1	RS	1	0	0	1	0	1	0	1	1	0	1	1	0	0	0	0	0	0
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676	B	2	1	RS	1	0	0	1	0	0	0	1	0	0	1	1	0	0	1	0	0	0
677	B	2	1	RS	1	0	0	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0
678	B	2	1	RS	0	0	0	1	1	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
679	B	2	1	RS	1	0	0	0	0	NA	NA	0	0	0	1	1	0	0	0	0	0	0
680	B	2	1	RS	1	1	0	0	1	1	0	0	0	0	1	1	0	0	0	0	0	0
681	B	2	2	RS	1	0	0	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0
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688	B	2	2	RS	1	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
689	B	2	2	RS	1	1	0	1	0	1	0	0	0	0	1	1	0	0	0	0	0	0
690	B	2	2	RS	1	1	0	0	0	NA	NA	1	0	0	1	1	0	0	1	0	0	0
691	B	2	2	RS	1	1	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
692	B	2	2	RS	1	1	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
693	B	2	2	RS	1	1	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
694	B	2	2	RS	1	1	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
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696	B	2	2	RS	1	1	0	0	0	1	0	1	0	0	1	1	0	0	1	0	0	0
697	B	2	2	RS	1	0	0	1	0	0	0	1	0	0	1	1	0	0	1	0	0	0
698	B	2	2	RS	1	1	0	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0
699	B	2	2	RS	1	1	0	0	0	NA	NA	0	0	0	1	1	0	0	0	0	0	0
700	B	2	2	RS	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
701	B	2	3	RS	0	0	0	0	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
702	B	2	3	RS	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
703	B	2	3	RS	1	0	0	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0
704	B	2	3	RS	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0	0	0
705	B	2	3	RS	1	0	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
706	B	2	3	RS	1	0	0	1	0	1	0	1	0	0	0	1	0	0	1	0	0	0
707	B	2	3	RS	1	1	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0
708	B	2	3	RS	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
709	B	2	3	RS	1	1	0	0	0	1	0	1	0	0	0	1	0	0	1	0	0	0
710	B	2	3	RS	1	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0
711	B	2	3	RS	0	0	0	1	0	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
712	B	2	3	RS	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
713	B	2	3	RS	0	0	0	1	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
714	B	2	3	RS	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
715	B	2	3	RS	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0

716	B	2	3	RS	1	0	0	0	0	NA	NA	1	0	0	0	0	0	0	0	0	0	0
717	B	2	3	RS	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1
718	B	2	3	RS	1	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0
719	B	2	3	RS	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
720	B	2	3	RS	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
721	B	4	1	RS	1	0	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0
722	B	4	1	RS	1	0	1	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0
723	B	4	1	RS	1	0	1	0	0	NA	NA	1	1	0	NA	NA	NA	NA	NA	NA	0	0
724	B	4	1	RS	1	1	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
725	B	4	1	RS	1	0	1	0	0	1	0	1	1	0	1	1	0	0	0	0	0	0
726	B	4	1	RS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
727	B	4	1	RS	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
728	B	4	1	RS	1	1	0	1	0	1	0	1	1	0	1	1	0	0	0	0	0	0
729	B	4	1	RS	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
730	B	4	1	RS	1	0	1	1	0	0	0	1	0	0	1	1	0	0	1	0	0	0
731	B	4	1	RS	1	1	0	0	0	NA	NA	0	0	0	1	1	0	0	0	0	0	0
732	B	4	1	RS	1	1	0	1	0	0	0	1	0	0	0	1	0	0	1	0	0	0
733	B	4	1	RS	1	0	0	1	0	1	0	1	0	0	0	0	0	0	1	0	NA	NA
734	B	4	1	RS	1	0	0	1	0	0	0	1	0	0	1	1	0	0	1	0	NA	NA
735	B	4	1	RS	1	0	0	0	0	1	0	1	0	0	1	0	0	0	1	0	NA	NA
736	B	4	1	RS	1	0	0	1	0	1	0	1	0	0	1	1	0	0	1	0	NA	NA
737	B	4	1	RS	1	1	0	0	0	NA	NA	0	0	0	0	0	0	1	1	0	0	0
738	B	4	1	RS	1	0	0	0	0	1	0	1	0	0	1	1	0	0	1	0	0	0
739	B	4	1	RS	1	0	0	1	0	0	0	1	0	0	1	1	0	0	0	0	0	0
740	B	4	1	RS	1	1	1	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
741	B	4	2	RS	1	0	0	NA	NA	NA	0	1	0	0	0	0	0	1	0	0	0	0
742	B	4	2	RS	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	NA	NA
743	B	4	2	RS	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0
744	B	4	2	RS	1	1	0	0	0	NA	NA	0	0	0	1	1	0	0	0	0	0	0
745	B	4	2	RS	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0
746	B	4	2	RS	1	1	0	1	0	0	0	1	0	0	1	1	0	0	1	0	0	0
747	B	4	2	RS	1	0	1	0	0	1	0	1	0	0	0	0	0	1	1	0	0	0
748	B	4	2	RS	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1
749	B	4	2	RS	1	0	0	NA	NA	NA	NA	1	0	0	1	1	0	0	1	0	0	0
750	B	4	2	RS	0	1	0	1	0	0	0	1	0	0	0	0	0	0	1	0	0	0
751	B	4	2	RS	0	0	0	1	0	0	0	0	0	0	NA	1	0	1	1	0	NA	NA
752	B	4	2	RS	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
753	B	4	2	RS	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
754	B	4	2	RS	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
755	B	4	2	RS	0	0	1	1	0	1	0	0	0	0	1	1	0	1	0	0	0	0
756	B	4	2	RS	0	1	0	NA	NA	NA	0	0	0	0	0	0	1	1	0	0	0	0
757	B	4	2	RS	1	1	0	0	0	NA	NA	0	0	0	1	1	0	0	0	0	0	0
758	B	4	2	RS	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
759	B	4	2	RS	1	1	0	0	0	NA	NA	1	1	0	1	1	0	0	0	0	0	0
760	B	4	2	RS	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
761	B	4	3	RS	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0
762	B	4	3	RS	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
763	B	4	3	RS	1	1	0	0	0	NA	NA	1	1	0	NA	NA	NA	NA	NA	NA	NA	NA

764	B	4	3	RS	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
765	B	4	3	RS	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	1
766	B	4	3	RS	1	0	0	0	0	NA	NA	1	0	0	0	1	0	0	1	0	0	0
767	B	4	3	RS	0	1	1	1	0	NA	NA	0	0	0	0	1	0	1	0	0	1	1
768	B	4	3	RS	NA	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
769	B	4	3	RS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
770	B	4	3	RS	1	0	0	0	0	1	0	1	0	0	1	1	0	0	1	0	0	0
771	B	4	3	RS	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0
772	B	4	3	RS	1	1	1	0	0	NA	NA	1	0	0	0	1	0	0	0	0	NA	NA
773	B	4	3	RS	1	1	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0
774	B	4	3	RS	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
775	B	4	3	RS	1	1	0	0	0	NA	NA	0	0	0	1	1	0	0	0	0	0	0
776	B	4	3	RS	1	1	0	0	0	1	0	1	1	0	0	1	0	0	0	0	0	0
777	B	4	3	RS	0	1	0	1	0	0	0	0	0	0	0	1	0	1	0	0	0	0
778	B	4	3	RS	0	1	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0
779	B	4	3	RS	1	1	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0
780	B	4	3	RS	0	1	0	1	0	1	0	0	0	0	1	1	0	0	0	0	0	0
781	B	5	1	RS	0	0	0	1	0	0	0	1	0	0	1	1	0	0	0	0	0	0
782	B	5	1	RS	1	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0
783	B	5	1	RS	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
784	B	5	1	RS	1	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0
785	B	5	1	RS	1	0	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
786	B	5	1	RS	1	0	0	1	0	0	0	1	0	0	1	1	0	0	0	0	0	0
787	B	5	1	RS	1	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	0	0
788	B	5	1	RS	1	1	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0
789	B	5	1	RS	1	0	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
790	B	5	1	RS	1	0	0	1	0	0	0	1	0	0	1	1	0	0	0	0	0	0
791	B	5	1	RS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
792	B	5	1	RS	1	0	0	0	0	1	0	1	0	0	1	1	0	0	0	0	0	0
793	B	5	1	RS	1	0	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
794	B	5	1	RS	0	0	0	1	1	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
795	B	5	1	RS	1	0	0	0	0	0	0	1	0	0	1	1	0	0	1	0	0	0
796	B	5	1	RS	1	1	0	1	0	0	0	1	0	0	1	1	0	0	0	0	0	0
797	B	5	1	RS	1	0	0	0	0	1	0	1	0	0	1	1	0	0	0	0	0	0
798	B	5	1	RS	0	1	0	0	0	NA	NA	1	1	0	1	1	0	0	0	0	0	0
799	B	5	1	RS	1	0	0	0	0	NA	NA	1	0	0	1	1	0	0	0	0	0	0
800	B	5	1	RS	1	0	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
801	B	5	2	RS	0	0	0	1	0	1	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
802	B	5	2	RS	1	0	0	0	0	NA	NA	1	0	0	0	1	0	0	0	0	0	0
803	B	5	2	RS	1	0	0	0	0	1	0	1	0	0	1	0	0	0	1	0	0	0
804	B	5	2	RS	1	0	0	0	0	1	0	1	0	0	1	1	0	0	0	0	0	0
805	B	5	2	RS	1	0	0	0	0	1	0	1	0	0	1	1	0	0	0	0	0	0
806	B	5	2	RS	1	0	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0
807	B	5	2	RS	1	1	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
808	B	5	2	RS	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
809	B	5	2	RS	1	1	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
810	B	5	2	RS	1	0	0	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0
811	B	5	2	RS	0	1	0	1	0	0	0	1	0	0	0	1	0	1	0	0	1	1

812	B	5	2	RS	NA	NA	NA	NA	NA	NA	0	NA	NA	NA	0	1	0	0	0	0	0	0
813	B	5	2	RS	1	0	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
814	B	5	2	RS	0	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
815	B	5	2	RS	1	1	0	0	0	1	0	1	0	0	1	1	0	0	0	0	0	0
816	B	5	2	RS	1	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0
817	B	5	2	RS	1	1	0	0	0	1	0	1	0	0	1	1	0	0	0	0	0	0
818	B	5	2	RS	1	0	0	1	0	1	0	1	0	0	0	1	0	0	1	0	0	0
819	B	5	2	RS	1	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0
820	B	5	2	RS	1	1	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
821	B	5	3	RS	1	0	1	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
822	B	5	3	RS	0	0	1	1	0	1	0	0	0	0	0	0	0	1	0	0	1	1
823	B	5	3	RS	0	1	0	0	0	NA	NA	0	0	0	0	1	0	0	0	0	0	0
824	B	5	3	RS	1	0	0	1	0	0	0	1	0	0	0	0	0	0	1	0	0	0
825	B	5	3	RS	1	0	0	1	0	0	0	1	0	0	1	1	0	0	1	0	0	0
826	B	5	3	RS	1	0	0	1	0	0	0	1	0	0	0	1	0	0	1	0	0	0
827	B	5	3	RS	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0
828	B	5	3	RS	0	1	0	1	0	0	0	1	0	0	1	1	0	0	0	0	0	0
829	B	5	3	RS	1	1	0	0	1	1	0	0	0	0	1	1	0	0	0	0	0	0
830	B	5	3	RS	1	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	0	0
831	B	5	3	RS	1	1	0	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0
832	B	5	3	RS	1	0	0	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0
833	B	5	3	RS	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0
834	B	5	3	RS	1	0	0	1	0	NA	NA	1	0	0	0	1	0	0	0	0	0	0
835	B	5	3	RS	1	1	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
836	B	5	3	RS	1	0	0	1	0	0	0	1	0	0	1	1	0	0	1	0	0	0
837	B	5	3	RS	1	1	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
838	B	5	3	RS	1	1	0	0	0	1	0	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
839	B	5	3	RS	1	0	0	1	0	1	0	1	0	0	0	1	0	0	0	0	0	0
840	B	5	3	RS	1	1	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
841	C	1	1	R	0	0	0	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0
842	C	1	1	R	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
843	C	1	1	R	0	0	1	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0
844	C	1	1	R	1	0	0	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0
845	C	1	1	R	1	0	0	1	0	1	1	1	0	0	1	1	0	0	0	0	0	0
846	C	1	1	R	1	1	1	0	0	NA	NA	1	1	0	1	1	0	1	0	0	0	0
847	C	1	1	R	0	0	1	1	0	1	0	0	0	0	NA	NA	NA	NA	NA	NA	0	0
848	C	1	1	R	0	0	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
849	C	1	1	R	0	0	0	0	0	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
850	C	1	1	R	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
851	C	1	1	R	0	1	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0
852	C	1	1	R	0	1	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0
853	C	1	1	R	1	1	1	1	0	1	0	0	0	0	1	1	0	0	1	0	0	0
854	C	1	1	R	0	1	0	1	0	0	0	1	1	0	1	1	0	0	0	0	0	0
855	C	1	1	R	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	1
856	C	1	1	R	1	1	0	1	0	NA	NA	0	0	0	0	1	0	0	1	0	0	0
857	C	1	1	R	0	0	0	1	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
858	C	1	1	R	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0	0	0	0
859	C	1	1	R	0	0	0	1	1	0	0	1	0	0	0	0	0	1	1	0	0	0

860	C	1	1	R	1	0	0	1	0	NA	NA	0	0	0	1	1	0	0	0	0	0	0
861	C	1	1	R	1	0	1	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0
862	C	1	1	R	0	1	0	1	0	NA	NA	1	0	0	1	1	0	0	0	0	0	0
863	C	1	1	R	1	1	0	1	0	NA	NA	1	0	0	1	1	0	0	0	0	0	0
864	C	1	1	R	1	0	0	1	0	NA	NA	1	0	0	1	1	0	0	0	0	0	0
865	C	1	1	R	0	0	0	1	0	1	0	0	0	0	1	1	0	0	0	0	0	0
866	C	1	1	R	1	0	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
867	C	1	1	R	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0
868	C	1	1	R	1	0	0	0	0	NA	NA	1	0	0	1	1	0	0	0	0	0	0
869	C	1	1	R	0	0	0	0	1	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
870	C	1	1	R	1	0	0	1	0	NA	NA	1	0	0	1	1	0	0	0	0	0	0
871	C	2	1	R	0	1	1	0	1	0	0	0	0	0	1	1	0	0	1	0	0	0
872	C	2	1	R	0	1	1	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0
873	C	2	1	R	1	1	1	0	0	NA	NA	0	0	0	1	1	0	0	0	0	0	0
874	C	2	1	R	0	0	0	0	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
875	C	2	1	R	0	1	0	1	0	0	0	1	1	0	1	0	0	0	1	1	0	0
876	C	2	1	R	0	0	1	0	0	NA	NA	0	0	0	0	1	0	0	0	0	1	1
877	C	2	1	R	0	0	0	1	1	0	0	1	0	0	0	0	1	0	1	0	0	0
878	C	2	1	R	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
879	C	2	1	R	0	0	0	0	0	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
880	C	2	1	R	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0	0	0
881	C	2	1	R	1	0	0	0	0	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
882	C	2	1	R	0	0	1	0	0	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA	0	0
883	C	2	1	R	1	0	1	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0
884	C	2	1	R	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0
885	C	2	1	R	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0
886	C	2	1	R	0	1	1	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
887	C	2	1	R	0	0	0	0	1	1	0	1	0	0	0	0	0	0	1	0	0	0
888	C	2	1	R	0	0	1	0	0	1	0	1	1	0	0	0	1	1	0	0	1	1
889	C	2	1	R	0	0	0	0	0	1	0	1	1	0	0	0	0	1	1	0	0	0
890	C	2	1	R	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
891	C	2	2	R	0	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0
892	C	2	2	R	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0
893	C	2	2	R	0	0	1	0	0	NA	NA	0	0	0	0	1	0	1	0	0	1	1
894	C	2	2	R	0	1	1	0	0	NA	NA	0	0	0	0	1	0	0	0	0	1	1
895	C	2	2	R	0	1	0	0	0	1	0	1	1	0	1	1	0	0	1	0	1	1
896	C	2	2	R	0	0	1	0	0	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
897	C	2	2	R	0	1	1	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0
898	C	2	2	R	0	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
899	C	2	2	R	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0
900	C	2	2	R	0	0	1	0	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
901	C	2	2	R	0	0	0	0	0	0	0	1	0	0	1	1	0	0	1	0	0	0
902	C	2	2	R	0	1	0	0	0	1	0	1	0	0	0	1	0	1	1	0	0	0
903	C	2	2	R	0	1	0	1	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
904	C	2	2	R	0	0	0	0	0	1	0	1	0	0	1	1	0	0	1	0	0	0
905	C	2	2	R	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0
906	C	2	2	R	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
907	C	2	2	R	0	1	0	0	0	1	0	1	1	0	0	0	0	1	1	0	0	0

908	C	2	2	R	0	0	0	0	1	1	0	1	1	0	0	0	1	0	1	0	0	0
909	C	2	2	R	0	0	1	0	0	1	0	1	0	0	1	1	0	0	1	0	0	0
910	C	2	2	R	0	0	0	0	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
911	C	2	3	R	0	1	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0
912	C	2	3	R	1	1	0	0	0	NA	NA	0	0	0	1	1	0	0	1	0	0	0
913	C	2	3	R	0	1	0	0	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
914	C	2	3	R	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
915	C	2	3	R	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
916	C	2	3	R	1	1	1	1	0	NA	NA	0	0	0	1	1	0	0	1	0	0	0
917	C	2	3	R	0	1	1	1	0	0	0	0	0	0	1	1	0	0	1	0	0	0
918	C	2	3	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
919	C	2	3	R	0	1	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0
920	C	2	3	R	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
921	C	2	3	R	0	0	1	0	0	NA	NA	1	1	0	NA	NA	NA	NA	NA	NA	NA	NA
922	C	2	3	R	0	0	1	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0
923	C	2	3	R	0	1	0	1	0	NA	NA	0	0	0	1	1	0	0	1	0	1	1
924	C	2	3	R	0	1	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0
925	C	2	3	R	0	1	1	1	0	0	0	0	0	0	1	1	0	0	0	0	1	1
926	C	2	3	R	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
927	C	2	3	R	0	1	1	1	0	1	0	0	0	0	1	1	0	0	0	0	0	0
928	C	2	3	R	0	1	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0
929	C	2	3	R	0	1	1	0	0	1	0	0	0	0	1	1	0	1	1	0	0	0
930	C	2	3	R	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
931	C	4	1	R	0	1	1	1	0	1	0	0	0	0	0	1	0	1	0	0	1	1
932	C	4	1	R	0	1	0	0	1	0	0	0	0	0	1	1	0	0	0	1	0	0
933	C	4	1	R	0	1	1	1	0	1	0	0	0	0	1	1	0	1	0	0	0	0
934	C	4	1	R	0	0	0	1	0	0	0	0	0	0	1	1	0	0	1	0	0	0
935	C	4	1	R	0	1	1	0	1	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
936	C	4	1	R	0	1	0	0	0	NA	NA	0	0	0	0	0	1	0	0	0	0	0
937	C	4	1	R	0	1	0	0	0	NA	NA	0	0	0	0	1	0	0	0	0	0	0
938	C	4	1	R	0	1	0	0	0	NA	NA	0	0	0	0	0	0	0	1	0	0	0
939	C	4	1	R	0	1	0	0	0	NA	NA	1	0	0	0	0	1	1	0	0	0	0
940	C	4	1	R	0	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
941	C	4	1	R	1	1	0	0	0	NA	NA	0	0	0	1	1	0	0	0	0	0	0
942	C	4	1	R	0	1	1	0	0	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
943	C	4	1	R	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
944	C	4	1	R	NA	NA	1	NA	NA	NA	0	NA	NA	NA	1	1	0	1	0	0	0	0
945	C	4	1	R	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
946	C	4	1	R	0	1	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1	1
947	C	4	1	R	0	0	0	1	0	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
948	C	4	1	R	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
949	C	4	1	R	0	0	1	1	0	0	0	1	0	0	1	1	0	0	0	0	0	0
950	C	4	1	R	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1
951	C	4	2	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
952	C	4	2	R	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
953	C	4	2	R	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0
954	C	4	2	R	0	1	0	1	0	NA	NA	0	0	0	0	0	0	0	0	0	0	0
955	C	4	2	R	0	0	0	0	0	NA	NA	0	0	0	0	0	0	0	0	0	0	0

956	C	4	2	R	0	1	1	0	0	0	0	1	1	0	0	0	0	1	1	0	1	1
957	C	4	2	R	0	1	0	0	1	0	0	0	0	0	1	1	0	0	0	1	0	0
958	C	4	2	R	0	1	0	1	1	0	0	0	0	0	1	1	0	0	0	0	0	0
959	C	4	2	R	0	1	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0
960	C	4	2	R	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0
961	C	4	2	R	0	1	0	0	1	0	0	0	0	0	1	1	0	0	0	1	0	0
962	C	4	2	R	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0
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964	C	4	2	R	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0
965	C	4	2	R	0	1	0	0	1	0	0	0	0	0	1	1	0	0	1	1	0	0
966	C	4	2	R	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
967	C	4	2	R	0	1	0	0	0	1	0	1	1	0	1	1	0	1	1	0	0	0
968	C	4	2	R	0	0	0	0	0	1	0	0	0	0	0	1	0	1	0	0	0	0
969	C	4	2	R	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
970	C	4	2	R	0	0	1	1	0	NA	NA	0	0	0	1	1	0	1	0	0	0	0
971	C	4	3	R	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0
972	C	4	3	R	0	1	1	1	0	1	0	0	0	0	1	1	0	0	0	0	0	0
973	C	4	3	R	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
974	C	4	3	R	0	1	1	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0
975	C	4	3	R	0	1	1	1	0	1	0	0	0	0	1	1	0	0	0	0	0	0
976	C	4	3	R	0	0	1	1	0	1	0	0	0	0	1	1	0	0	0	0	0	0
977	C	4	3	R	0	1	1	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
978	C	4	3	R	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
979	C	4	3	R	0	0	1	1	0	1	0	0	0	0	1	1	0	0	0	0	0	0
980	C	4	3	R	0	0	1	1	0	NA	NA	0	0	0	1	1	0	0	0	0	0	0
981	C	4	3	R	0	1	1	0	0	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
982	C	4	3	R	0	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
983	C	4	3	R	0	0	1	0	0	NA	NA	0	0	0	1	1	0	0	0	0	0	0
984	C	4	3	R	0	1	1	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
985	C	4	3	R	0	0	1	1	0	NA	NA	0	0	0	1	1	0	0	0	0	0	0
986	C	4	3	R	1	1	0	0	0	NA	NA	1	0	0	0	0	0	0	0	0	0	0
987	C	4	3	R	0	0	0	0	0	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
988	C	4	3	R	1	1	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
989	C	4	3	R	0	1	0	0	1	0	0	0	0	0	1	1	0	0	0	1	0	0
990	C	4	3	R	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0	0	0	0
991	C	5	1	R	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0
992	C	5	1	R	1	0	0	0	0	1	0	1	0	0	1	1	0	0	0	0	0	0
993	C	5	1	R	1	1	0	0	1	1	0	0	0	0	1	1	0	0	0	0	0	0
994	C	5	1	R	0	1	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0
995	C	5	1	R	0	1	1	1	1	0	0	0	0	0	1	1	0	0	0	1	0	0
996	C	5	1	R	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
997	C	5	1	R	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
998	C	5	1	R	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0
999	C	5	1	R	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
1000	C	5	1	R	1	0	0	0	0	1	0	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1001	C	5	1	R	1	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
1002	C	5	1	R	1	1	0	0	1	1	0	0	0	0	1	1	0	0	0	0	0	0
1003	C	5	1	R	1	0	0	1	0	1	0	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA

1004	C	5	1	R	1	0	0	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0
1005	C	5	1	R	1	1	0	1	0	NA	NA	0	0	0	0	0	0	0	0	0	0	0
1006	C	5	1	R	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
1007	C	5	1	R	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
1008	C	5	1	R	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
1009	C	5	1	R	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
1010	C	5	1	R	1	1	0	0	1	1	0	0	0	0	1	1	0	0	0	0	0	0
1011	C	5	2	R	1	0	0	0	0	NA	NA	0	0	0	0	0	0	0	0	0	0	0
1012	C	5	2	R	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
1013	C	5	2	R	1	0	0	0	1	1	0	1	0	0	0	0	0	1	0	0	0	0
1014	C	5	2	R	0	1	0	0	0	NA	NA	0	0	0	0	0	0	0	0	0	0	0
1015	C	5	2	R	1	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0
1016	C	5	2	R	0	1	1	1	0	1	0	0	0	0	0	1	0	1	0	0	0	0
1017	C	5	2	R	0	1	1	1	0	NA	NA	0	0	0	0	1	0	0	0	0	0	0
1018	C	5	2	R	1	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0
1019	C	5	2	R	1	1	0	0	0	NA	NA	0	0	0	0	0	0	0	0	0	0	0
1020	C	5	2	R	1	0	0	0	1	1	0	1	0	0	1	0	0	1	0	0	0	0
1021	C	5	2	R	1	1	0	0	1	1	0	0	0	0	1	1	0	0	0	0	0	0
1022	C	5	2	R	1	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0
1023	C	5	2	R	0	0	0	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0
1024	C	5	2	R	0	0	0	0	0	NA	NA	0	0	0	0	0	0	0	0	0	0	0
1025	C	5	2	R	1	1	0	0	0	NA	NA	1	0	0	1	1	0	0	1	0	0	0
1026	C	5	2	R	0	1	0	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0
1027	C	5	2	R	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1028	C	5	2	R	0	1	0	0	0	0	0	1	0	0	0	0	1	0	1	0	1	1
1029	C	5	2	R	1	0	0	0	0	1	0	1	0	0	1	0	0	0	0	0	0	0
1030	C	5	2	R	1	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0
1031	C	5	3	R	1	1	0	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0
1032	C	5	3	R	1	1	0	0	0	NA	NA	0	0	0	0	0	0	0	0	0	0	0
1033	C	5	3	R	1	1	0	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0
1034	C	5	3	R	1	0	0	0	0	1	0	1	0	0	NA	NA	NA	NA	NA	NA	0	0
1035	C	5	3	R	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
1036	C	5	3	R	1	0	0	1	0	1	0	1	0	0	1	1	0	0	0	0	NA	NA
1037	C	5	3	R	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1038	C	5	3	R	1	0	0	0	0	NA	NA	0	0	0	0	0	0	0	0	0	0	0
1039	C	5	3	R	1	0	0	1	0	0	0	1	0	0	NA	NA	NA	NA	NA	NA	0	0
1040	C	5	3	R	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
1041	C	5	3	R	1	1	0	0	0	1	0	1	0	0	NA	NA	NA	NA	NA	NA	0	0
1042	C	5	3	R	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
1043	C	5	3	R	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0
1044	C	5	3	R	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
1045	C	5	3	R	1	1	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0
1046	C	5	3	R	1	0	0	1	0	0	0	1	0	0	1	1	0	0	0	0	0	0
1047	C	5	3	R	1	0	0	0	0	1	0	1	0	0	1	1	0	0	1	0	0	0
1048	C	5	3	R	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0
1049	C	5	3	R	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0
1050	C	5	3	R	1	1	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0
1051	C	1	1	RS	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0

1052	C	1	1	RS	1	0	0	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0
1053	C	1	1	RS	1	0	0	1	0	0	0	1	0	0	1	1	0	0	1	0	0	0
1054	C	1	1	RS	0	0	0	0	1	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1055	C	1	1	RS	1	0	0	0	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1056	C	1	1	RS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1057	C	1	1	RS	1	0	0	0	0	NA	NA	0	0	0	1	1	0	0	0	0	0	0
1058	C	1	1	RS	0	0	1	1	0	1	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1059	C	1	1	RS	1	0	1	0	0	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1060	C	1	1	RS	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
1061	C	1	1	RS	1	1	0	0	0	NA	NA	0	0	1	1	1	0	0	0	0	0	0
1062	C	1	1	RS	1	1	0	1	0	NA	NA	0	0	0	0	1	0	0	0	0	0	0
1063	C	1	1	RS	1	0	0	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0
1064	C	1	1	RS	1	1	0	1	0	NA	NA	0	0	0	1	1	0	0	0	0	0	0
1065	C	1	1	RS	0	1	1	1	0	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1066	C	1	1	RS	0	1	0	1	0	0	0	1	0	0	0	0	0	0	1	0	0	0
1067	C	1	1	RS	0	0	0	0	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1068	C	1	1	RS	1	0	0	1	0	NA	NA	1	1	0	NA	NA	NA	NA	NA	NA	NA	NA
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1071	C	1	1	RS	1	0	1	0	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1072	C	1	1	RS	1	1	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0
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1074	C	1	1	RS	1	1	0	1	0	NA	NA	0	0	0	1	1	0	0	0	0	0	0
1075	C	1	1	RS	0	1	1	0	0	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1076	C	1	1	RS	0	0	0	0	0	NA	NA	0	0	0	0	0	0	0	0	0	0	0
1077	C	1	1	RS	0	1	0	0	0	NA	NA	0	0	0	0	0	0	0	0	0	0	0
1078	C	1	1	RS	0	0	0	0	1	NA	NA	0	0	1	1	1	0	0	0	0	0	0
1079	C	1	1	RS	1	1	0	1	0	1	0	1	1	0	1	1	0	0	0	0	0	0
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1081	C	2	1	RS	0	0	0	1	0	NA	NA	0	0	0	1	1	0	0	0	0	0	0
1082	C	2	1	RS	1	0	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
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1089	C	2	1	RS	1	0	0	1	1	1	0	0	0	0	1	1	0	0	0	0	0	0
1090	C	2	1	RS	1	0	0	0	0	NA	NA	1	1	0	NA	NA	NA	NA	NA	NA	NA	NA
1091	C	2	1	RS	1	0	0	1	0	NA	NA	1	1	0	1	1	0	0	0	0	0	0
1092	C	2	1	RS	1	0	0	0	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1093	C	2	1	RS	0	0	0	1	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1094	C	2	1	RS	0	0	1	1	0	1	0	1	1	0	0	0	1	0	0	0	1	1
1095	C	2	1	RS	0	0	1	0	0	NA	NA	0	0	0	1	1	0	1	0	0	0	0
1096	C	2	1	RS	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1097	C	2	1	RS	1	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	0	0
1098	C	2	1	RS	1	0	1	0	0	NA	NA	0	0	0	0	0	0	1	0	0	0	0
1099	C	2	1	RS	1	0	0	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0

1100	C	2	1	RS	1	0	0	1	0	NA	NA	1	1	0	1	1	0	0	0	0	0	0
1101	C	2	2	RS	1	1	0	0	0	NA	NA	1	0	0	1	1	0	0	0	0	0	0
1102	C	2	2	RS	0	1	1	1	0	0	0	1	0	0	NA	NA	NA	NA	NA	NA	0	0
1103	C	2	2	RS	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1104	C	2	2	RS	0	0	1	0	0	NA	NA	0	0	0	1	1	0	0	0	0	0	0
1105	C	2	2	RS	0	1	0	0	0	NA	NA	1	0	0	0	0	0	0	1	0	0	0
1106	C	2	2	RS	0	0	0	1	0	NA	NA	1	0	0	1	1	0	0	1	0	0	0
1107	C	2	2	RS	0	0	1	0	0	NA	NA	0	0	0	1	1	0	0	0	0	0	0
1108	C	2	2	RS	0	0	1	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0
1109	C	2	2	RS	0	1	1	0	0	NA	NA	0	0	0	0	1	0	1	0	0	0	0
1110	C	2	2	RS	0	0	0	0	0	NA	NA	0	0	0	0	1	0	0	0	0	1	1
1111	C	2	2	RS	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
1112	C	2	2	RS	0	0	1	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0
1113	C	2	2	RS	0	0	0	1	0	1	0	0	0	0	1	1	0	0	1	0	0	0
1114	C	2	2	RS	1	0	0	1	0	1	0	1	0	0	0	0	0	0	1	0	0	0
1115	C	2	2	RS	1	0	0	1	0	NA	NA	1	1	0	1	1	0	0	1	0	0	0
1116	C	2	2	RS	1	0	0	1	0	NA	NA	1	0	0	0	0	0	0	1	0	0	0
1117	C	2	2	RS	0	1	0	1	1	NA	NA	1	1	0	NA	NA	NA	NA	NA	NA	NA	NA
1118	C	2	2	RS	0	0	1	1	0	NA	NA	0	0	0	1	1	0	0	0	0	0	0
1119	C	2	2	RS	0	1	0	1	0	0	0	1	0	0	1	1	0	0	1	0	0	0
1120	C	2	2	RS	0	1	0	1	0	NA	NA	1	1	0	1	1	0	0	0	0	0	0
1121	C	2	3	RS	1	0	0	1	0	0	0	1	0	0	1	1	0	0	0	0	0	0
1122	C	2	3	RS	1	1	0	1	0	NA	NA	1	0	0	1	1	0	0	1	0	0	0
1123	C	2	3	RS	0	1	1	1	0	1	0	1	1	0	0	0	1	1	0	0	1	1
1124	C	2	3	RS	1	0	0	0	0	NA	NA	0	0	0	1	1	0	0	1	0	0	0
1125	C	2	3	RS	0	1	1	1	0	1	0	1	1	0	0	0	0	0	0	0	0	0
1126	C	2	3	RS	0	0	0	1	1	0	0	1	0	0	0	0	0	1	0	0	0	0
1127	C	2	3	RS	1	0	0	1	0	1	0	1	1	0	1	1	0	0	0	0	0	0
1128	C	2	3	RS	1	1	0	0	0	1	0	1	1	0	0	0	0	1	0	0	0	0
1129	C	2	3	RS	1	1	1	1	0	NA	NA	0	0	0	1	1	0	0	1	0	0	0
1130	C	2	3	RS	0	1	0	0	0	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1131	C	2	3	RS	0	1	0	1	0	NA	NA	0	0	0	1	0	0	0	0	0	0	0
1132	C	2	3	RS	1	0	1	1	0	NA	NA	0	0	0	1	1	0	1	0	0	0	0
1133	C	2	3	RS	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0
1134	C	2	3	RS	0	1	0	0	0	NA	NA	0	0	0	1	1	0	0	1	0	0	0
1135	C	2	3	RS	1	1	0	0	0	NA	NA	0	0	0	1	1	0	0	0	0	0	0
1136	C	2	3	RS	0	0	1	0	0	1	0	0	0	0	1	1	0	1	0	0	0	0
1137	C	2	3	RS	0	0	1	0	0	NA	NA	0	0	0	1	1	0	1	0	0	0	0
1138	C	2	3	RS	0	0	1	1	1	NA	NA	1	1	0	NA	NA	NA	NA	NA	NA	NA	NA
1139	C	2	3	RS	0	1	1	0	0	NA	NA	1	0	0	1	1	0	0	0	0	0	0
1140	C	2	3	RS	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0
1141	C	4	1	RS	1	0	0	0	1	1	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1142	C	4	1	RS	1	1	0	1	0	NA	NA	0	0	0	1	1	0	0	1	0	0	0
1143	C	4	1	RS	0	0	0	1	0	1	0	1	0	0	0	0	0	0	1	0	0	0
1144	C	4	1	RS	1	1	0	1	0	1	0	1	1	0	1	1	0	0	0	0	0	0
1145	C	4	1	RS	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0
1146	C	4	1	RS	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0
1147	C	4	1	RS	0	1	1	1	0	1	0	0	0	1	1	1	0	0	0	0	0	0

1148	C	4	1	RS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1149	C	4	1	RS	0	1	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0
1150	C	4	1	RS	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
1151	C	4	1	RS	0	1	0	0	0	0	0	1	0	0	1	0	0	1	0	0	0	0
1152	C	4	1	RS	1	1	0	0	1	1	0	1	0	0	1	1	0	0	0	0	0	0
1153	C	4	1	RS	1	0	0	1	0	1	0	1	1	0	1	1	0	0	0	0	0	0
1154	C	4	1	RS	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1155	C	4	1	RS	1	0	0	0	0	NA	NA	1	0	0	0	0	0	1	0	0	0	0
1156	C	4	1	RS	1	1	0	0	0	1	0	1	1	0	0	0	0	1	0	0	0	0
1157	C	4	1	RS	1	1	0	0	0	NA	NA	1	1	0	1	1	0	0	1	0	0	0
1158	C	4	1	RS	0	1	0	1	0	NA	NA	0	0	0	1	1	0	1	0	0	1	1
1159	C	4	1	RS	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
1160	C	4	1	RS	1	1	0	1	0	1	0	1	1	0	1	1	0	0	0	0	0	0
1161	C	4	2	RS	0	0	0	1	0	NA	NA	0	0	0	1	1	0	0	1	0	0	0
1162	C	4	2	RS	0	0	0	0	0	NA	NA	1	1	0	1	1	0	0	0	0	0	0
1163	C	4	2	RS	NA	1	0	1	0	1	0	1	1	0	1	1	0	0	0	0	0	0
1164	C	4	2	RS	0	0	0	1	1	0	0	0	0	0	1	1	0	0	1	1	0	0
1165	C	4	2	RS	0	0	0	1	0	1	0	1	0	0	0	0	0	0	1	0	0	0
1166	C	4	2	RS	1	0	0	0	0	1	0	1	1	0	0	0	0	1	0	0	0	0
1167	C	4	2	RS	1	1	0	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0
1168	C	4	2	RS	1	1	1	0	1	0	0	0	0	0	1	1	0	0	1	0	0	0
1169	C	4	2	RS	0	0	1	1	0	0	0	1	1	0	1	1	0	0	0	0	0	0
1170	C	4	2	RS	0	0	1	0	0	1	0	1	1	0	1	1	0	0	0	0	0	0
1171	C	4	2	RS	1	0	0	1	0	NA	NA	1	1	0	NA	NA	NA	NA	NA	NA	NA	NA
1172	C	4	2	RS	1	0	0	0	0	NA	NA	1	1	0	NA	NA	NA	NA	NA	NA	NA	NA
1173	C	4	2	RS	1	1	0	1	0	1	0	1	1	0	1	1	0	0	1	0	0	0
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1175	C	4	2	RS	1	0	0	0	0	1	0	1	1	0	1	1	0	0	0	0	0	0
1176	C	4	2	RS	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0
1177	C	4	2	RS	0	0	0	1	0	0	0	0	0	0	1	1	0	0	1	1	0	0
1178	C	4	2	RS	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0
1179	C	4	2	RS	1	0	0	1	0	0	0	1	0	0	0	1	0	0	1	0	0	0
1180	C	4	2	RS	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0
1181	C	4	3	RS	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	1	0	0
1182	C	4	3	RS	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1183	C	4	3	RS	1	1	0	0	1	1	0	0	0	0	1	1	0	0	0	0	0	0
1184	C	4	3	RS	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1185	C	4	3	RS	1	0	0	0	0	1	0	1	0	0	1	1	0	0	1	0	0	0
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1187	C	4	3	RS	1	0	1	0	0	NA	NA	1	1	0	0	0	0	1	0	0	0	0
1188	C	4	3	RS	0	1	0	1	0	0	0	1	0	0	1	1	0	0	1	0	0	0
1189	C	4	3	RS	1	0	0	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0
1190	C	4	3	RS	1	1	1	0	0	NA	NA	1	0	0	1	1	0	1	0	0	0	0
1191	C	4	3	RS	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
1192	C	4	3	RS	0	1	0	1	0	NA	NA	1	0	0	1	1	0	0	1	0	0	0
1193	C	4	3	RS	0	1	0	0	0	0	0	1	0	0	1	1	0	0	0	1	0	0
1194	C	4	3	RS	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	1	0	0
1195	C	4	3	RS	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0

1196	C	4	3	RS	1	0	0	0	0	NA	NA	1	0	0	0	1	0	1	1	0	0	0
1197	C	4	3	RS	1	1	0	0	0	NA	NA	1	0	0	1	1	0	1	1	0	0	0
1198	C	4	3	RS	1	1	0	0	1	1	0	0	0	0	1	1	0	0	0	0	0	0
1199	C	4	3	RS	0	0	0	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0
1200	C	4	3	RS	1	1	0	1	0	NA	NA	0	0	0	1	1	0	0	0	0	0	0
1201	C	5	1	RS	1	0	0	1	0	0	0	1	0	0	1	1	0	0	1	0	0	0
1202	C	5	1	RS	1	0	0	1	0	NA	NA	1	0	0	1	1	0	0	0	0	0	0
1203	C	5	1	RS	1	0	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0
1204	C	5	1	RS	1	1	0	0	0	1	0	1	0	0	1	1	0	0	1	0	NA	NA
1205	C	5	1	RS	1	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
1206	C	5	1	RS	1	0	0	0	0	NA	NA	1	0	0	1	1	0	0	1	0	NA	NA
1207	C	5	1	RS	1	0	0	0	0	1	0	0	0	0	1	1	0	0	1	0	0	0
1208	C	5	1	RS	1	1	0	1	0	0	0	1	0	0	1	1	0	0	1	0	0	0
1209	C	5	1	RS	0	1	1	1	1	0	0	0	0	0	1	1	0	0	1	1	0	0
1210	C	5	1	RS	1	1	0	0	0	NA	NA	0	0	0	1	0	0	0	0	0	0	0
1211	C	5	1	RS	1	0	0	0	NA	NA	0	1	0	0	1	1	0	0	1	0	0	0
1212	C	5	1	RS	0	1	0	0	NA	NA	0	0	0	0	0	1	1	1	1	0	0	0
1213	C	5	1	RS	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
1214	C	5	1	RS	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
1215	C	5	1	RS	1	1	0	0	1	1	0	0	0	0	1	1	0	0	0	0	0	0
1216	C	5	1	RS	1	0	0	0	0	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1217	C	5	1	RS	0	1	0	0	0	1	0	1	0	0	0	0	1	0	1	0	0	0
1218	C	5	1	RS	1	0	0	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0
1219	C	5	1	RS	1	1	0	1	0	NA	NA	0	0	0	1	1	0	0	0	0	0	0
1220	C	5	1	RS	1	0	0	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0
1221	C	5	2	RS	1	1	0	0	0	NA	NA	0	0	0	1	0	0	0	0	0	0	0
1222	C	5	2	RS	0	0	0	1	0	0	0	0	0	0	1	1	0	1	1	0	NA	NA
1223	C	5	2	RS	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	NA	NA
1224	C	5	2	RS	NA	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
1225	C	5	2	RS	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
1226	C	5	2	RS	1	1	0	0	0	NA	NA	0	0	0	0	0	0	0	0	0	0	0
1227	C	5	2	RS	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
1228	C	5	2	RS	0	1	0	1	1	0	0	1	0	0	0	1	0	1	0	0	0	0
1229	C	5	2	RS	1	0	0	0	0	NA	NA	0	0	0	1	1	0	0	0	0	0	0
1230	C	5	2	RS	1	0	0	1	0	NA	NA	1	0	0	1	1	0	0	0	0	0	0
1231	C	5	2	RS	1	1	0	0	0	1	0	1	0	0	1	1	0	0	0	0	0	0
1232	C	5	2	RS	1	0	0	0	0	1	0	1	0	0	1	1	0	0	0	0	0	0
1233	C	5	2	RS	1	0	0	1	0	1	0	1	0	0	0	1	0	0	1	0	0	0
1234	C	5	2	RS	1	1	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
1235	C	5	2	RS	0	0	0	1	0	0	0	0	0	0	NA	NA	NA	NA	NA	NA	0	0
1236	C	5	2	RS	0	0	0	1	0	1	0	0	0	0	NA	NA	NA	NA	NA	NA	0	0
1237	C	5	2	RS	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1238	C	5	2	RS	1	1	0	1	0	0	0	1	0	0	1	1	0	0	1	0	0	0
1239	C	5	2	RS	1	1	0	0	0	1	0	1	0	0	1	1	0	0	1	0	0	0
1240	C	5	2	RS	0	0	0	0	0	1	0	0	0	0	0	1	0	1	1	0	0	0
1241	C	5	3	RS	1	1	0	1	0	NA	NA	0	0	0	0	0	0	0	0	0	NA	NA
1242	C	5	3	RS	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0
1243	C	5	3	RS	1	0	0	0	0	NA	NA	1	0	0	1	1	0	0	1	0	0	0

1244	C	5	3	RS	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	1	1
1245	C	5	3	RS	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	1	1
1246	C	5	3	RS	0	0	0	1	0	NA	NA	0	0	0	0	0	0	0	0	0	1	1
1247	C	5	3	RS	1	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
1248	C	5	3	RS	1	0	0	0	0	1	0	1	0	0	1	1	0	0	1	0	0	0
1249	C	5	3	RS	0	0	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
1250	C	5	3	RS	0	0	0	0	0	NA	NA	1	0	0	0	0	0	0	0	0	NA	NA
1251	C	5	3	RS	0	0	0	1	1	NA	NA	0	0	0	0	0	0	1	0	0	0	0
1252	C	5	3	RS	1	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
1253	C	5	3	RS	1	0	0	0	0	1	0	1	0	0	1	0	0	0	1	0	0	0
1254	C	5	3	RS	1	0	0	1	0	0	0	1	0	0	0	1	0	0	1	0	0	0
1255	C	5	3	RS	0	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	0	0
1256	C	5	3	RS	1	1	0	1	0	NA	NA	0	0	0	0	1	0	0	0	0	0	0
1257	C	5	3	RS	1	1	0	0	1	1	0	0	0	0	1	1	0	0	0	0	0	0
1258	C	5	3	RS	1	0	0	1	0	1	1	1	0	0	1	1	0	0	0	0	0	0
1259	C	5	3	RS	0	0	0	0	0	NA	NA	0	0	0	0	0	0	0	0	0	0	0
1260	C	5	3	RS	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	1	1
1261	L	1	1	R	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
1262	L	1	1	R	0	1	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0
1263	L	1	1	R	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0
1264	L	1	1	R	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0
1265	L	1	1	R	0	1	1	0	1	0	0	0	0	0	1	1	0	0	0	0	0	1
1266	L	1	1	R	1	0	0	0	1	1	0	0	0	0	1	0	0	0	0	0	0	0
1267	L	1	1	R	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
1268	L	1	1	R	1	1	1	0	0	1	0	1	0	0	1	0	0	0	1	0	0	0
1269	L	1	1	R	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
1270	L	1	1	R	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
1271	L	1	1	R	0	0	1	1	1	0	0	1	0	0	0	0	0	1	0	0	0	0
1272	L	1	1	R	0	0	1	0	1	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1273	L	1	1	R	0	0	1	0	1	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1274	L	1	1	R	0	0	0	1	1	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1275	L	1	1	R	0	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
1276	L	1	1	R	1	0	0	0	0	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1277	L	1	1	R	0	1	1	0	1	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1278	L	1	1	R	0	0	1	0	0	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1279	L	1	1	R	0	1	1	0	0	1	0	1	0	0	0	0	0	1	1	0	0	0
1280	L	1	1	R	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1281	L	1	1	R	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
1282	L	1	1	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1283	L	1	1	R	1	1	0	0	0	1	0	1	0	0	1	1	0	0	0	0	0	0
1284	L	1	1	R	0	0	1	1	1	NA	NA	1	1	0	NA	NA	NA	NA	NA	NA	NA	NA
1285	L	1	1	R	0	0	0	0	0	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1286	L	1	1	R	0	0	1	0	1	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1287	L	1	1	R	1	1	0	1	0	1	0	1	0	0	1	0	0	0	0	0	0	0
1288	L	1	1	R	0	0	0	0	0	1	0	1	0	0	0	1	0	0	0	0	0	0
1289	L	1	1	R	0	1	1	1	1	0	0	0	0	0	1	1	0	0	1	0	0	0
1290	L	1	1	R	0	0	1	0	1	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1291	L	2	1	R	0	0	1	0	0	1	0	0	0	0	1	1	0	0	0	0	1	1

1292	L	2	1	R	1	1	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
1293	L	2	1	R	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0
1294	L	2	1	R	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1295	L	2	1	R	1	1	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
1296	L	2	1	R	0	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
1297	L	2	1	R	0	1	1	0	0	1	0	0	0	0	1	1	0	0	0	0	1	1
1298	L	2	1	R	0	1	1	1	1	0	0	0	0	0	1	1	0	0	1	0	0	0
1299	L	2	1	R	0	1	0	0	1	1	0	0	0	0	1	0	0	0	0	0	0	0
1300	L	2	1	R	0	0	0	0	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1301	L	2	1	R	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
1302	L	2	1	R	0	0	0	0	0	NA	NA	0	0	0	1	1	0	1	0	0	0	0
1303	L	2	1	R	1	0	1	0	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1304	L	2	1	R	0	0	0	1	0	0	0	1	0	0	NA	NA	NA	NA	NA	NA	0	0
1305	L	2	1	R	0	0	0	0	0	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1306	L	2	1	R	0	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
1307	L	2	1	R	0	1	0	0	0	NA	NA	1	0	0	1	1	0	0	1	0	0	0
1308	L	2	1	R	0	1	1	0	0	NA	NA	0	0	0	1	1	0	1	0	0	0	0
1309	L	2	1	R	0	1	1	0	0	NA	NA	0	0	0	1	1	0	1	0	0	1	1
1310	L	2	1	R	0	1	1	1	1	0	0	0	0	0	1	1	0	0	1	0	0	0
1311	L	2	2	R	0	1	0	0	0	NA	NA	0	0	0	1	1	0	0	0	0	0	0
1312	L	2	2	R	0	1	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0
1313	L	2	2	R	0	0	1	1	0	0	0	1	0	0	1	1	0	0	1	0	0	0
1314	L	2	2	R	0	0	1	0	1	0	0	0	0	0	0	0	1	1	1	0	0	0
1315	L	2	2	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1316	L	2	2	R	NA	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0
1317	L	2	2	R	0	0	0	0	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1318	L	2	2	R	0	0	0	0	0	NA	NA	0	0	0	1	1	0	0	0	0	0	0
1319	L	2	2	R	0	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
1320	L	2	2	R	1	0	0	0	0	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1321	L	2	2	R	0	0	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
1322	L	2	2	R	0	0	1	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
1323	L	2	2	R	0	0	0	0	0	NA	NA	1	1	0	1	1	0	0	0	0	0	0
1324	L	2	2	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1325	L	2	2	R	0	0	0	0	0	NA	NA	0	0	0	1	1	0	0	0	0	0	0
1326	L	2	2	R	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	1	1
1327	L	2	2	R	0	0	1	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
1328	L	2	2	R	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0
1329	L	2	2	R	0	0	1	0	0	1	0	0	0	0	1	1	0	1	0	0	0	0
1330	L	2	2	R	1	0	0	1	0	1	0	1	1	0	1	1	0	0	0	0	0	0
1331	L	2	3	R	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
1332	L	2	3	R	0	1	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0
1333	L	2	3	R	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1334	L	2	3	R	0	0	1	0	0	0	0	1	0	0	0	1	0	1	1	0	0	0
1335	L	2	3	R	1	1	0	0	0	NA	NA	1	0	0	0	0	0	1	0	0	0	0
1336	L	2	3	R	1	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0
1337	L	2	3	R	0	1	1	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0
1338	L	2	3	R	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
1339	L	2	3	R	0	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0

1340	L	2	3	R	0	1	1	0	0	1	0	1	1	0	0	0	0	0	1	0	0	0
1341	L	2	3	R	0	0	0	0	0	1	0	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1342	L	2	3	R	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1343	L	2	3	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1344	L	2	3	R	0	1	0	1	0	1	0	0	0	0	0	0	0	1	1	0	0	0
1345	L	2	3	R	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
1346	L	2	3	R	0	1	1	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0
1347	L	2	3	R	1	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
1348	L	2	3	R	0	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
1349	L	2	3	R	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0
1350	L	2	3	R	0	1	0	0	0	NA	NA	1	0	0	1	1	0	1	1	0	0	0
1351	L	4	1	R	1	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
1352	L	4	1	R	0	1	0	1	1	0	0	0	0	0	0	1	1	0	0	0	0	0
1353	L	4	1	R	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0
1354	L	4	1	R	0	0	1	0	0	1	0	0	0	0	1	1	0	1	0	0	0	0
1355	L	4	1	R	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1356	L	4	1	R	0	0	1	0	0	1	0	1	1	0	0	0	0	1	0	0	1	1
1357	L	4	1	R	0	0	0	0	1	1	0	0	0	0	1	1	0	1	0	0	0	0
1358	L	4	1	R	0	0	1	1	0	1	0	0	0	0	1	1	0	1	0	0	0	0
1359	L	4	1	R	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0
1360	L	4	1	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1361	L	4	1	R	NA	NA	1	NA	NA	NA	0	NA	NA	NA	1	1	0	1	0	0	0	0
1362	L	4	1	R	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	NA	NA
1363	L	4	1	R	1	0	0	0	0	1	0	1	0	0	1	1	0	0	1	0	0	0
1364	L	4	1	R	0	1	1	1	0	1	0	0	0	0	0	1	0	1	0	0	1	1
1365	L	4	1	R	0	0	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0
1366	L	4	1	R	0	1	0	0	0	1	0	0	0	0	0	1	0	1	1	0	0	0
1367	L	4	1	R	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0
1368	L	4	1	R	0	1	1	1	0	0	0	0	0	0	0	1	0	1	0	0	1	1
1369	L	4	1	R	1	0	0	1	0	0	0	1	0	0	1	1	0	0	1	0	0	0
1370	L	4	1	R	0	0	0	0	1	1	0	0	0	0	1	1	0	1	1	0	0	0
1371	L	4	2	R	0	1	1	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0
1372	L	4	2	R	0	1	1	1	0	1	0	0	0	0	1	1	0	0	0	0	0	0
1373	L	4	2	R	1	0	0	0	0	1	0	0	0	0	1	0	0	1	0	0	0	0
1374	L	4	2	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
1375	L	4	2	R	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0
1376	L	4	2	R	1	0	0	1	0	0	0	1	0	0	0	1	0	0	1	0	0	0
1377	L	4	2	R	0	1	0	0	0	1	0	1	0	0	1	1	0	0	0	0	0	0
1378	L	4	2	R	0	1	0	0	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1379	L	4	2	R	0	1	0	0	0	1	0	1	0	0	1	1	0	0	0	0	0	0
1380	L	4	2	R	1	1	0	0	1	1	0	0	0	0	1	1	0	0	0	0	0	0
1381	L	4	2	R	1	0	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
1382	L	4	2	R	0	1	1	1	0	NA	NA	0	0	0	0	1	0	1	0	0	0	0
1383	L	4	2	R	0	1	1	1	0	1	0	0	0	0	1	1	0	0	0	0	0	0
1384	L	4	2	R	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
1385	L	4	2	R	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0	0	0
1386	L	4	2	R	0	0	1	1	0	1	0	0	0	0	1	1	0	0	0	0	0	0
1387	L	4	2	R	0	1	1	1	0	1	0	0	0	0	0	1	0	0	0	0	1	1

1388	L	4	2	R	0	0	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
1389	L	4	2	R	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NA	NA
1390	L	4	2	R	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
1391	L	4	3	R	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1392	L	4	3	R	0	1	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0
1393	L	4	3	R	0	1	1	1	0	1	0	0	0	0	0	1	0	0	0	0	1	1
1394	L	4	3	R	0	1	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0
1395	L	4	3	R	0	1	0	1	0	0	0	0	0	0	0	0	1	1	1	0	0	0
1396	L	4	3	R	0	1	1	1	0	0	0	0	0	0	0	1	0	0	0	0	1	1
1397	L	4	3	R	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	NA	NA
1398	L	4	3	R	0	1	0	1	0	0	0	0	0	0	0	0	1	1	1	0	0	0
1399	L	4	3	R	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
1400	L	4	3	R	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
1401	L	4	3	R	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0
1402	L	4	3	R	0	1	0	1	0	0	0	1	0	0	0	0	0	0	1	0	0	0
1403	L	4	3	R	0	1	1	1	0	0	0	1	1	0	0	0	0	0	1	0	0	0
1404	L	4	3	R	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
1405	L	4	3	R	NA	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
1406	L	4	3	R	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
1407	L	4	3	R	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
1408	L	4	3	R	0	1	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0
1409	L	4	3	R	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
1410	L	4	3	R	0	0	0	1	0	0	0	1	0	0	0	0	0	0	1	0	0	0
1411	L	5	1	R	0	0	0	1	0	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1412	L	5	1	R	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0
1413	L	5	1	R	0	1	1	0	0	1	0	1	1	0	0	0	1	0	0	0	1	1
1414	L	5	1	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1415	L	5	1	R	0	1	0	1	0	1	0	1	0	0	NA	NA	NA	NA	NA	NA	0	0
1416	L	5	1	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1417	L	5	1	R	0	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
1418	L	5	1	R	1	0	0	0	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1419	L	5	1	R	1	0	0	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0
1420	L	5	1	R	1	0	0	0	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1421	L	5	1	R	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0
1422	L	5	1	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1423	L	5	1	R	0	1	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0
1424	L	5	1	R	1	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
1425	L	5	1	R	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1426	L	5	1	R	0	1	1	0	0	1	1	1	1	0	0	0	0	0	0	0	1	1
1427	L	5	1	R	0	0	0	1	0	1	0	0	0	0	1	1	0	0	0	0	0	0
1428	L	5	1	R	1	0	0	1	0	0	0	1	0	0	1	1	0	0	1	0	0	0
1429	L	5	1	R	0	1	0	0	0	1	0	0	0	1	1	1	0	0	0	0	0	0
1430	L	5	1	R	0	1	0	1	0	0	0	0	0	0	0	1	1	0	1	0	0	0
1431	L	5	2	R	NA	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
1432	L	5	2	R	0	1	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0
1433	L	5	2	R	0	1	0	0	0	1	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1434	L	5	2	R	0	0	0	1	0	NA	NA	0	0	0	1	1	0	0	0	0	0	0
1435	L	5	2	R	1	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0

1436	L	5	2	R	1	1	0	1	0	1	0	0	0	0	1	1	0	0	0	0	0	0
1437	L	5	2	R	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0
1438	L	5	2	R	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
1439	L	5	2	R	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0
1440	L	5	2	R	1	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
1441	L	5	2	R	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0
1442	L	5	2	R	0	1	1	0	0	1	0	1	1	0	0	0	0	0	1	0	1	1
1443	L	5	2	R	1	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0
1444	L	5	2	R	1	0	0	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0
1445	L	5	2	R	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0
1446	L	5	2	R	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0
1447	L	5	2	R	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0
1448	L	5	2	R	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0
1449	L	5	2	R	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0
1450	L	5	2	R	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0
1451	L	5	3	R	1	0	0	0	0	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1452	L	5	3	R	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0
1453	L	5	3	R	NA	NA	0	NA	NA	NA	0	NA	NA	NA	0	0	0	0	0	0	0	0
1454	L	5	3	R	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
1455	L	5	3	R	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
1456	L	5	3	R	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
1457	L	5	3	R	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1
1458	L	5	3	R	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
1459	L	5	3	R	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
1460	L	5	3	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
1461	L	5	3	R	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0	0	0	0
1462	L	5	3	R	1	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	0	0
1463	L	5	3	R	0	0	1	0	1	1	0	0	0	0	1	0	0	0	0	0	0	0
1464	L	5	3	R	1	0	0	0	0	1	0	1	0	0	1	1	0	0	1	0	0	0
1465	L	5	3	R	1	0	0	0	0	1	0	1	0	0	1	1	0	0	0	0	NA	NA
1466	L	5	3	R	1	0	0	1	0	1	0	1	0	0	1	1	0	0	0	0	NA	NA
1467	L	5	3	R	0	0	1	0	1	1	0	0	0	0	1	0	0	0	0	0	NA	NA
1468	L	5	3	R	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
1469	L	5	3	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NA	NA
1470	L	5	3	R	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0
1471	L	1	1	RS	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0
1472	L	1	1	RS	0	1	1	1	1	0	0	1	1	0	0	0	0	0	1	0	0	0
1473	L	1	1	RS	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0
1474	L	1	1	RS	0	0	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
1475	L	1	1	RS	0	0	1	1	0	1	0	1	0	0	0	1	0	1	1	0	0	0
1476	L	1	1	RS	0	1	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0
1477	L	1	1	RS	0	0	0	0	0	NA	NA	0	0	0	1	0	0	0	0	0	0	0
1478	L	1	1	RS	1	1	0	1	0	NA	NA	1	1	0	1	1	0	0	0	0	0	0
1479	L	1	1	RS	0	0	0	1	0	NA	NA	0	0	0	0	1	0	0	0	0	0	0
1480	L	1	1	RS	1	1	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
1481	L	1	1	RS	1	1	1	1	0	0	0	1	0	0	0	1	0	0	1	0	0	0
1482	L	1	1	RS	0	0	1	1	0	NA	NA	0	0	1	0	1	0	0	0	0	0	0
1483	L	1	1	RS	0	0	1	1	0	0	0	1	0	0	1	1	0	0	1	0	0	0

1484	L	1	1	RS	1	1	0	0	0	NA	NA	0	0	0	0	0	0	0	0	0	0
1485	L	1	1	RS	0	0	0	1	1	0	0	1	0	0	0	0	0	0	1	0	0	0
1486	L	1	1	RS	1	1	0	1	0	1	0	1	0	0	1	1	0	0	0	0	NA	NA
1487	L	1	1	RS	0	1	1	1	0	0	0	1	0	0	1	1	0	0	1	0	0	0
1488	L	1	1	RS	0	0	0	1	0	0	0	1	0	0	1	1	0	0	1	0	0	0
1489	L	1	1	RS	0	1	1	0	1	1	0	1	0	0	0	0	1	1	1	0	0	0
1490	L	1	1	RS	1	1	1	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0
1491	L	1	1	RS	1	1	1	0	0	NA	NA	1	0	0	0	1	0	0	0	0	0	0
1492	L	1	1	RS	0	0	0	0	1	1	0	1	0	0	0	1	0	0	0	0	0	0
1493	L	1	1	RS	1	0	1	0	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	0	0
1494	L	1	1	RS	0	0	0	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0
1495	L	1	1	RS	0	0	0	1	0	NA	NA	0	0	0	0	1	0	0	0	0	0	0
1496	L	1	1	RS	1	1	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
1497	L	1	1	RS	1	1	0	1	0	NA	NA	1	0	0	1	1	0	0	0	0	0	0
1498	L	1	1	RS	1	1	0	0	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1499	L	1	1	RS	0	0	0	1	0	NA	NA	0	0	0	0	1	0	0	0	0	0	0
1500	L	1	1	RS	0	0	1	1	0	1	0	0	0	0	1	1	0	0	1	0	0	0
1501	L	2	1	RS	0	1	1	0	0	NA	NA	0	0	0	1	1	0	0	0	0	0	0
1502	L	2	1	RS	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
1503	L	2	1	RS	1	0	1	1	0	NA	NA	1	0	0	1	1	0	0	1	0	0	0
1504	L	2	1	RS	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0
1505	L	2	1	RS	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
1506	L	2	1	RS	0	0	1	0	0	1	0	1	1	0	0	0	0	0	1	0	1	1
1507	L	2	1	RS	1	1	0	1	0	NA	NA	0	0	0	1	1	0	0	0	0	0	0
1508	L	2	1	RS	0	0	0	0	0	1	0	1	0	0	0	0	0	0	1	0	1	1
1509	L	2	1	RS	0	1	0	0	0	1	0	1	1	0	1	1	0	0	1	0	0	0
1510	L	2	1	RS	1	1	1	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0
1511	L	2	1	RS	1	1	0	1	0	NA	NA	1	0	0	0	0	0	0	1	0	0	0
1512	L	2	1	RS	1	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
1513	L	2	1	RS	0	1	1	0	0	NA	NA	0	0	0	1	1	0	0	0	0	0	0
1514	L	2	1	RS	NA	1	0	1	0	NA	NA	1	1	0	1	1	0	0	0	0	0	0
1515	L	2	1	RS	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
1516	L	2	1	RS	1	1	0	0	1	1	0	0	0	0	1	1	0	0	0	0	NA	NA
1517	L	2	1	RS	0	1	0	0	0	NA	NA	1	1	0	0	0	0	0	1	0	0	0
1518	L	2	1	RS	0	0	0	0	0	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA	0	0
1519	L	2	1	RS	1	1	0	0	0	NA	NA	1	0	0	0	1	0	1	0	0	0	0
1520	L	2	1	RS	0	0	0	0	0	NA	NA	0	0	0	1	1	0	0	0	0	0	0
1521	L	2	2	RS	1	0	0	1	0	NA	NA	1	1	0	1	1	0	0	0	0	0	0
1522	L	2	2	RS	0	0	1	1	1	1	0	1	1	0	0	0	0	1	0	0	0	0
1523	L	2	2	RS	1	0	1	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0
1524	L	2	2	RS	0	0	0	1	0	0	0	1	0	0	1	0	0	0	0	0	0	0
1525	L	2	2	RS	0	0	1	1	0	0	0	1	1	0	0	0	0	0	1	0	0	0
1526	L	2	2	RS	1	0	0	0	0	NA	NA	1	0	0	0	0	0	1	1	0	0	0
1527	L	2	2	RS	1	0	1	1	0	NA	NA	1	0	0	1	0	0	0	1	0	0	0
1528	L	2	2	RS	1	0	0	1	0	1	0	1	0	0	1	0	0	0	1	0	0	0
1529	L	2	2	RS	1	0	0	0	0	1	0	1	0	0	1	1	0	0	0	0	0	0
1530	L	2	2	RS	1	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
1531	L	2	2	RS	0	0	1	1	1	0	0	1	1	0	1	1	1	1	0	0	0	0

1532	L	2	2	RS	1	0	0	1	0	1	0	1	1	0	1	1	0	0	0	0	0	0
1533	L	2	2	RS	1	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	0	0
1534	L	2	2	RS	1	0	1	0	0	1	0	1	0	0	1	1	0	0	1	0	0	0
1535	L	2	2	RS	1	1	0	0	0	NA	NA	1	0	0	1	1	0	0	0	0	0	0
1536	L	2	2	RS	1	1	1	1	0	0	0	1	0	0	1	1	0	0	1	0	0	0
1537	L	2	2	RS	1	0	1	1	0	0	0	1	0	0	1	1	0	0	1	0	0	0
1538	L	2	2	RS	1	0	0	1	0	NA	NA	1	1	0	1	1	0	0	0	0	0	0
1539	L	2	2	RS	1	1	0	1	0	NA	NA	0	0	0	1	1	0	0	0	0	0	0
1540	L	2	2	RS	1	0	0	1	0	0	0	1	0	0	1	1	0	0	1	0	0	0
1541	L	2	3	RS	1	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
1542	L	2	3	RS	1	0	0	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0
1543	L	2	3	RS	1	1	0	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0
1544	L	2	3	RS	0	0	0	1	0	NA	NA	1	1	0	1	1	0	0	0	0	0	0
1545	L	2	3	RS	0	0	1	1	0	1	0	1	1	0	1	1	0	0	0	0	1	1
1546	L	2	3	RS	1	1	0	0	1	1	0	0	0	0	1	1	0	0	0	0	0	0
1547	L	2	3	RS	0	0	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
1548	L	2	3	RS	1	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0
1549	L	2	3	RS	0	1	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0
1550	L	2	3	RS	1	1	0	1	0	0	0	1	0	0	1	1	0	0	0	0	0	0
1551	L	2	3	RS	1	1	0	1	0	NA	NA	1	0	0	0	0	0	0	1	0	0	0
1552	L	2	3	RS	0	0	0	1	0	NA	NA	1	1	0	NA	NA	NA	NA	NA	NA	NA	NA
1553	L	2	3	RS	1	1	0	1	0	1	0	1	1	0	1	1	0	0	0	0	0	0
1554	L	2	3	RS	1	1	0	1	0	1	0	1	1	0	1	1	0	0	0	0	0	0
1555	L	2	3	RS	1	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
1556	L	2	3	RS	1	1	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
1557	L	2	3	RS	1	0	0	1	1	1	0	0	0	0	1	1	0	0	0	0	0	0
1558	L	2	3	RS	1	1	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
1559	L	2	3	RS	1	1	0	1	1	1	0	1	1	0	1	1	0	0	0	0	0	0
1560	L	2	3	RS	1	1	0	1	0	NA	NA	1	1	0	1	1	0	0	0	0	0	0
1561	L	4	1	RS	1	0	0	1	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1562	L	4	1	RS	1	0	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
1563	L	4	1	RS	1	1	0	1	0	1	0	1	1	0	1	1	0	0	0	0	0	0
1564	L	4	1	RS	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1565	L	4	1	RS	0	0	0	1	0	1	0	0	0	0	1	1	0	0	0	0	0	0
1566	L	4	1	RS	1	0	1	0	0	1	0	1	0	0	0	1	0	1	1	0	0	0
1567	L	4	1	RS	1	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
1568	L	4	1	RS	1	0	0	0	0	1	0	1	0	0	1	1	0	0	0	0	0	0
1569	L	4	1	RS	1	1	0	1	0	1	0	1	1	0	1	1	0	0	0	0	0	0
1570	L	4	1	RS	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
1571	L	4	1	RS	0	1	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
1572	L	4	1	RS	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
1573	L	4	1	RS	1	0	0	0	0	1	0	1	0	0	1	1	0	0	1	0	0	0
1574	L	4	1	RS	0	0	0	0	0	NA	NA	1	1	0	NA	NA	NA	NA	NA	NA	NA	NA
1575	L	4	1	RS	1	0	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
1576	L	4	1	RS	1	0	0	1	0	0	0	1	0	0	1	1	0	0	0	0	0	0
1577	L	4	1	RS	0	1	1	1	0	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1578	L	4	1	RS	0	0	0	0	0	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1579	L	4	1	RS	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0

1580	L	4	1	RS	1	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
1581	L	4	2	RS	1	1	0	1	0	1	0	1	1	0	1	1	0	0	0	0	0	0
1582	L	4	2	RS	1	1	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
1583	L	4	2	RS	1	0	0	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0
1584	L	4	2	RS	1	1	0	1	0	0	0	1	1	0	1	1	0	0	0	0	0	0
1585	L	4	2	RS	1	0	0	1	0	NA	NA	1	0	0	1	1	0	0	1	0	0	0
1586	L	4	2	RS	1	1	0	0	1	1	0	NA	NA	NA	1	1	0	0	0	0	0	0
1587	L	4	2	RS	1	0	0	1	0	0	0	1	0	0	1	0	0	0	1	0	0	0
1588	L	4	2	RS	1	0	0	0	1	1	0	0	0	0	1	0	0	1	0	0	0	0
1589	L	4	2	RS	1	0	0	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0
1590	L	4	2	RS	1	1	0	1	0	1	0	1	1	0	1	1	0	0	0	0	0	0
1591	L	4	2	RS	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0
1592	L	4	2	RS	1	0	0	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0
1593	L	4	2	RS	1	0	0	0	0	1	0	1	0	0	1	1	0	0	1	0	0	0
1594	L	4	2	RS	1	0	0	0	0	1	0	1	1	0	1	1	0	0	0	0	0	0
1595	L	4	2	RS	0	1	1	1	0	0	0	1	1	0	0	0	1	0	0	0	1	1
1596	L	4	2	RS	0	1	1	1	0	1	0	1	1	0	0	0	1	0	0	0	1	1
1597	L	4	2	RS	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1
1598	L	4	2	RS	1	1	0	0	0	1	0	1	1	0	1	1	0	0	0	0	0	0
1599	L	4	2	RS	0	1	1	1	0	1	0	1	1	0	0	0	1	0	0	0	1	1
1600	L	4	2	RS	1	1	0	1	0	1	0	1	1	0	1	1	0	0	0	0	0	0
1601	L	4	3	RS	1	1	0	0	0	NA	NA	1	1	0	NA	NA	NA	NA	NA	NA	NA	NA
1602	L	4	3	RS	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
1603	L	4	3	RS	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
1604	L	4	3	RS	1	1	0	0	0	1	0	1	0	0	1	1	0	0	0	0	0	0
1605	L	4	3	RS	1	0	0	1	0	1	0	1	0	0	0	1	0	0	1	0	0	0
1606	L	4	3	RS	1	0	1	0	0	NA	NA	1	0	0	1	1	0	1	1	0	0	0
1607	L	4	3	RS	1	1	0	0	0	0	0	1	1	0	0	0	1	1	0	0	0	0
1608	L	4	3	RS	0	1	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0
1609	L	4	3	RS	1	0	0	1	0	1	0	1	1	0	1	1	0	0	0	0	0	0
1610	L	4	3	RS	0	1	0	1	1	0	0	0	0	0	1	1	0	0	0	1	0	0
1611	L	4	3	RS	0	1	0	0	1	0	0	0	0	0	1	1	0	0	1	0	0	0
1612	L	4	3	RS	1	1	0	0	1	1	0	0	0	0	1	1	0	0	0	0	0	0
1613	L	4	3	RS	1	0	0	1	0	1	0	1	1	0	1	1	0	0	0	0	0	0
1614	L	4	3	RS	0	0	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0
1615	L	4	3	RS	0	1	0	0	1	0	0	0	0	0	1	1	0	0	0	1	0	0
1616	L	4	3	RS	0	1	0	0	0	1	0	1	0	0	1	1	0	0	1	0	0	0
1617	L	4	3	RS	0	1	0	1	0	0	0	1	0	0	0	1	0	1	1	0	1	1
1618	L	4	3	RS	0	1	0	0	1	0	0	0	0	0	1	1	0	0	0	1	0	0
1619	L	4	3	RS	1	1	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
1620	L	4	3	RS	0	1	0	0	1	1	0	0	0	1	1	1	0	0	0	0	0	0
1621	L	5	1	RS	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	1	1	0	0	0	0	0	0
1622	L	5	1	RS	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0	0	0	0
1623	L	5	1	RS	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
1624	L	5	1	RS	1	0	0	1	0	NA	NA	1	0	0	1	1	0	0	0	0	0	0
1625	L	5	1	RS	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0
1626	L	5	1	RS	0	0	0	0	1	1	0	1	0	0	0	0	0	0	1	0	0	0
1627	L	5	1	RS	NA	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

1628	L	5	1	RS	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0
1629	L	5	1	RS	1	1	0	1	0	NA	NA	1	1	0	NA	NA	NA	NA	NA	NA	NA	NA
1630	L	5	1	RS	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0
1631	L	5	1	RS	0	0	0	0	0	1	0	1	0	0	1	0	0	0	1	0	0	0
1632	L	5	1	RS	0	1	0	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0
1633	L	5	1	RS	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
1634	L	5	1	RS	1	1	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
1635	L	5	1	RS	1	0	0	0	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1636	L	5	1	RS	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0
1637	L	5	1	RS	1	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0
1638	L	5	1	RS	1	1	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
1639	L	5	1	RS	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	1	1	0	0	0
1640	L	5	1	RS	0	0	0	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0
1641	L	5	2	RS	1	0	0	1	0	0	0	1	0	0	1	1	0	0	1	0	0	0
1642	L	5	2	RS	1	0	0	1	0	1	0	1	0	0	1	0	0	0	1	0	0	0
1643	L	5	2	RS	1	0	0	0	0	NA	NA	1	0	0	0	1	0	0	1	0	0	0
1644	L	5	2	RS	1	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0	0	0
1645	L	5	2	RS	1	0	0	0	0	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1646	L	5	2	RS	1	0	0	0	0	NA	NA	0	0	0	0	0	0	0	0	0	0	0
1647	L	5	2	RS	1	0	0	0	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1648	L	5	2	RS	1	0	0	1	0	NA	NA	1	0	0	1	1	0	0	1	0	0	0
1649	L	5	2	RS	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	1	1	0	0	0	0	0	0
1650	L	5	2	RS	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0
1651	L	5	2	RS	1	1	0	1	0	0	0	1	0	0	1	1	0	0	0	0	0	0
1652	L	5	2	RS	1	0	0	0	0	NA	NA	0	0	0	0	1	0	0	0	0	0	0
1653	L	5	2	RS	1	0	0	1	0	0	0	1	0	0	1	1	0	0	1	0	0	0
1654	L	5	2	RS	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
1655	L	5	2	RS	1	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
1656	L	5	2	RS	0	1	0	0	0	NA	NA	0	0	0	1	1	0	0	1	0	0	0
1657	L	5	2	RS	1	0	0	0	0	NA	NA	1	0	0	0	0	0	1	1	0	0	0
1658	L	5	2	RS	1	1	0	0	0	NA	NA	1	0	0	0	0	0	0	1	0	0	0
1659	L	5	2	RS	1	1	0	0	0	NA	NA	0	0	0	0	1	0	0	1	0	0	0
1660	L	5	2	RS	1	0	0	1	0	0	0	1	0	0	1	1	0	0	1	0	0	0
1661	L	5	3	RS	0	0	0	1	0	NA	NA	0	0	0	1	1	0	0	0	0	0	0
1662	L	5	3	RS	0	1	1	1	1	0	0	1	1	0	0	0	0	0	1	0	0	0
1663	L	5	3	RS	0	0	0	1	0	NA	NA	0	0	0	0	1	0	0	0	0	0	0
1664	L	5	3	RS	1	0	0	0	0	1	0	0	0	0	1	0	0	1	0	0	0	0
1665	L	5	3	RS	1	0	0	0	0	NA	NA	1	1	0	0	0	0	0	0	0	0	0
1666	L	5	3	RS	1	0	0	0	0	1	0	1	0	0	1	0	0	0	1	0	0	0
1667	L	5	3	RS	1	0	0	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0
1668	L	5	3	RS	1	0	0	0	0	0	0	1	0	0	0	1	0	0	1	0	0	0
1669	L	5	3	RS	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
1670	L	5	3	RS	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0	0	0	0
1671	L	5	3	RS	1	0	0	0	0	1	0	1	0	0	1	1	0	0	0	0	0	0
1672	L	5	3	RS	1	0	0	1	0	0	0	1	0	0	NA	NA	NA	NA	NA	NA	0	0
1673	L	5	3	RS	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
1674	L	5	3	RS	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
1675	L	5	3	RS	1	1	0	0	0	NA	NA	1	0	0	1	1	0	1	1	0	0	0

1676	L	5	3	RS	1	0	0	1	0	1	0	1	0	0	0	0	0	0	1	0	0	0
1677	L	5	3	RS	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1678	L	5	3	RS	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
1679	L	5	3	RS	0	1	0	0	1	1	0	0	0	0	1	0	0	0	0	0	0	0
1680	L	5	3	RS	0	0	0	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0
1681	T	1	1	S	0	0	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
1682	T	1	1	S	0	0	0	0	0	1	0	1	0	0	1	1	0	0	1	0	0	0
1683	T	1	1	S	0	0	0	0	0	1	0	0	0	0	0	1	0	1	1	0	0	0
1684	T	1	1	S	1	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
1685	T	1	1	S	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0
1686	T	1	1	S	1	0	0	1	0	NA	NA	1	0	0	0	0	0	1	0	0	0	0
1687	T	1	1	S	0	0	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
1688	T	1	1	S	0	0	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
1689	T	1	1	S	0	1	1	1	0	1	0	0	0	0	0	0	0	1	0	0	0	0
1690	T	1	1	S	0	1	0	0	0	0	0	1	0	0	1	1	0	0	1	0	1	1
1691	T	1	1	S	1	1	0	0	0	NA	NA	1	0	0	0	0	0	0	0	0	0	0
1692	T	1	1	S	0	1	1	0	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1693	T	1	1	S	0	0	0	1	1	1	0	1	0	0	1	1	0	0	0	0	0	0
1694	T	1	1	S	0	0	1	1	0	0	0	0	0	0	1	1	0	1	0	0	0	0
1695	T	1	1	S	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
1696	T	1	1	S	0	1	0	0	1	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1697	T	1	1	S	0	0	0	1	0	NA	NA	1	1	0	NA	NA	NA	NA	NA	NA	NA	NA
1698	T	1	1	S	0	1	1	1	1	1	0	1	0	0	1	1	0	0	1	0	0	0
1699	T	1	1	S	1	0	0	0	0	1	0	1	0	0	1	1	0	0	0	0	0	0
1700	T	1	1	S	0	0	0	1	0	0	0	0	0	0	1	1	0	1	1	0	0	0
1701	T	1	1	S	1	1	0	1	0	NA	NA	1	0	0	1	1	0	0	0	0	0	0
1702	T	1	1	S	0	0	0	1	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1703	T	1	1	S	1	1	0	0	0	NA	NA	1	0	0	1	1	0	1	0	0	0	0
1704	T	1	1	S	0	0	0	1	0	1	0	1	0	0	1	1	0	1	1	0	0	0
1705	T	1	1	S	1	1	0	0	0	NA	NA	0	0	0	1	1	0	1	1	0	0	0
1706	T	1	1	S	0	1	1	1	0	1	0	0	0	0	1	1	0	1	0	0	0	0
1707	T	1	1	S	0	1	0	1	0	0	0	1	0	0	1	1	1	0	0	1	0	0
1708	T	1	1	S	0	1	1	1	1	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1709	T	1	1	S	0	0	0	1	0	0	0	1	0	0	1	1	0	0	1	0	0	0
1710	T	1	1	S	1	1	0	0	0	1	0	1	0	0	1	1	0	0	0	0	0	0
1711	T	1	2	S	0	0	0	1	0	0	0	1	0	0	1	1	0	0	1	0	0	0
1712	T	1	2	S	0	0	0	1	1	NA	NA	1	1	0	0	0	0	0	0	0	0	0
1713	T	1	2	S	1	1	0	0	0	NA	NA	1	1	0	0	1	0	0	0	0	0	0
1714	T	1	2	S	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1715	T	1	2	S	1	0	0	1	0	NA	NA	1	0	0	0	0	0	0	1	0	0	0
1716	T	1	2	S	1	0	1	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0
1717	T	1	2	S	0	0	1	1	1	1	0	1	0	0	0	0	0	1	0	0	0	0
1718	T	1	2	S	1	0	1	1	0	0	0	1	0	0	0	1	0	0	1	0	0	0
1719	T	1	2	S	1	0	1	1	0	1	0	0	0	0	1	1	0	0	0	0	0	0
1720	T	1	2	S	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
1721	T	1	2	S	1	0	0	1	0	0	0	1	0	0	1	1	0	0	1	0	0	0
1722	T	1	2	S	1	0	0	0	0	1	0	1	0	0	1	1	0	0	0	0	0	0
1723	T	1	2	S	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0

1724	T	1	2	S	1	1	0	0	0	NA	NA	1	0	0	0	1	0	0	1	0	0	0
1725	T	1	2	S	0	0	1	1	0	1	0	1	1	0	0	0	1	1	0	0	1	1
1726	T	1	2	S	0	0	1	1	1	1	0	0	0	0	0	0	0	0	1	0	0	0
1727	T	1	2	S	1	1	0	1	0	NA	NA	0	0	0	1	1	0	0	1	0	0	0
1728	T	1	2	S	0	0	1	1	0	NA	NA	1	1	0	0	0	1	1	0	0	1	1
1729	T	1	2	S	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
1730	T	1	2	S	1	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
1731	T	1	2	S	0	0	0	1	0	0	0	0	0	0	0	1	0	0	1	0	0	0
1732	T	1	2	S	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
1733	T	1	2	S	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
1734	T	1	2	S	0	0	1	0	1	1	0	1	0	0	1	1	0	1	0	0	0	0
1735	T	1	2	S	1	1	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0
1736	T	1	2	S	1	0	0	1	1	0	0	0	0	0	1	1	0	0	1	0	0	0
1737	T	1	2	S	1	1	1	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0
1738	T	1	2	S	0	0	0	1	0	1	0	0	0	0	1	1	0	0	1	0	0	0
1739	T	1	2	S	1	0	0	0	1	1	0	0	0	0	0	1	0	1	0	0	0	0
1740	T	1	2	S	0	1	0	0	0	NA	NA	0	0	0	0	1	0	0	0	0	0	0
1741	T	1	3	S	1	0	0	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0
1742	T	1	3	S	1	0	0	0	0	NA	NA	0	0	0	1	1	0	0	1	0	0	0
1743	T	1	3	S	1	1	0	1	0	NA	NA	0	0	0	1	1	0	0	0	0	0	0
1744	T	1	3	S	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
1745	T	1	3	S	1	1	0	1	0	NA	NA	1	0	0	0	1	0	0	1	0	0	0
1746	T	1	3	S	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0
1747	T	1	3	S	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
1748	T	1	3	S	1	1	0	1	0	NA	NA	1	0	0	1	1	0	0	0	0	0	0
1749	T	1	3	S	1	1	1	1	0	0	0	1	1	0	0	0	0	1	1	0	0	0
1750	T	1	3	S	1	0	0	1	0	1	0	1	0	0	0	1	0	0	0	0	0	0
1751	T	1	3	S	0	0	0	0	0	NA	NA	0	0	0	0	1	0	1	0	0	0	0
1752	T	1	3	S	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0
1753	T	1	3	S	1	0	0	0	0	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1754	T	1	3	S	1	1	0	0	0	NA	NA	1	0	0	1	0	0	0	0	0	0	0
1755	T	1	3	S	1	0	0	1	1	1	0	0	0	0	1	1	0	0	1	0	0	0
1756	T	1	3	S	1	1	0	1	0	NA	NA	0	0	0	0	1	0	0	0	0	0	0
1757	T	1	3	S	1	0	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0
1758	T	1	3	S	1	0	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
1759	T	1	3	S	1	1	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0
1760	T	1	3	S	0	0	0	1	0	1	0	0	0	0	1	1	0	0	0	0	0	0
1761	T	1	3	S	0	0	0	0	0	1	0	1	0	0	1	1	0	1	0	0	0	0
1762	T	1	3	S	1	0	0	1	0	0	0	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1763	T	1	3	S	1	0	1	1	0	NA	NA	1	0	0	1	1	0	0	0	0	0	0
1764	T	1	3	S	1	1	0	0	0	0	0	1	0	0	0	1	0	0	1	0	0	0
1765	T	1	3	S	1	1	0	0	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1766	T	1	3	S	0	0	0	1	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1767	T	1	3	S	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0
1768	T	1	3	S	1	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0
1769	T	1	3	S	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
1770	T	1	3	S	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1771	T	2	1	S	1	1	0	1	0	NA	NA	1	0	0	1	1	0	0	0	0	0	0

1772	T	2	1	S	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
1773	T	2	1	S	1	0	0	0	0	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA	NA
1774	T	2	1	S	0	0	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0
1775	T	2	1	S	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
1776	T	2	1	S	0	0	0	0	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA
1777	T	2	1	S	1	0	0	0	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA
1778	T	2	1	S	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
1779	T	2	1	S	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
1780	T	2	1	S	0	0	0	1	0	0	0	1	0	0	1	1	0	0	0	0	0
1781	T	2	1	S	1	1	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0
1782	T	2	1	S	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
1783	T	2	1	S	1	0	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0
1784	T	2	1	S	0	1	0	1	1	0	0	1	0	0	NA	NA	NA	NA	NA	NA	NA
1785	T	2	1	S	0	0	0	1	0	1	0	0	0	0	1	1	0	1	1	0	0
1786	T	2	1	S	1	1	0	0	0	NA	NA	1	0	0	1	1	0	0	0	0	0
1787	T	2	1	S	0	1	0	0	0	NA	NA	0	0	0	0	1	0	0	0	0	0
1788	T	2	1	S	1	1	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0
1789	T	2	1	S	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
1790	T	2	1	S	1	1	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0
1791	T	2	2	S	1	1	0	1	0	1	0	0	0	0	0	0	0	0	1	0	0
1792	T	2	2	S	1	0	0	1	0	1	0	1	0	0	1	1	0	0	1	0	0
1793	T	2	2	S	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0
1794	T	2	2	S	1	1	0	1	0	1	0	1	1	0	1	1	0	0	0	0	0
1795	T	2	2	S	1	0	1	0	0	1	0	0	0	0	1	1	0	0	0	0	0
1796	T	2	2	S	1	0	0	0	0	NA	NA	1	1	0	0	0	0	1	0	0	0
1797	T	2	2	S	1	0	0	0	1	1	0	1	0	0	1	1	0	0	1	0	0
1798	T	2	2	S	1	0	0	0	0	NA	NA	1	1	0	0	0	0	0	0	0	0
1799	T	2	2	S	1	0	0	0	0	NA	NA	1	0	0	0	0	0	0	0	0	0
1800	T	2	2	S	0	1	1	0	0	0	0	1	1	0	0	0	0	1	0	0	0
1801	T	2	2	S	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
1802	T	2	2	S	0	1	0	1	0	1	0	0	0	0	1	1	0	1	0	0	0
1803	T	2	2	S	1	0	0	1	0	1	0	1	0	0	1	1	0	0	1	0	0
1804	T	2	2	S	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
1805	T	2	2	S	0	0	0	0	0	0	0	1	0	0	1	1	0	0	1	0	0
1806	T	2	2	S	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0
1807	T	2	2	S	0	0	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0
1808	T	2	2	S	1	1	0	0	0	NA	NA	1	0	0	1	1	0	0	0	0	0
1809	T	2	2	S	1	0	0	1	0	1	0	1	0	0	1	0	0	0	0	0	0
1810	T	2	2	S	1	0	0	1	0	1	0	1	0	0	1	1	0	0	1	0	0
1811	T	2	3	S	1	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0
1812	T	2	3	S	1	1	0	1	0	1	0	1	0	0	0	0	0	0	1	0	0
1813	T	2	3	S	1	0	0	1	0	NA	NA	1	0	0	1	0	0	0	0	0	0
1814	T	2	3	S	0	0	0	0	0	1	0	1	0	0	0	0	0	0	1	0	0
1815	T	2	3	S	1	1	0	1	0	1	0	1	1	0	1	1	0	0	0	0	0
1816	T	2	3	S	1	0	0	1	0	1	0	1	1	0	1	1	0	0	0	0	0
1817	T	2	3	S	1	0	0	1	0	1	0	1	1	0	1	1	0	0	1	0	0
1818	T	2	3	S	1	1	0	1	0	0	0	1	0	0	0	1	0	0	1	0	0
1819	T	2	3	S	1	1	0	1	0	0	0	1	1	0	1	1	0	0	1	0	0

1820	T	2	3	S	1	1	0	1	0	NA	NA	1	0	0	1	1	0	0	0	0	0	0
1821	T	2	3	S	1	0	0	1	0	1	0	1	0	0	1	0	0	0	0	0	0	0
1822	T	2	3	S	1	0	0	0	1	1	0	1	0	0	1	1	0	1	0	0	0	0
1823	T	2	3	S	1	1	0	0	0	NA	NA	1	1	0	1	1	0	0	1	0	0	0
1824	T	2	3	S	1	1	0	1	1	1	0	1	0	0	1	1	0	0	0	0	0	0
1825	T	2	3	S	0	1	0	0	0	NA	NA	1	0	0	1	0	0	0	0	0	0	0
1826	T	2	3	S	0	0	0	1	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1827	T	2	3	S	0	1	0	1	0	1	0	0	0	1	1	1	0	0	1	0	0	0
1828	T	2	3	S	1	1	0	1	0	NA	NA	1	1	0	1	1	0	0	0	0	0	0
1829	T	2	3	S	1	1	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0
1830	T	2	3	S	0	1	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0
1831	T	4	1	S	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0
1832	T	4	1	S	1	0	0	1	0	1	0	1	0	0	1	0	0	0	1	0	0	0
1833	T	4	1	S	1	1	0	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0
1834	T	4	1	S	1	0	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
1835	T	4	1	S	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0
1836	T	4	1	S	1	1	0	0	0	NA	NA	1	1	0	1	1	0	0	1	0	0	0
1837	T	4	1	S	1	0	0	1	0	0	0	1	0	0	1	1	0	0	1	0	0	0
1838	T	4	1	S	1	1	0	1	0	0	0	1	0	0	1	1	0	0	1	0	0	0
1839	T	4	1	S	1	0	0	1	0	0	0	0	0	0	0	0	0	1	1	0	0	0
1840	T	4	1	S	1	0	0	1	0	1	0	1	0	0	0	0	0	1	0	0	0	0
1841	T	4	1	S	1	1	0	0	1	1	0	0	0	0	1	1	0	0	0	0	0	0
1842	T	4	1	S	1	0	0	1	0	1	0	1	0	0	1	0	0	0	1	0	0	0
1843	T	4	1	S	1	1	0	0	0	1	0	1	0	0	1	1	0	0	0	0	0	0
1844	T	4	1	S	1	1	0	0	0	NA	NA	1	0	0	0	0	0	0	0	0	0	0
1845	T	4	1	S	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1	0	0	0
1846	T	4	1	S	1	1	0	0	0	0	0	1	0	0	NA	NA	NA	NA	NA	NA	0	0
1847	T	4	1	S	1	1	0	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0
1848	T	4	1	S	0	1	0	0	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1849	T	4	1	S	1	1	0	1	0	NA	NA	1	0	0	1	1	0	0	0	0	0	0
1850	T	4	1	S	1	1	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0
1851	T	4	2	S	1	1	0	1	0	0	0	1	0	0	1	0	0	0	1	0	0	0
1852	T	4	2	S	1	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	0	0
1853	T	4	2	S	1	1	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
1854	T	4	2	S	1	0	0	1	0	0	0	1	0	0	1	1	0	0	1	0	0	0
1855	T	4	2	S	1	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0
1856	T	4	2	S	1	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0
1857	T	4	2	S	1	0	0	1	0	1	0	1	0	0	0	1	0	0	0	0	0	0
1858	T	4	2	S	0	1	0	0	0	NA	NA	0	0	0	0	0	0	0	1	0	0	0
1859	T	4	2	S	1	1	0	0	1	1	0	0	0	0	1	1	0	0	0	0	0	0
1860	T	4	2	S	1	0	0	1	0	0	0	1	0	0	0	1	0	0	1	0	0	0
1861	T	4	2	S	1	0	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
1862	T	4	2	S	1	1	0	1	0	NA	NA	1	1	0	1	1	0	0	0	0	0	0
1863	T	4	2	S	1	1	0	0	1	1	0	0	0	0	1	1	0	0	0	0	0	0
1864	T	4	2	S	1	0	0	0	0	NA	NA	1	1	0	1	1	0	0	1	0	0	0
1865	T	4	2	S	1	0	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
1866	T	4	2	S	1	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0
1867	T	4	2	S	1	0	0	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0

1868	T	4	2	S	1	1	0	1	0	NA	NA	1	1	0	1	1	0	0	0	0	0	0
1869	T	4	2	S	1	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	0	0
1870	T	4	2	S	1	0	0	1	0	1	0	1	0	0	0	1	0	0	1	0	0	0
1871	T	4	3	S	1	1	0	1	0	NA	NA	1	0	0	NA	NA	NA	NA	NA	NA	NA	NA
1872	T	4	3	S	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
1873	T	4	3	S	1	0	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
1874	T	4	3	S	1	0	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
1875	T	4	3	S	1	0	0	1	0	NA	NA	1	1	0	1	1	0	0	0	0	0	0
1876	T	4	3	S	1	0	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
1877	T	4	3	S	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0
1878	T	4	3	S	1	0	0	1	0	0	0	1	0	0	1	1	0	0	1	0	0	0
1879	T	4	3	S	1	0	0	1	0	0	0	1	0	0	1	1	0	0	0	0	0	0
1880	T	4	3	S	1	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
1881	T	4	3	S	1	0	0	0	0	NA	NA	1	0	0	0	0	0	0	0	0	0	0
1882	T	4	3	S	1	0	0	1	0	NA	NA	1	0	0	1	1	0	0	0	0	0	0
1883	T	4	3	S	1	0	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
1884	T	4	3	S	1	0	0	1	0	1	0	1	0	0	0	0	0	0	1	0	0	0
1885	T	4	3	S	1	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0
1886	T	4	3	S	1	0	0	1	0	1	0	1	0	0	0	1	0	0	1	0	0	0
1887	T	4	3	S	1	0	0	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0
1888	T	4	3	S	1	1	1	0	0	NA	NA	0	0	0	0	0	0	0	0	0	1	1
1889	T	4	3	S	1	0	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
1890	T	4	3	S	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
1891	T	5	1	S	1	1	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
1892	T	5	1	S	1	0	0	0	0	NA	NA	0	0	0	1	1	0	0	0	0	0	0
1893	T	5	1	S	0	1	0	0	1	1	0	1	0	0	0	0	0	0	1	0	0	0
1894	T	5	1	S	1	0	0	1	0	1	0	1	0	0	1	0	0	0	1	0	0	0
1895	T	5	1	S	1	1	0	0	0	1	0	1	0	0	1	1	0	0	0	0	0	0
1896	T	5	1	S	0	1	0	1	0	0	0	1	0	0	1	1	0	0	0	1	0	0
1897	T	5	1	S	1	0	0	0	0	NA	NA	0	0	0	0	0	0	0	1	0	0	0
1898	T	5	1	S	0	1	1	0	0	0	0	1	1	0	0	0	1	1	0	0	1	1
1899	T	5	1	S	1	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
1900	T	5	1	S	1	0	0	1	0	0	0	1	0	0	1	1	0	0	1	0	0	0
1901	T	5	1	S	1	1	0	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0
1902	T	5	1	S	1	1	0	1	0	NA	NA	1	0	0	1	1	0	0	0	0	0	0
1903	T	5	1	S	1	1	0	0	1	1	0	0	0	0	1	1	0	0	0	0	0	0
1904	T	5	1	S	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
1905	T	5	1	S	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
1906	T	5	1	S	1	0	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
1907	T	5	1	S	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
1908	T	5	1	S	1	0	0	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0
1909	T	5	1	S	1	1	0	0	0	NA	NA	0	0	0	1	1	0	0	0	0	0	0
1910	T	5	1	S	1	0	0	1	0	NA	NA	1	0	0	1	1	0	0	0	0	0	0
1911	T	5	2	S	1	1	0	1	1	1	0	1	0	0	1	1	0	0	0	0	0	0
1912	T	5	2	S	1	0	0	0	1	1	0	0	0	0	0	0	0	1	1	0	0	0
1913	T	5	2	S	1	1	0	1	0	1	0	1	0	0	0	1	0	0	1	0	0	0
1914	T	5	2	S	1	1	0	1	1	1	0	1	0	0	1	1	0	0	0	0	0	0
1915	T	5	2	S	1	0	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0

1916	T	5	2	S	1	1	1	1	1	0	0	1	0	0	1	1	0	0	0	0	0	0
1917	T	5	2	S	1	1	0	1	0	NA	NA	1	0	0	1	1	0	0	1	0	0	0
1918	T	5	2	S	1	1	0	1	0	0	0	1	0	0	1	1	0	0	0	0	0	0
1919	T	5	2	S	1	1	0	0	1	0	0	0	0	0	1	1	0	0	1	0	0	0
1920	T	5	2	S	1	0	0	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0
1921	T	5	2	S	1	0	0	1	0	1	0	1	0	0	1	0	0	0	1	0	0	0
1922	T	5	2	S	1	0	0	0	0	NA	NA	1	0	0	1	1	0	0	0	0	0	0
1923	T	5	2	S	1	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
1924	T	5	2	S	1	1	0	1	0	NA	NA	1	0	0	1	1	0	0	0	0	0	0
1925	T	5	2	S	1	1	0	0	0	NA	NA	0	0	0	0	0	0	0	1	0	0	0
1926	T	5	2	S	1	1	0	0	0	NA	NA	0	0	0	0	1	0	0	1	0	0	0
1927	T	5	2	S	1	0	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
1928	T	5	2	S	1	1	0	1	0	NA	NA	1	0	0	1	1	0	0	0	0	0	0
1929	T	5	2	S	1	0	0	1	0	1	0	1	0	0	0	1	0	0	1	0	0	0
1930	T	5	2	S	1	1	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
1931	T	5	3	S	1	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
1932	T	5	3	S	1	1	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
1933	T	5	3	S	1	1	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
1934	T	5	3	S	1	0	0	1	0	0	0	1	0	0	0	1	0	0	1	0	0	0
1935	T	5	3	S	1	0	0	0	0	1	0	1	0	0	1	1	0	0	0	0	0	0
1936	T	5	3	S	1	1	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
1937	T	5	3	S	1	1	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
1938	T	5	3	S	1	1	0	1	0	0	0	1	0	0	1	1	0	0	1	0	0	0
1939	T	5	3	S	1	1	0	1	0	0	0	1	0	0	0	0	0	0	1	0	0	0
1940	T	5	3	S	1	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
1941	T	5	3	S	1	1	0	0	0	NA	NA	1	0	0	1	1	0	0	0	0	0	0
1942	T	5	3	S	1	0	0	1	0	NA	NA	1	0	0	1	1	0	0	0	0	0	0
1943	T	5	3	S	1	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
1944	T	5	3	S	1	0	0	1	0	0	0	1	0	0	0	1	0	0	1	0	0	0
1945	T	5	3	S	1	0	0	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0
1946	T	5	3	S	1	1	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
1947	T	5	3	S	1	1	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
1948	T	5	3	S	1	0	0	0	0	1	0	0	0	0	NA	NA	NA	NA	NA	NA	0	0
1949	T	5	3	S	1	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0
1950	T	5	3	S	1	0	0	1	0	0	0	1	0	0	1	1	0	0	0	0	0	0

FREQUENCY OF BACTERIAL FUNCTIONS

Number of positive strains , total number of isolates tested (Total), and proportion of positive isolates (%Pos), for each bacterial trait tested

Cultivars	Anaconda (A), Bastion (B), Cavia (C), Lipresso (L), Control without plant (T)
Fraction	Root (R), rhizosphere soil (RS), bulk soil (S)
Plant development stage	Stem apparition (1), four leaves (2), ear development (3), ear maturity (4)

Enzymatic activities				
(1)	Gram+		Gram positive (No Aminopeptidase activity)	
(2)	Oxid+		Oxydase activity	
(3)	Rednit+		Reduction of nitrate in nitrite	
(4)	Denit+		Reduction of nitrate in gazeous nitrogen (denitrification)	
(5)	Nitram+		Reduction of nitrate in ammonium (nitrammonification)	
(6)	Case+		Casein hydrolysis	
(7)	Gelat+		Gelatin hydrolysis	
(8)	Sulf+		Production of Aryl-sulfatases in presence or absence of sulfate	
(9)	PhyG+		Production of phytases (total PhyNC + PhyC)	
	----------PhyNC		Production of phytases	No clearing-zone
	----------PhyC		Production of phytases (total PhyCRd + PhyCRa + PhyCEd + PhyCEa + PhyCEm)	Clearing-zone
		----------PhyCRd	Production of phytases (ectoparietal)	Clearing-zone restricted to the underface of colonies / disapeared after cobal addition
		----------PhyCRa	Production of phytases (ectoparietal)	Clearing-zone restricted to the underface of colonies / attenuated after cobal addition
		----------PhyCEd	Production of phytases (extracellular)	Clearing-zone extended around colonies / disapeared after cobal addition
		----------PhyCEa	Production of phytases (extracellular)	Clearing-zone extended around colonies / attenuated after cobal addition
		----------PhyCEm	Production of phytases (extracellular) and to mineralise phytate	Clearing-zone extended around colonies / maintained after cobal addition
Secretion activities				
(10)	Psb+		Ca-phosphate solubilisation	
(11)	Sid+		Production of siderophores	
(12)	Iaa+		Production of auxin	
(13)	Hcn+		Production of hydrogen cyanide	
(14)	Eps+		Production of glucan	
(15)	NahI+ C4-C8		Production of oxo- or C3 non-substituted short carbon chain N-acylhomosérine lactones (C4 to C8)	
(16)	NahI+ C6-C12		Production of C3 substituted or non-substituted long carbon chain N-acylhomosérine lactones (C6 to C12)	

Gram+

cultivar	fraction	stage	Nb+	Total	% Pos
A	R	1	8	30	26.7
		2	3	56	5.4
		4	17	57	29.8
		5	19	60	31.7
	Total R		47	203	23.2
	RS	1	14	30	46.7
		2	37	58	63.8
		4	40	58	69.0
		5	50	60	83.3
	Total RS		141	206	68.4
Total A			188	409	**46.0**
B	R	1	1	28	3.6
		2	7	59	11.9
		4	14	59	23.7
		5	29	60	48.3
	Total R		51	206	24.8
	RS	1	14	27	51.9
		2	44	58	75.9
		4	34	59	57.6
		5	46	58	79.3
	Total RS		138	202	68.3
Total B			189	408	**46.3**
C	R	1	12	30	40.0
		2	5	59	8.5
		4	4	59	6.8
		5	34	57	59.6
	Total R		55	205	26.8
	RS	1	17	30	56.7
		2	28	60	46.7
		4	27	59	45.8
		5	36	57	63.2
	Total RS		108	206	52.4
Total C			163	411	**39.7**
L	R	1	7	30	23.3
		2	8	59	13.6
		4	8	57	14.0
		5	15	54	27.8
	Total R		38	200	19.0
	RS	1	11	30	36.7
		2	38	59	64.4
		4	36	59	61.0
		5	34	52	65.4
	Total RS		119	200	59.5
Total L			157	400	**39.3**
T	S	1	44	88	50.0
		2	38	57	66.7
		4	55	58	94.8
		5	55	60	91.7
	Total S		192	263	**73.0**
Total T			192	263	
TOTAL			889	1891	

Oxydase+

cultivar	fraction	stage	Nb+	Total	% Pos
A	R	1	8	30	26.7
		2	21	55	38.2
		4	28	57	49.1
		5	41	60	68.3
	Total R		98	202	48.5
	RS	1	2	30	6.7
		2	28	58	48.3
		4	27	58	46.6
		5	26	60	43.3
	Total RS		83	206	40.3
Total A			181	408	**44.4**
B	R	1	11	28	39.3
		2	15	59	25.4
		4	36	59	61.0
		5	21	60	35.0
	Total R		83	206	40.3
	RS	1	16	27	59.3
		2	25	58	43.1
		4	30	59	50.8
		5	17	58	29.3
	Total RS		88	202	43.6
Total B			171	408	**41.9**
C	R	1	8	30	26.7
		2	29	59	49.2
		4	37	59	62.7
		5	26	57	45.6
	Total R		100	205	48.8
	RS	1	16	30	53.3
		2	19	60	31.7
		4	28	60	46.7
		5	19	57	33.3
	Total RS		82	207	39.6
Total C			182	412	**44.2**
L	R	1	13	30	43.3
		2	24	59	40.7
		4	30	57	52.6
		5	16	54	29.6
	Total R		83	200	41.5
	RS	1	14	30	46.7
		2	25	60	41.7
		4	30	59	50.8
		5	12	52	23.1
	Total RS		81	201	40.3
Total L			164	401	**40.9**
T	S	1	31	88	35.2
		2	28	57	49.1
		4	22	58	37.9
		5	33	60	55.0
	Total S		114	263	**43.3**
Total T			114	263	
TOTAL			812	1892	

Rechit+

cultivar	fraction	stage	Nb+	Total	% Pos
A	R	1	17	30	56.7
		2	22	56	39.3
		4	22	57	38.6
		5	39	60	65.0
	Total R		100	203	49.3
	RS	1	24	30	80.0
		2	36	58	62.1
		4	37	58	63.8
		5	46	60	76.7
	Total RS		143	206	69.4
Total A			243	409	**59.4**
B	R	1	10	28	35.7
		2	22	59	37.3
		4	15	59	25.4
		5	20	60	33.3
	Total R		67	206	32.5
	RS	1	19	27	70.4
		2	32	58	55.2
		4	28	59	47.5
		5	43	58	74.1
	Total RS		122	202	60.4
Total B			189	408	**46.3**
C	R	1	13	30	43.3
		2	26	59	44.1
		4	9	59	15.3
		5	28	57	49.1
	Total R		76	205	37.1
	RS	1	12	30	40.0
		2	32	60	53.3
		4	37	60	61.7
		5	29	57	50.9
	Total RS		110	207	53.1
Total C			186	412	**45.1**
L	R	1	14	30	46.7
		2	13	59	22.0
		4	13	57	22.8
		5	14	54	25.9
	Total R		54	200	27.0
	RS	1	19	30	63.3
		2	40	60	66.7
		4	38	58	65.5
		5	30	52	57.7
	Total RS		127	200	63.5
Total L			181	400	**45.3**
T	S	1	52	88	59.1
		2	44	57	77.2
		4	44	58	75.9
		5	40	60	66.7
	Total S		180	263	**68.4**
Total T			180	263	
TOTAL			979	1892	

Denit+

cultivar	fraction	stage	Nb+	Total	% Pos
A	R	1	2	30	6.7
		2	4	56	7.1
		4	6	57	10.5
		5	15	60	25.0
	Total R		27	203	13.3
	RS	1	5	30	16.7
		2	7	58	12.1
		4	13	58	22.4
		5	12	60	20.0
	Total RS		37	206	18.0
Total A			64	409	**15.6**
B	R	1	3	28	10.7
		2	7	59	11.9
		4	1	59	1.7
		5	2	60	3.3
	Total R		13	206	6.3
	RS	1	7	27	25.9
		2	1	58	1.7
		4	7	59	11.9
		5	1	58	1.7
	Total RS		16	202	7.9
Total B			29	408	**7.1**
C	R	1	2	30	6.7
		2	8	59	13.6
		4	3	59	5.1
		5	1	57	1.8
	Total R		14	205	6.8
	RS	1	4	30	13.3
		2	12	60	20.0
		4	16	60	26.7
		5		57	0.0
	Total RS		32	207	15.5
Total C			46	412	**11.2**
L	R	1	2	30	6.7
		2	3	59	5.1
		4	2	57	3.5
		5	3	54	5.6
	Total R		10	200	5.0
	RS	1	2	30	6.7
		2	19	60	31.7
		4	17	58	29.3
		5	3	52	5.8
	Total RS		41	200	20.5
Total L			51	400	**12.8**
T	S	1	6	88	6.8
		2	11	57	19.3
		4	6	58	10.3
		5	1	60	1.7
	Total S		24	263	**9.1**
Total T			24	263	
TOTAL			214	1892	

			Nitram+					Case+					Gelat+					Sulf+				
cultivar	fraction	stage	Nb+	Total	% Pos	cultivar	fraction	stage	Nb+	Total	% Pos	cultivar	fraction	stage	Nb+	Total	% Pos	cultivar	fraction	stage	Nb+	Total	% Pos
A	R	1		30	0.0	A	R	1	15	28	53.6	A	R	1	13	28	46.4	A	R	1	1	25	4.0
		2	2	56	3.6			2	7	42	16.7			2	12	42	28.6			2	7	41	17.1
		4		57	0.0			4	28	57	49.1			4	26	57	45.6			4	3	56	5.4
		5		60	0.0			5	18	58	31.0			5	20	58	34.5			5	7	53	13.2
	Total R		2	203	1.0		Total R		68	185	36.8		Total R		71	185	38.4		Total R		18	175	10.3
	RS	1		30	0.0		RS	1	14	28	50.0		RS	1	13	28	46.4		RS	1		19	0.0
		2		58	0.0			2	34	55	61.8			2	27	55	49.1			2		55	0.0
		4		58	0.0			4	34	53	64.2			4	25	53	47.2			4	5	45	11.1
		5		60	0.0			5	36	56	64.3			5	39	56	69.6			5	3	56	5.4
	Total RS			206	0.0		Total RS		118	192	61.5		Total RS		104	192	54.2		Total RS		8	175	4.6
Total A			2	409	**0.5**	Total A			186	377	**49.3**	Total A			175	377	**46.4**	Total A			26	350	**7.4**
B	R	1		28	0.0	B	R	1	7	25	28.0	B	R	1	7	25	28.0	B	R	1	4	25	16.0
		2		59	0.0			2	17	52	32.7			2	18	52	34.6			2	5	50	10.0
		4		59	0.0			4	15	49	30.6			4	26	49	53.1			4	8	45	17.8
		5		60	0.0			5	26	58	44.8			5	38	58	65.5			5	2	57	3.5
	Total R			206	0.0		Total R		65	184	35.3		Total R		89	184	48.4		Total R		19	177	10.7
	RS	1		27	0.0		RS	1	10	19	52.6		RS	1	7	19	36.8		RS	1	4	19	21.1
		2		58	0.0			2	36	54	66.7			2	41	54	75.9			2	1	54	1.9
		4		59	0.0			4	23	56	41.1			4	30	57	52.6			4	3	51	5.9
		5		58	0.0			5	37	56	66.1			5	47	56	83.9			5	2	56	3.6
	Total RS			202	0.0		Total RS		106	185	57.3		Total RS		125	186	67.2		Total RS		10	180	5.6
Total B				408	**0.0**	Total B			171	369	**46.3**	Total B			214	370	**57.8**	Total B			29	357	**8.1**
C	R	1	1	30	3.3	C	R	1	19	26	73.1	C	R	1	19	26	73.1	C	R	1	1	27	3.7
		2		59	0.0			2	21	49	42.9			2	22	49	44.9			2	7	50	14.0
		4		59	0.0			4	27	55	49.1			4	34	55	61.8			4	4	55	7.3
		5		57	0.0			5	23	52	44.2			5	23	52	44.2			5	1	54	1.9
	Total R		1	205	0.5		Total R		90	182	49.5		Total R		98	182	53.8		Total R		13	186	7.0
	RS	1	3	30	10.0		RS	1	12	21	57.1		RS	1	13	21	61.9		RS	1		21	0.0
		2		60	0.0			2	36	53	67.9			2	37	53	69.8			2	3	54	5.6
		4	1	60	1.7			4	36	57	63.2			4	38	57	66.7			4	1	57	1.8
		5		57	0.0			5	31	54	57.4			5	36	54	66.7			5	4	50	8.0
	Total RS		4	207	1.9		Total RS		115	185	62.2		Total RS		124	185	67.0		Total RS		8	182	4.4
Total C			5	412	**1.2**	Total C			205	367	**55.9**	Total C			222	367	**60.5**	Total C			21	368	**5.7**
L	R	1		30	0.0	L	R	1	8	20	40.0	L	R	1	7	20	35.0	L	R	1		20	0.0
		2		59	0.0			2	31	52	59.6			2	33	52	63.5			2	4	53	7.5
		4		57	0.0			4	23	57	40.4			4	32	57	56.1			4	7	54	13.0
		5	2	54	3.7			5	19	49	38.8			5	27	49	55.1			5	4	46	8.7
	Total R		2	200	1.0		Total R		81	178	45.5		Total R		99	178	55.6		Total R		15	173	8.7
	RS	1	1	30	3.3		RS	1	13	28	46.4		RS	1	20	28	71.4		RS	1		28	0.0
		2		60	0.0			2	43	58	74.1			2	41	58	70.7			2	3	58	5.2
		4	1	58	1.7			4	39	54	72.2			4	39	54	72.2			4	5	54	9.3
		5		52	0.0			5	23	50	46.0			5	27	50	54.0			5		51	0.0
	Total RS		2	200	1.0		Total RS		118	190	62.1		Total RS		127	190	66.8		Total RS		8	191	4.2
Total L			4	400	**1.0**	Total L			199	368	**54.1**	Total L			226	368	**61.4**	Total L			23	364	**6.3**
T	S	1		88	0.0	T	S	1	41	79	51.9	T	S	1	53	79	67.1	T	S	1	3	79	3.8
		2	1	57	1.8			2	33	52	63.5			2	32	52	61.5			2		52	0.0
		4		58	0.0			4	37	55	67.3			4	40	55	72.7			4	1	56	1.8
		5		60	0.0			5	44	59	74.6			5	47	59	79.7			5	1	60	1.7
	Total S		1	263	**0.4**		Total S		155	245	**63.3**		Total S		172	245	**70.2**		Total S		5	247	**2.0**
Total T			1	263		Total T			155	245		Total T			172	245		Total T			5	247	
TOTAL			12	1892		TOTAL			916	1726		TOTAL			1009	1727		TOTAL			104	1686	

PhyG+

cultivar	fraction	stage	Nb+	Total	% Pos	PhyNC	% Pos	PhyC	% Pos	PhyCRd	% Pos	PhyCRa	% Pos	PhyCEd	% Pos	PhyCEa	% Pos	PhyCEm	% Pos
A	R	1	24	29	82.8	13	44.8	11	37.9	0	0.0	0	0.0	8	27.6	3	10.3	0	0.0
		2	35	42	83.3	19	45.2	16	38.1	0	0.0	0	0.0	9	21.4	4	9.5	3	7.1
		4	52	57	91.2	25	43.9	27	47.4	2	3.5	0	0.0	6	10.5	9	15.8	10	17.5
		5	51	58	87.9	34	58.6	17	29.3	3	5.2	2	3.4	5	8.6	5	8.6	2	3.4
	Total R		162	186	87.1	91	48.9	71	38.2	5	2.7	2	1.1	28	15.1	21	11.3	15	8.1
	RS	1	25	28	89.3	12	42.9	13	46.4	5	17.9	0	0.0	7	25.0	0	0.0	1	3.6
		2	47	55	85.5	16	29.1	31	56.4	3	5.5	0	0.0	26	47.3	2	3.6	0	0.0
		4	47	54	87.0	21	38.9	26	48.1	5	9.3	1	1.9	20	37.0	0	0.0	0	0.0
		5	46	58	79.3	15	25.9	31	53.4	5	8.6	0	0.0	26	44.8	0	0.0	0	0.0
	Total RS		165	195	84.6	64	32.8	101	51.8	18	9.2	1	0.5	79	40.5	2	1.0	1	0.5
Total A			**327**	**381**	**85.8**	**155**	**40.7**	**172**	**45.1**	**23**	**6.0**	**3**	**0.8**	**107**	**28.1**	**23**	**6.0**	**16**	**4.2**
B	R	1	24	25	96.0	14	56.0	10	40.0	1	4.0	0	0.0	3	12.0	5	20.0	1	4.0
		2	47	53	88.7	34	64.2	13	24.5	1	1.9	3	5.7	3	5.7	3	5.7	3	5.7
		4	44	51	86.3	22	43.1	22	43.1	1	2.0	1	2.0	6	11.8	12	23.5	2	3.9
		5	56	59	94.9	19	32.2	37	62.7	6	10.2	1	1.7	25	42.4	4	6.8	1	1.7
	Total R		171	188	91.0	89	47.3	82	43.6	9	4.8	5	2.7	37	19.7	24	12.8	7	3.7
	RS	1	17	19	89.5	8	42.1	9	47.4	4	21.1	0	0.0	5	26.3	0	0.0	0	0.0
		2	48	55	87.3	17	30.9	31	56.4	4	7.3	0	0.0	26	47.3	1	1.8	0	0.0
		4	45	55	81.8	29	52.7	16	29.1	1	1.8	0	0.0	15	27.3	0	0.0	0	0.0
		5	52	57	91.2	19	33.3	33	57.9	2	3.5	0	0.0	31	54.4	0	0.0	0	0.0
	Total RS		162	186	87.1	73	39.2	89	47.8	11	5.9	0	0.0	77	41.4	1	0.5	0	0.0
Total B			**333**	**374**	**89.0**	**162**	**43.3**	**171**	**45.7**	**20**	**5.3**	**5**	**1.3**	**114**	**30.5**	**25**	**6.7**	**7**	**1.9**
C	R	1	19	27	70.4	8	29.6	11	40.7	0	0.0	0	0.0	8	29.6	2	7.4	1	3.7
		2	42	49	85.7	19	38.8	23	46.9	1	2.0	0	0.0	21	42.9	1	2.0	0	0.0
		4	42	54	77.8	25	46.3	17	31.5	0	0.0	0	0.0	15	27.8	2	3.7	0	0.0
		5	48	57	84.2	20	35.1	28	49.1	1	1.8	2	3.5	23	40.4	2	3.5	0	0.0
	Total R		151	187	80.7	72	38.5	79	42.2	2	1.1	2	1.1	67	35.8	7	3.7	1	0.5
	RS	1	13	23	56.5	6	26.1	7	30.4	1	4.3	0	0.0	6	26.1	0	0.0	0	0.0
		2	29	54	53.7	11	20.4	18	33.3	2	3.7	0	0.0	14	25.9	2	3.7	0	0.0
		4	45	58	77.6	23	39.7	22	37.9	3	5.2	0	0.0	17	29.3	2	3.4	0	0.0
		5	39	54	72.2	13	24.1	26	48.1	5	9.3	1	1.9	16	29.6	3	5.6	1	1.9
	Total RS		126	189	66.7	53	28.0	73	38.6	11	5.8	1	0.5	53	28.0	7	3.7	1	0.5
Total C			**277**	**376**	**73.7**	**125**	**33.2**	**152**	**40.4**	**13**	**3.5**	**3**	**0.8**	**120**	**31.9**	**14**	**3.7**	**2**	**0.5**
L	R	1	20	20	100.0	8	40.0	12	60.0	0	0.0	0	0.0	12	60.0	0	0.0	0	0.0
		2	44	54	81.5	24	44.4	20	37.0	0	0.0	0	0.0	19	35.2	0	0.0	1	1.9
		4	55	56	98.2	28	50.0	27	48.2	4	7.1	0	0.0	22	39.3	0	0.0	1	1.8
		5	49	52	94.2	24	46.2	25	48.1	3	5.8	0	0.0	15	28.8	6	11.5	1	1.9
	Total R		168	182	92.3	84	46.2	84	46.2	7	3.8	0	0.0	68	37.4	6	3.3	3	1.6
	RS	1	19	29	65.5	10	34.5	9	31.0	0	0.0	0	0.0	9	31.0	0	0.0	0	0.0
		2	40	59	67.8	10	16.9	30	50.8	3	5.1	1	1.7	24	40.7	2	3.4	0	0.0
		4	52	53	98.1	20	37.7	32	60.4	2	3.8	1	1.9	26	49.1	3	5.7	0	0.0
		5	35	47	74.5	18	38.3	17	36.2	0	0.0	0	0.0	16	34.0	1	2.1	0	0.0
	Total RS		146	188	77.7	58	30.9	88	46.8	5	2.7	2	1.1	75	39.9	6	3.2	0	0.0
Total L			**314**	**370**	**84.9**	**142**	**38.4**	**172**	**46.5**	**12**	**3.2**	**2**	**0.5**	**143**	**38.6**	**12**	**3.2**	**3**	**0.8**
T	S	1	60	80	75.0	28	35.0	32	40.0	6	7.5	0	0.0	25	31.3	1	1.3	0	0.0
		2	41	53	77.4	13	24.5	28	52.8	1	1.9	1	1.9	24	45.3	2	3.8	0	0.0
		4	45	56	80.4	15	26.8	30	53.6	0	0.0	2	3.6	20	35.7	8	14.3	0	0.0
		5	47	60	78.3	17	28.3	30	50.0	1	1.7	0	0.0	22	36.7	7	11.7	0	0.0
	Total S		**193**	**249**	**77.5**	**73**	**29.3**	**120**	**48.2**	**8**	**3.2**	**3**	**1.2**	**91**	**36.5**	**18**	**7.2**	**0**	**0.0**
Total T			193	249	77.5	73	29.3	120	48.2	8	3.2	3	1.2	91	36.5	18	7.2	0	0.0
TOTAL			1444	1750	82.5	657	37.5	787	45.0	76	4.3	16	0.9	575	32.9	92	5.3	28	1.6

Psb+

cultivar	fraction	stage	Nb+	Total	% Pos
A	R	1	9	30	30.0
		2	9	55	16.4
		4	15	57	26.3
		5	13	60	21.7
	Total R		46	202	22.8
	RS	1	6	30	20.0
		2	14	57	24.6
		4	7	58	12.1
		5	4	60	6.7
	Total RS		31	205	15.1
Total A			77	407	**18.9**
B	R	1	9	28	32.1
		2	10	59	16.9
		4	5	59	8.5
		5	23	60	38.3
	Total R		47	206	22.8
	RS	1	11	27	40.7
		2	4	58	6.9
		4	1	56	1.8
		5	6	58	10.3
	Total RS		22	199	11.1
Total B			69	405	**17.0**
C	R	1	7	30	23.3
		2	6	59	10.2
		4	10	59	16.9
		5	8	57	14.0
	Total R		31	205	15.1
	RS	1	3	30	10.0
		2	7	60	11.7
		4	9	60	15.0
		5	7	55	12.7
	Total RS		26	205	12.7
Total C			57	410	**13.9**
L	R	1	15	30	50.0
		2	5	59	8.5
		4	7	57	12.3
		5	4	54	7.4
	Total R		31	200	15.5
	RS	1	6	30	20.0
		2	8	60	13.3
		4	11	59	18.6
		5	5	52	9.6
	Total RS		30	201	14.9
Total L			61	401	**15.2**
T	S	1	13	88	14.8
		2	7	57	12.3
		4	6	58	10.3
		5	8	60	13.3
	Total S		34	263	**12.9**
Total T			34	263	
TOTAL			298	1886	

Sid+

cultivar	fraction	stage	Nb+	Total	% Pos
A	R	1	14	30	46.7
		2	29	55	52.7
		4	28	57	49.1
		5	28	60	46.7
	Total R		99	202	49.0
	RS	1	16	30	53.3
		2	29	57	50.9
		4	27	58	46.6
		5	30	60	50.0
	Total RS		102	205	49.8
Total A			201	407	**49.4**
B	R	1	7	28	25.0
		2	24	59	40.7
		4	29	59	49.2
		5	14	60	23.3
	Total R		74	206	35.9
	RS	1	14	27	51.9
		2	29	58	50.0
		4	24	56	42.9
		5	29	58	50.0
	Total RS		96	199	48.2
Total B			170	405	**42.0**
C	R	1	19	30	63.3
		2	18	59	30.5
		4	22	59	37.3
		5	19	57	33.3
	Total R		78	205	38.0
	RS	1	15	30	50.0
		2	31	60	51.7
		4	28	60	46.7
		5	26	57	45.6
	Total RS		100	207	48.3
Total C			178	412	**43.2**
L	R	1	8	30	26.7
		2	11	59	18.6
		4	30	57	52.6
		5	17	54	31.5
	Total R		66	200	33.0
	RS	1	20	30	66.7
		2	32	60	53.3
		4	27	59	45.8
		5	23	52	44.2
	Total RS		102	201	50.7
Total L			168	401	**41.9**
T	S	1	52	88	59.1
		2	31	57	54.4
		4	37	58	63.8
		5	34	60	56.7
	Total S		154	263	**58.6**
Total T			154	263	
TOTAL			871	1888	

baa+

cultivar	fraction	stage	Nb+	Total	% Pos
A	R	1	12	28	42.9
		2	13	42	31.0
		4	14	57	24.6
		5	17	58	29.3
	Total R		56	185	30.3
	RS	1	15	28	53.6
		2	28	55	50.9
		4	22	53	41.5
		5	20	56	35.7
	Total RS		85	192	44.3
Total A			141	377	**37.4**
B	R	1	1	25	4.0
		2	7	52	13.5
		4	7	49	14.3
		5	1	58	1.7
	Total R		16	184	8.7
	RS	1	6	19	31.6
		2	20	54	37.0
		4	20	57	35.1
		5	9	56	16.1
	Total RS		55	186	29.6
Total B			71	370	**19.2**
C	R	1	6	26	23.1
		2	21	49	42.9
		4	11	55	20.0
		5	11	52	21.2
	Total R		49	182	26.9
	RS	1	6	21	28.6
		2	13	53	24.5
		4	22	57	38.6
		5	20	54	37.0
	Total RS		61	185	33.0
Total C			110	367	**30.0**
L	R	1	3	20	15.0
		2	11	52	21.2
		4	12	57	21.1
		5	12	49	24.5
	Total R		38	178	21.3
	RS	1	11	28	39.3
		2	18	58	31.0
		4	13	54	24.1
		5	24	50	48.0
	Total RS		66	190	34.7
Total L			104	368	**28.3**
T	S	1	28	79	35.4
		2	16	52	30.8
		4	24	55	43.6
		5	20	59	33.9
	Total S		88	245	**35.9**
Total T			88	245	
TOTAL			514	1727	

Hcn+

cultivar	fraction	stage	Nb+	Total	% Pos
A	R	1		28	0.0
		2	1	42	2.4
		4	1	57	1.8
		5	2	58	3.4
	Total R		4	185	2.2
	RS	1		28	0.0
		2		55	0.0
		4		53	0.0
		5	1	56	1.8
	Total RS		1	192	0.5
Total A			5	377	**1.3**
B	R	1		25	0.0
		2	2	52	3.8
		4	2	49	4.1
		5		58	0.0
	Total R		4	184	2.2
	RS	1		19	0.0
		2		54	0.0
		4		57	0.0
		5		56	0.0
	Total RS			186	0.0
Total B			4	370	**1.1**
C	R	1		26	0.0
		2	1	49	2.0
		4	5	55	9.1
		5	1	52	1.9
	Total R		7	182	3.8
	RS	1		21	0.0
		2		53	0.0
		4	6	57	10.5
		5	1	54	1.9
	Total RS		7	185	3.8
Total C			14	367	**3.8**
L	R	1		20	0.0
		2		52	0.0
		4		57	0.0
		5		49	0.0
	Total R			178	0.0
	RS	1		28	0.0
		2		58	0.0
		4	3	54	5.6
		5		50	0.0
	Total RS		3	190	1.6
Total L			3	368	**0.8**
T	S	1	1	79	1.3
		2		52	0.0
		4		55	0.0
		5	1	59	1.7
	Total S		2	245	**0.8**
Total T			2	245	
TOTAL			28	1727	

Eps+

cultivar	fraction	stage	Nb+	Total	% Pos
A	R	1	10	30	33.3
		2	12	59	20.3
		4	13	60	21.7
		5	16	60	26.7
	Total R		51	209	24.4
	RS	1	3	30	10.0
		2	6	60	10.0
		4	9	60	15.0
		5	10	60	16.7
	Total RS		28	210	13.3
Total A			79	419	**18.9**
B	R	1	10	30	33.3
		2	17	60	28.3
		4	24	60	40.0
		5	2	60	3.3
	Total R		53	210	25.2
	RS	1	9	30	30.0
		2	4	60	6.7
		4	17	60	28.3
		5	3	59	5.1
	Total RS		33	209	15.8
Total B			86	419	**20.5**
C	R	1	6	30	20.0
		2	24	60	40.0
		4	27	60	45.0
		5	3	60	5.0
	Total R		60	210	28.6
	RS	1	11	30	36.7
		2	21	60	35.0
		4	8	60	13.3
		5	3	60	5.0
	Total RS		43	210	20.5
Total C			103	420	**24.5**
L	R	1	13	30	43.3
		2	22	60	36.7
		4	22	60	36.7
		5	7	60	11.7
	Total R		64	210	30.5
	RS	1	13	30	43.3
		2	17	60	28.3
		4	11	60	18.3
		5	3	58	5.2
	Total RS		44	208	21.2
Total L			108	418	**25.8**
T	S	1	20	90	22.2
		2	2	60	3.3
		4	1	60	1.7
		5	2	60	3.3
	Total S		25	270	**9.3**
Total T			25	270	
TOTAL			401	1946	

Nahl+ C4-C8

cultivar	fraction	stage	Nb+	Total	% Pos
A	R	1	6	28	21.4
		2	13	42	31.0
		4	2	57	3.5
		5	10	58	17.2
	Total R		31	185	16.8
	RS	1	1	28	3.6
		2	1	55	1.8
		4	4	53	7.5
		5	2	56	3.6
	Total RS		8	192	4.2
Total A			39	377	**10.3**
B	R	1	2	25	8.0
		2	7	52	13.5
		4	3	49	6.1
		5	4	58	6.9
	Total R		16	184	8.7
	RS	1	4	19	21.1
		2		54	0.0
		4	2	57	3.5
		5		56	0.0
	Total RS		6	186	3.2
Total B			22	370	**5.9**
C	R	1		26	0.0
		2	6	49	12.2
		4	2	55	3.6
		5	2	52	3.8
	Total R		10	182	5.5
	RS	1	1	21	4.8
		2	2	53	3.8
		4	1	57	1.8
		5	2	54	3.7
	Total RS		6	185	3.2
Total C			16	367	**4.4**
L	R	1	1	20	5.0
		2	4	52	7.7
		4	5	57	8.8
		5	3	49	6.1
	Total R		13	178	7.3
	RS	1	1	28	3.6
		2	1	58	1.7
		4	4	54	7.4
		5		50	0.0
	Total RS		6	190	3.2
Total L			19	368	**5.2**
T	S	1	3	79	3.8
		2		52	0.0
		4		55	0.0
		5	1	59	1.7
	Total S		4	245	**1.6**
Total T			4	245	
TOTAL			100	1727	

Nahl+ C6-C12

cultivar	fraction	stage	Nb+	Total	% Pos
A	R	1	8	28	28.6
		2	18	42	42.9
		4	14	57	24.6
		5	17	58	29.3
	Total R		57	185	30.8
	RS	1	9	28	32.1
		2	10	55	18.2
		4	9	53	17.0
		5	6	56	10.7
	Total RS		34	192	17.7
Total A			91	377	**24.1**
B	R	1	14	25	56.0
		2	19	52	36.5
		4	18	49	36.7
		5	13	58	22.4
	Total R		64	184	34.8
	RS	1	6	19	31.6
		2	6	54	11.1
		4	12	57	21.1
		5	4	56	7.1
	Total RS		28	186	15.1
Total B			92	370	**24.9**
C	R	1	2	26	7.7
		2	7	49	14.3
		4	11	55	20.0
		5	7	52	13.5
	Total R		27	182	14.8
	RS	1	1	21	4.8
		2	9	53	17.0
		4	12	57	21.1
		5	5	54	9.3
	Total RS		27	185	14.6
Total C			54	367	**14.7**
L	R	1	3	20	15.0
		2	13	52	25.0
		4	15	57	26.3
		5	2	49	4.1
	Total R		33	178	18.5
	RS	1	3	28	10.7
		2	6	58	10.3
		4	6	54	11.1
		5	10	50	20.0
	Total RS		25	190	13.2
Total L			58	368	**15.8**
T	S	1	22	79	27.8
		2	7	52	13.5
		4	4	55	7.3
		5	3	59	5.1
	Total S		36	245	**14.7**
Total T			36	245	
TOTAL			331	1727	