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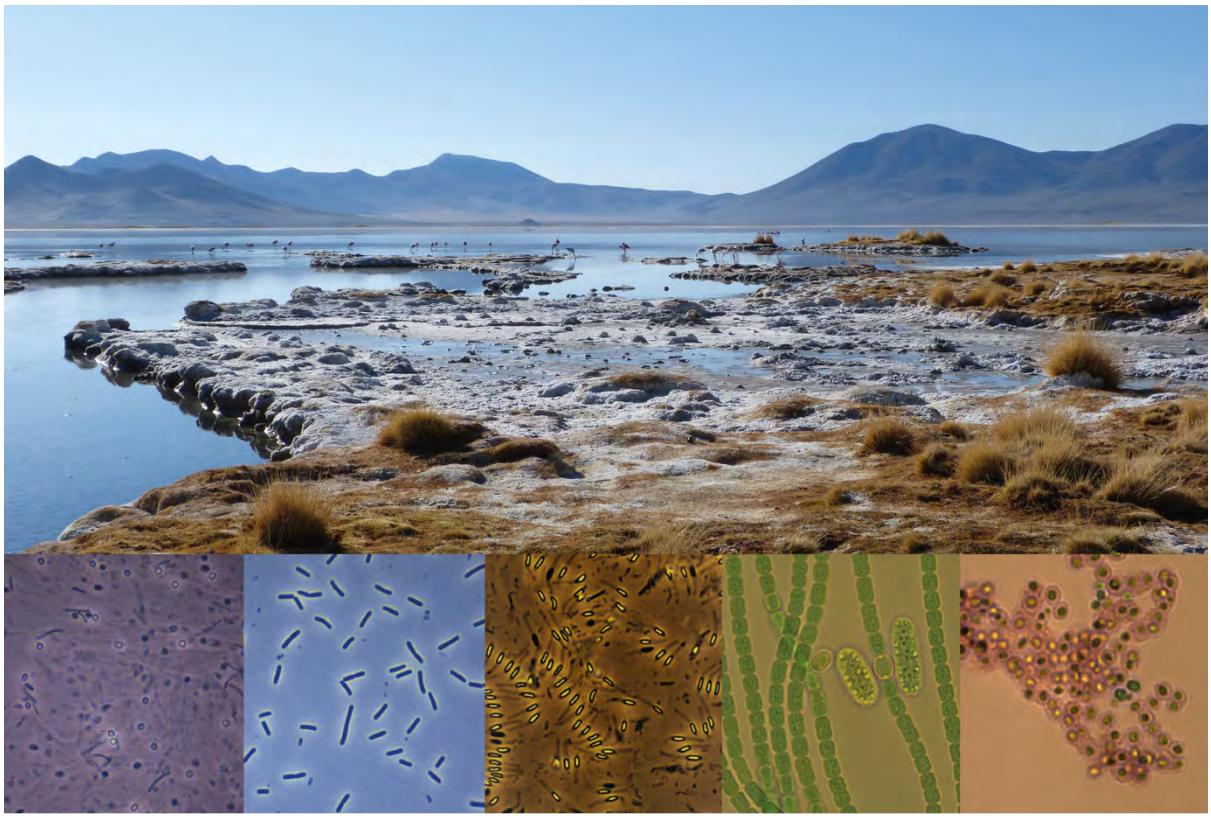
A step closer to Unveiling the Environmental diversity of Spores and Lysis-resistant cells in Bacteria, Archaea and Fungi



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September 2022
Neuchâtel, Switzerland

A step closer to Unveiling the Environmental diversity of Spores and Lysis-resistant cells in Bacteria, Archaea and Fungi

A dissertation submitted to the
University of Neuchâtel
by
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Neuchâtel, le 15 septembre 2022 Le Doyen, Prof. R. Bshary

“It is our responsibility as scientists, knowing the great progress and great value of a satisfactory philosophy of ignorance, the great progress that is the fruit of freedom of thought, to proclaim the value of this freedom, to teach how doubt is not to be feared but welcomed and discussed, and to demand this freedom as our duty to all coming generations.”

Richard Feynman,
on the Universal Responsibility of Scientists

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Summary

Dormancy and the production of specialized resistant structures (e.g., spores, cysts, sclerotia, etc.) are survival strategies used by many organisms to endure environmental conditions that are not ideal for growth and reproduction. In this doctoral dissertation we focus on the dormant fraction (i.e., dormant, and specialized resistant structures) produced by bacteria, fungi, and archaea. Dormant and specialized resistant cells rest in the environment, forming what it denominated, the microbial seedbank. The microbial seedbank is an important reservoir of phenotypical and genetical diversity which is also known to influence the evolution of populations and microbial processes. Given the importance of the microbial seedbank it would be important to understand its composition and dynamics. However, until now the methods used to assess the microbial environmental diversity, are not able to distinguish between the active and dormant fraction and specially specialized resistant structures that form part of the dormant fraction. Here we use lysis-resistance as a proxy to target the diversity of specialized resistant cells of bacteria, fungi, and archaea. Through the application of the “lysis-resistant enrichment method” we show that lysis-resistance in bacteria is limited to a few taxa, however not limited to the well described sporogenic genus. On the other hand, lysis-resistance in fungi and archaea was observed to be broadly distributes. In the case of fungi this was to be expected as the production of spores and other resistant structures are an integral part of most fungi’s life cycle. In the case of archaea, the broad distribution of lysis-resistance might be a result of the cell’s composition which might make them more resistance to lysis than bacteria, however it would be feasible that archaea are also able to enter dormancy and produce spore-like structures.

Here we also systematically evaluated the morphological (optical and cryo-EM microscopy) and chemical composition (Raman microspectroscopy) of model bacterial spores, in the search of a universal spore marker. This to develop a spore sorting platform to isolate spores from environmental samples. Though the systematic evaluation of the model sporulating bacteria, the only characteristic that was shared among all spores, was the enlargement of the cell wall compared to the vegetative cell. On the other hand, the cell wall of each spore type differed in the number and density of the layers from the other spore types. The chemical composition of each

spore type was also unique; thus, no spore universal marker could be identified. Nonetheless, unique chemical markers were identified for each spore type that could be used for the isolation of bacterial spores from environmental samples.

To conclude, although we were not able to develop a universal sorting platform for the isolation of dormant and specialized resistant cells. We have now moved a step closer to be able to assess the microbial seedbank.

Key words:

Bacteria | Fungi | Archaea | Microbial seedbank | dormancy | lysis-resistance | diversity | spores | endospore | exospore | myxospore | cysts | akinete | Raman spectroscopy | Cryo-EM|

Résumé

La dormance et la production de structures résistantes spécialisées, comme par exemple les spores, les kystes, ou les sclérotes, sont des stratégies de survie utilisées par de nombreux organismes pour résister à des conditions environnementales qui ne sont pas idéales pour la croissance et la reproduction. Dans cette thèse de doctorat, nous nous concentrons sur la fraction dormante (c'est-à-dire les structures dormantes et résistantes spécialisées) produite par les bactéries, les champignons et les archées. Les cellules dormantes et résistantes spécialisées reposent dans l'environnement, et forment ce que l'on appelle une «banque de graines microbiennes». Cette banque de graines microbiennes est un réservoir de diversité phénotypique et génétique important qui est également connu pour influencer l'évolution des populations et des processus microbiens. Compte tenu de l'importance de la banque de graines microbiennes, il serait important de comprendre sa composition et sa dynamique. Cependant, jusqu'à présent, les méthodes utilisées pour évaluer la diversité microbienne environnementale ne sont pas en mesure de distinguer la fraction active de celle dormante, comprenant également les structures résistantes spécialisées. Ici, nous utilisons la résistance à la lyse comme proxy pour cibler la diversité des cellules résistantes spécialisées des bactéries, des champignons et des archées. Grâce à l'application de la «méthode d'enrichissement résistant à la lyse», nous montrons que la résistance à la lyse chez les bactéries est non seulement limitée à quelques taxons, mais également pas uniquement limitée au genres sporogènes bien décrits. D'autre part, la résistance à la lyse chez les champignons et les archées s'est avérée largement distribuée. Dans le cas des champignons, il fallait s'y attendre car la production de spores et autres structures résistantes fait partie intégrante du cycle de vie de la plupart des champignons. Dans le cas des archées, la large distribution de la résistance à la lyse pourrait être le résultat de la composition de la cellule qui pourrait les rendre plus résistantes à la lyse que les bactéries, mais il serait possible que les archées soient également capables d'entrer en dormance et de produire des structures ressemblant aux spores.

Ici, nous avons également évalué systématiquement la composition morphologique (microscopie optique et cryo-EM) et chimique (microspectroscopie Raman) de spores bactériennes modèles afin d'identifier un marqueur de spores universel. Ceci afin de

développer une plateforme de tri de spores pour isoler les spores des échantillons environnementaux. A travers l'évaluation systématique des bactéries sporulantes modèles, la seule caractéristique commune à toutes les spores était l'élargissement de la paroi cellulaire par rapport à la cellule végétative. D'autre part, la paroi cellulaire de chaque type de spores différait par le nombre et la densité des couches des autres types de spores. La composition chimique de chaque type de spore était également unique; ainsi, aucun marqueur universel de spore n'a pu être identifié. Néanmoins, des marqueurs chimiques uniques ont été identifiés pour chaque type de spore qui pourraient être utilisés pour l'isolement des spores bactériennes spécifiques à partir d'échantillons environnementaux.

Pour conclure, bien que nous n'ayons pas été en mesure de développer une plateforme de tri universelle pour l'isolement des cellules résistantes dormantes et spécialisées, nous avons maintenant fait un pas de plus vers l’évaluation des banques de graines microbiennes.

Mots clés:

Bactéries | Champignons | Archées | Banque de graines microbiennes | dormance | résistance à la lyse | diversité | spores | endospore | exopore | myxospores | kystes | akinète | Spectroscopie Raman | Cryo-EM|

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Chapter 1 — General Introduction

Introduction

1. Environmental microbiology, the challenges in the environment and dormancy as a survival strategy

Microorganisms are everywhere, impacting life on Earth and geochemical processes in the environment. As time passes, we understand more and more the importance of the role microorganisms play in human, animal, and plant health, in the cycles of carbon, phosphorus, and nitrogen, among others; and thus, their influence in climate, food production, and environmental stability. In the field of environmental microbiology, we aim to better understand not only the diversity of microorganisms in the environment, but also their abundance, their interactions, their life cycles, as well as their interface with the environment.

One characteristic of natural environments is the fluctuation of biotic and abiotic conditions, which can impact the growth and survival of the organism inhabiting them. Therefore, to survive in the environment, organisms must develop strategies to confront these environmental fluctuations (Gray et al., 2019; Yang et al., 2016). One common response used not only by microorganisms, but also by many other organisms such as mammals (Horii et al., 2018; Nasoori et al., 2020), amphibians (Gao et al., 2015; G. S. Rossi et al., 2020), viruses (Li et al., 2021; P. Rossi et al., 2003), and plants (Fadón et al., 2020) is dormancy. Dormancy consists in the decrease of the metabolic activity of the organism, which can be achieved through different mechanisms (Hice et al., 2018; Jones & Lennon, 2010; Lennon & Jones, 2011), like the production of specialized cells (Cáceres & Tessier, 2003). Examples of this can be found in many branches of the tree of life. For instance, parasitic and free-living protists form cysts, which are highly resistant structures (Aguilar-Díaz et al., 2011; Lambrecht et al., 2015). Others such as ferns, produce spores that are resistant to desiccation and temperature (Ballesteros et al., 2017). Furthermore, in the case of bacteria, members of the Firmicutes phyla produce endospores, one of the most resistant specialized structures known to date. Endospores are able to resist high temperatures, ionizing radiation, chemical solvents, detergents, and also to stay dormant in the environment for very long periods of time (Cano & Borucki, 1995; Nicholson et al., 2000; Setlow, 2014). Apart from endospores, four additional types of resistant bacterial cells are widely recognized: exospores, produced by members of

the Actinobacteria phyla; myxospores, produced by members of the Myxococcales order (Kroos, 2007; Wireman, 1979); cysts, produced by the Azotobacteraceae order (J. Moreno et al., 1986; Vela, 1974); and akinetes produces by members of the Cyanobacteria phyla (Adams & Duggan, 1999; Kaplan-Levy, Hadas, Summers, & Sukenik, 2010). The mechanism of formation of each one of these resistant structures is different as shown in Chapter 4.

Spore formation is also a characteristic of fungi. Fungal spores differ from those of bacteria, as they not only aid in long-term survival, but also participate in the sexual/asexual reproduction, and long range dispersal of fungi (Dijksterhuis, 2017; Wyatt et al., 2013). Moreover, there is a larger diversity of fungal spores than of those of bacteria. The broad taxonomic and morphological diversity of fungi (going from unicellular to multicellular) and their diverse life cycles is reflected in the wide variety of fungal spores. Fungal spores vary in shape, mode of formation, state of dormancy, and their level of stress resistance (Dijksterhuis, 2017) (Fig.1). Although dormancy and the production of specialized cells appears to be a common strategy for survival in many microorganisms, little information is available about the formation of specialized resistant cells in archaea.

2. Ecological importance of dormant fractions

As it was exemplified in the previous section, the transition into dormancy has evolved independently several times in many branches of the tree of life. However, in this dissertation, we will be focusing only on dormant cells in microorganisms. While dormancy enables microorganisms to survive suboptimal growth conditions and enhance dispersal, dormancy is a costly strategy (Cáceres & Tessier, 2003; Locey et al., 2020; Mestre & Höfer, 2021; Wisnoski et al., 2019). The organisms that enter dormancy do not only need to invest their energy in the production of resting structures, but they also might miss opportunities to reproduce if the conditions become favorable once more (Cáceres & Tessier, 2003; Ratcliff et al., 2013). In spite of the high cost of dormancy, many microorganisms enter into dormancy in the environment, forming a reservoir often referred to as a seed bank (Lennon & Jones, 2011). Microbial seed banks are an important reservoir of phenotypic and genetic diversity, and are known to influence the evolution of populations and microbial processes (Joergensen & Wichern, 2018; Jones & Lennon, 2010; Shoemaker &

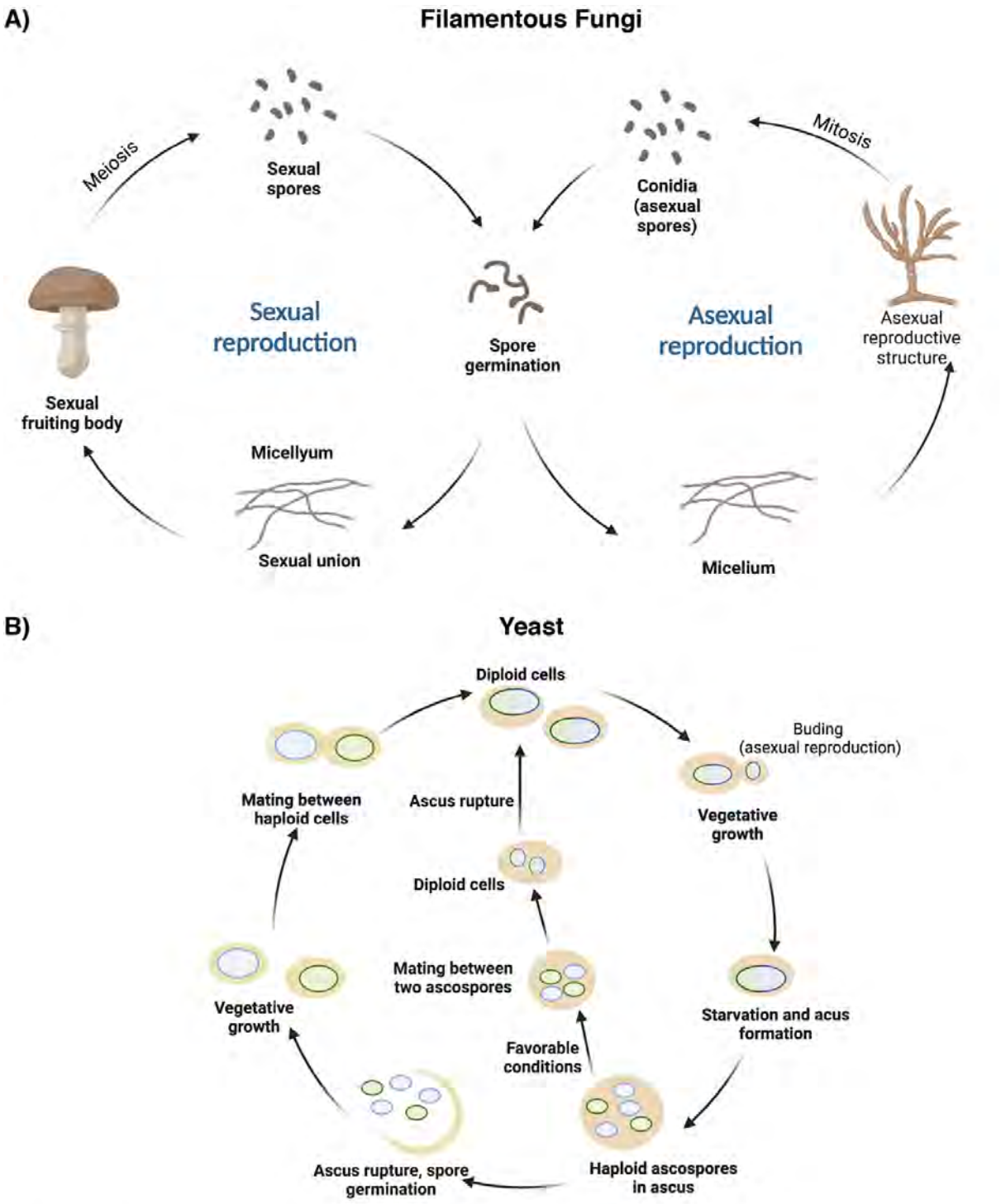

Figure 1. Fungal life cycles. Graphic representation of two different life cycles of fungi. **A)** Filamentous fungi life cycle, left sexual reproduction and right asexual reproduction. **B)** Yeast life cycle, representing the sexual and asexual reproduction of yeast.

Lennon, 2018; Sorensen & Shade, 2020). Moreover, it has been estimated that at least 90% of the fungi and bacteria in soils are dormant (Blagodatskaya & Kuzyakov,

2013; Lennon & Jones, 2011). Also, it is estimated that there are 10^{29} endospores in marine sediments (Wörmer et al., 2019), which means that a very large fraction of the microbial biomass on Earth might be dormant. Thus, giving the abundance and importance of the microbial seed banks, and their influence in population dynamics and evolution, being able to assess the dormant microbial fraction, would allow a better understanding of the ecological role of these populations. This would allow disentangling the active versus dormant fractions of the microbial community. In addition, the dynamic changes among the dormant and non-dormant fractions could be investigated as well. Furthermore, now that our planet is facing major ecological challenges in the form of climatic changes, the resistant dormant microbial community might represent a seed bank from which new communities can emerge. One example of this took place in 2016, when the temperatures in the northwest of Siberia raised above 35°C causing an anthrax outbreak, that killed hundreds of reindeer and two people (Gross, 2019). Moreover, the effect of climate change in soil microbial communities and permafrost has been an important research topic in the last years. This studies not only show that the microbial community composition and taxonomic composition change with rising temperatures and the frequency of soil freeze/thaw events, but also that the increase or decrease in the function of terrestrial microbial communities is directly and indirectly affected by climate change (Douglas & Waldrop, 2019; Garcia et al., 2020; Jansson & Taş, 2014; Potapowicz et al., 2019; Wahid et al., 2020). Thus, the ability to assess the dormant microbial diversity in the environment might help us better predict the response of microbial communities to climate change and the effect they might have in the environment.

3. Methods to assess diversity of dormant versus non-dormant cells in the environment and knowledge gaps

Assessing the diversity and abundance of the dormant microbial community and specifically of the specialized resistant structures, such as spores, presents a few challenges. Until now many of the studies assessing microbial abundance and diversity, rely on molecular techniques. The development of molecular biology has enabled the unveiling of a large microbial diversity previously unknown. Nonetheless, this technology still presents a few setbacks. First, it requires extraction of the genetic material (i.e., DNA or RNA) of the organisms, which might be inherently difficult in the case of specialized resistant cells, as exemplified by endospores (Filippidou et al.,

2015; Knüpfer et al., 2020). Indeed, specialized resistance cells such as endospores show higher resistance to lysis than the cells from the same organism in its vegetative stage, thus a normal DNA extraction protocol might not be sufficient to extract its genetical material. Second, contamination during the extraction or amplification steps might lead to misinterpretation of the data. Finally, the identification of the microorganisms depends on database used, thus when assessing less explored environments, or archaeal and fungal diversity, molecular studies often result in a high percentage of unidentified sequences. In spite of these setbacks, molecular biology is a powerful tool that in combination with other culture-dependent and -independent methods can help us to better understand the diversity, functions, and interactions of these microorganisms in the environment.

Many studies assessing microbial environmental diversity follow the same methodology: selecting environmental samples, extracting the DNA, and amplifying and sequencing a target region (often the 16S rDNA or ITS region) (Bach et al., 2018; Deng et al., 2018; L. Qiu et al., 2021). However, these studies do not distinguish between dead or alive microorganisms or between active and dormant microorganisms, and since it has been shown that in soils, an average of 40% of the microbial DNA is extracellular DNA (Carini et al., 2017), distinguishing between active, dormant and dead microorganism would yield a different picture of the composition and structure of microbial communities. Different alternatives have been proposed to differentiate between dead and alive cells. Some methods target the RNA of the microbial population (Menni et al., 2018; Salgar-Chaparro & Machuca, 2019; Yan et al., 2017), as transcription takes place only in living cells and RNA has a shorter average half-life than DNA. Therefore, the RNA extracted from environmental samples belong, most probably, to a living microbe. Furthermore, dyes have been developed to assess the viable bacteria and archaea in environmental samples (Biggerstaff et al., 2006; Leuko et al., 2004). For example, flow-cytometric single-cell metabolic assays uses two dyes, DAPI (4’,6-diamidino-2- phenylindole) to stain all cells, and CTC (5-Cyano-2,3-ditolyl-tetrazolium chloride) to stain only respirating bacteria. The stained bacteria are then analyzed using a flow-cytometer to quantify the abundance of active and dormant microbial fractions (Salazar et al., 2019). Although these methods are able to differentiate between dead and alive bacteria, they all lack the

ability to differentiate between active, specialized resistant cells and dormant bacteria and specially, they lack the ability to identify resistant cells from fungi and archaea.

Alternative methods have been developed to target bacterial endospores produced by Firmicutes or spores from fungi. For endospores, the quantification of specific chemical markers, such as calcium-dipicolinic acid (CaDPA) (Rattray et al., 2021; Wörmer et al., 2019) or the gene encoding the regulator protein Spo0A (Bueche et al., 2013; Filippidou et al., 2016), are often used. Moreover, specialized DNA extraction methods have been developed to extract DNA from bacterial endospores (Knüpfer et al., 2020; Mauchline et al., 2010). The method of Knüpfe *et al.* 2020 (Fig. 2), uses mechanical lysis to break the endospores and after centrifugation the supernatant is treated with the MasterPure Complete DNA & RNA Purification kit (Lucigen, Middleton, WI, United States) to extract the DNA. The debris pellet from the centrifugation step is also used for DNA extraction, this time with the modified MasterPure protocol as follows: The cells pellet is resuspended in 150 μl TE buffer containing 1250 U of Ready-Lyse Lysozyme Solution (Lucigen, Middleton, WI, USA) and then incubated at 37°C for 60 min. Then 150 μl of Tissue and Cell Lysis Solution (with 1 μL of 50 μg/μl Proteinase K) are added and the mixture is incubated for 15 min, shaking at 900 rpm at 65°C. Then, the sample is place on ice for 5 min after this time, 175 μl of MPC Protein Precipitation Reagent are added. Later the sample is centrifuged at 4°C for 10 min at 13,100× g and the supernatant is collected. Then, 5 μl of Roti-Pink (Carl Roth, Karlsruhe, Germany), 10 μl glycogen solution (5 mg/ml, Carl Roth, Karlsruhe, Germany), and 500 μl isopropanol (Carl Roth, Karlsruhe, Germany) are added to the supernatant. Finally, the precipitated DNA is pelleted by centrifugation at 4°C for 10 min at 13,100× g. The DNA obtained from both extractions is then pull together. The method of Mauchline *et al.* 2010 uses the microLysis-PLUS (Microzone Ltd, Haywords Heath, W. Sussex, UK) and a thermal cycler to lyse the bacterial endospores as follows: The bacterial cells are resuspended in 20 μl of microLYSIS-PLUS and placed in a thermal cycler with the following temperature conditions: 65°C 15 min; 96°C for 2 min; 65°C for 4 min; 96°C for 1 min; 65°C for 1 min and 96°C for 30 s. After this, an equal volume of microCLEAN (Microzone Ltd) solution is added to the sample and is incubated at ambient temperature for 5 min.

Then the sample is centrifugated at 10'392 g for 7 min, the supernatant removed, and the pellet resuspended in 20 µl H_2O.

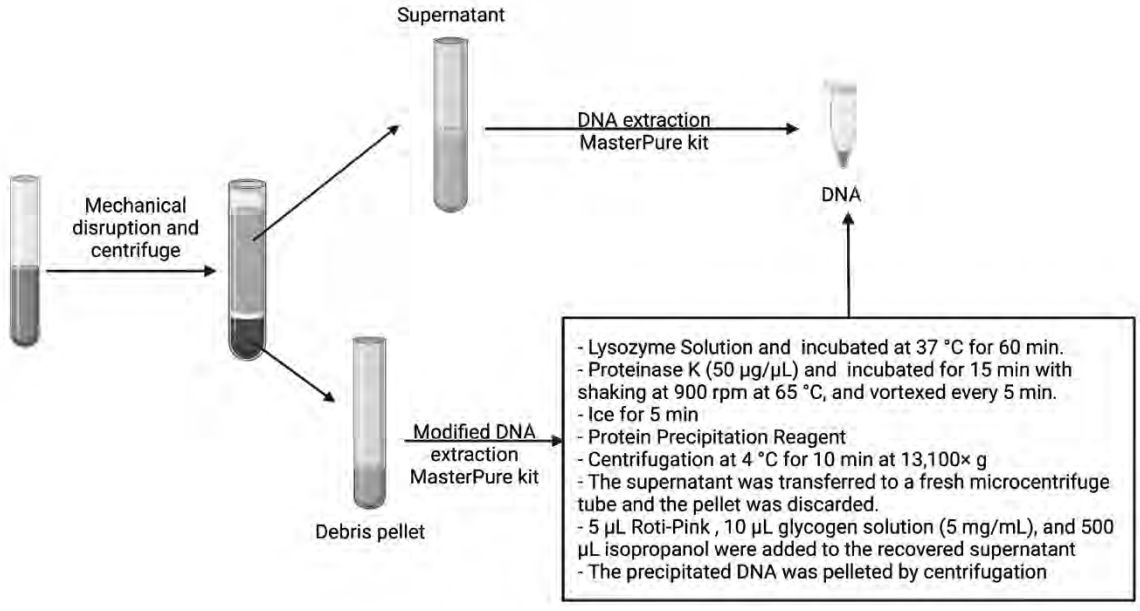

Figure 2. DNA extraction method for *Bacillus anthracis* endospores (Knüpfe *et al.* 2020). Specialliced method for the extraction of *B. anthracis*. After the mechanical disruption of the spores, the DNA is extracted from the supernatant and debries in two separate steps.

Nonetheless, although these methods are able to extract DNA from endospores, they were developed for pure cultures and not environmental samples. Concerning methods targeting the environmental diversity of fungal spores, the majority of them have focused on the fraction of fungal spores suspended in the air (Abrego et al., 2018; Banchi et al., 2018; Kauserud et al., 2005; Roy & Gupta Bhattacharya, 2020), while studies that target fungal spore diversity in soils, apply methods such as a spore washing, which is mainly used to assess Arbuscular Mycorrhizal Fungi (AMF) (Júnior et al., 2019; Soka & Ritchie, 2018a). This method is time consuming, as it requires several steps of washing, centrifugation, and filtrations.

A method to target the diversity of endospores in the environment was developed by Wunderlin et al., 2016. This method uses an indirect DNA extraction by first removing the sample matrix and then applying physical and chemical lysis to break the vegetative cells and isolate the endospores (Fig. 3). Once the endospores have been isolated, a soil DNA extraction kit with three consecutive bead beating steps is used to lyse the resistant endospore cells. The method was extensively validated to assess the diversity of bacterial endospores (Wunderlin et al., 2014), and has been used in

permafrost (Douglas & Waldrop, 2019), and the human gut microbiome (Kearney et al., 2018). The application of this method in environmental samples showed that not only endospores are enriched, but also other spore forming phyla, such as Actinobacteria and Proteobacteria. In addition to spore forming bacteria, also some asporogenic (non-spore forming) bacteria were enriched, and thus this method appears to select not only for endospores, but also for similarly highly lysis-resistant bacteria. The enrichment of asporogenic bacteria in the dataset hints to the existence of cells that exhibit high lysis resistance (Junier et al., 2022). However, proving the existence of these spore-like cells presents additional challenges, as often reports of spore-like structures produced by taxa other that the thoroughly studied ones are received with skepticism. Some reasons for this are that microscopic evidence showing the existence of a modified cell might be insufficient proof (Ajithkumar et al., 2003), or the modified structures might be the result of contamination (Ghosh et al., 2009), or it might correspond to the mistaken interpretation of cellular inclusions as cysts or spores (Beskrovnaya et al., 2020; Girija et al., 2010). Furthermore, the production of specialized resistant cells in some bacteria, and probably in archaea, might only be triggered by a specific set of environmental conditions, that could be too difficult to replicate in the laboratory. Thus, showing the sporulation capabilities of these organisms by inducing sporulation in the laboratory might not be possible. In the case of fungi although the generalized production of specialized resistant structures is recognized, assessing their distribution in the environment is mainly done in aerial samples. Therefore, investigating the environmental diversity of specialized resistant cells in bacteria, archaea, and fungi, requires a functional proxy.

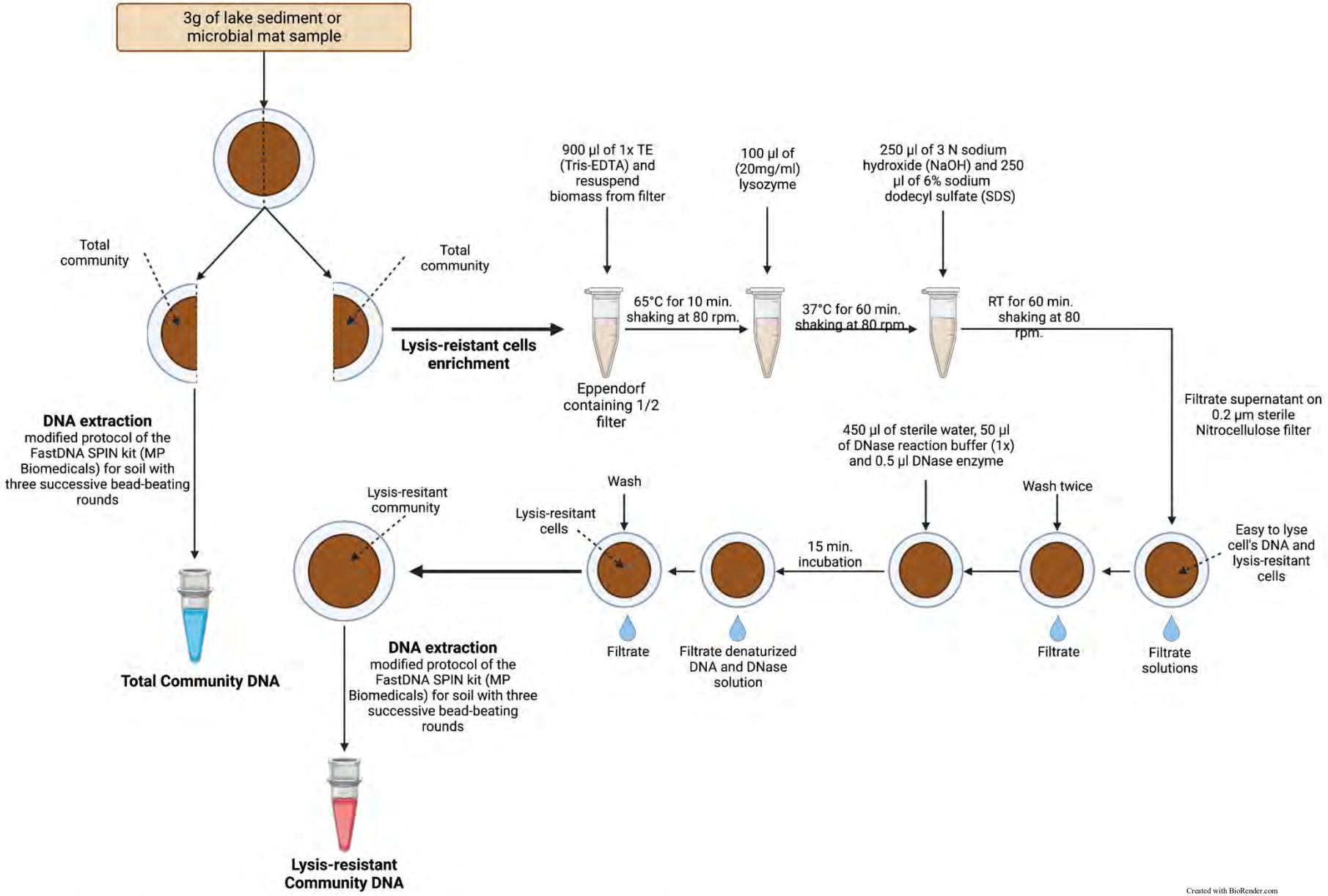

Figure 3. Lysis-resistant enrichment method. Method developed for the isolation of bacterial endospores Wunderlin et al., 2016. The total community and the lysis-resistant fraction are obtained from the same filter.

4. Goals and scope

4.1. Further evaluation of the spore separation method (a.k.a. lysis-resistant enrichment method)

The first goal of this doctoral research was to assess if lysis-resistance is a good parameter not only to assess the diversity of resistant bacterial cells (spore-like cells), but also if it could be extended to investigating lysis-resistant (putative dormant) fractions in other microbial groups such as Archaea and Fungi. To this end, the Wunderlin *et al.* 2016 "Spore separation method" was tested in environmental samples to assess its effect on environmental communities of Archaea and Fungi alongside to Bacteria. By applying the spore separation method, we use lysis-resistance as a proxy to target spore-like cells. It is important to clarify that lysis-resistance does not necessary equals dormancy, however, some of the most lysis-resistant cells known are spores-like structures. Additionally, the spore separation method combines physical and chemical lysis to maximize the lysis-pressure. Nonetheless, it is feasible that non-dormant cells express lysis-resistance (Fig. 4).

The samples analyzed were collected in the polyextreme environment of the Salar de Huasco. This environment was selected as the formation of dormant cells has been shown to affect the fitness and ecological distribution of bacteria (Mutlu et al., 2020). Hence, polyextreme environments might be more selective for microorganisms able to produce dormant cells (Filippidou et al., 2016). The diversity of bacterial and archaeal lysis-resistant cells was explored in the second chapter of this dissertation. For archaea, although no evidence of spore-like cells has been found, we hypothesized that archaea would present a similar distribution of lysis resistance as bacteria. Some genes encoding programmed cell death and dormancy in bacteria have been found in archaea (Makarova et al., 2012), and the formation of persister cells has been found in *Haloferax volcanii* in response to stress (Megaw & Gilmore, 2017). Thus, it is plausible that archaea would produce specialized resistant structures as a mean to increase survival in a similar way as bacteria do.

In the case of the lysis-resistant diversity in fungi, this was explored in the third chapter of this dissertation. Due to the diversity in morphology, function, formation, and resistance of fungal specialized resistant structures (spores, sclerotia, and zoospores), we hypothesized that the application of the lysis-resistant method in environmental samples would result in a widespread distribution of lysis-resistance,

which would reflect not only the presence of resistance structures, but also the multitude of life cycles in fungi. Indeed, apart from producing spores and specialized structures, some fungi have developed morpho-physiological adaptations to increase their resistance. These include switching among different growth forms (i.e., mycelia and yeast) or the production of melanin. One example of this is the ecological group of black fungi. These fungi are considered to be among the most stress-resistant eukaryotes on Earth (Gostinčar et al., 2010; Tesei, 2022). To the best of our knowledge, this is the first time that the lysis-resistant diversity of Archaea and Fungi are assessed in environmental samples.

4.2. Development of a universal spore isolation method

A major limitation of the Wunderlin *et al.* 2016 method is that it uses lysis-resistance as a proxy for specialized resistant cells (spores or spore-like cells), and the resistance to lysis is often not enough to show the existence of specialized resistant cells, specially without microscopical evidence and/or molecular markers. To overcome these limitations, the second goal of this dissertation was to develop a method to identify and isolate bacterial spores from environmental samples that was not based on lysis-resistance. This method would enable the identification and isolation of model bacterial spores as well as spore-like cells and would also allow further assessment of the isolated cells. Additionally, it would differ from the spore-separation method by being a non-destructive method with single cell resolution. For the development of the universal spore separation method, two different technologies were considered as possible sorting platforms, Atomic Force Microscopy (AFM) and Raman spectroscopy. These two technologies were considered as they have single cell resolution, thus allowing to assess individual cells in a mix community and small differences in the composition of the cell could be detected. In addition to the sorting platforms, Cryo-Electron Microscopy (Cryo-EM) was used to identify shared morphological characteristics among spores, that could also be used as sorting parameters. The application of Raman spectroscopy and Cryo-EM are presented in chapter 4. In this chapter, the search for a universal chemical and morphological spore marker is presented, as well as specific markers that could be used for the isolation of different spore types in bacteria. Additionally, the proof of concept for the isolation of bacterial endospores is presented too.

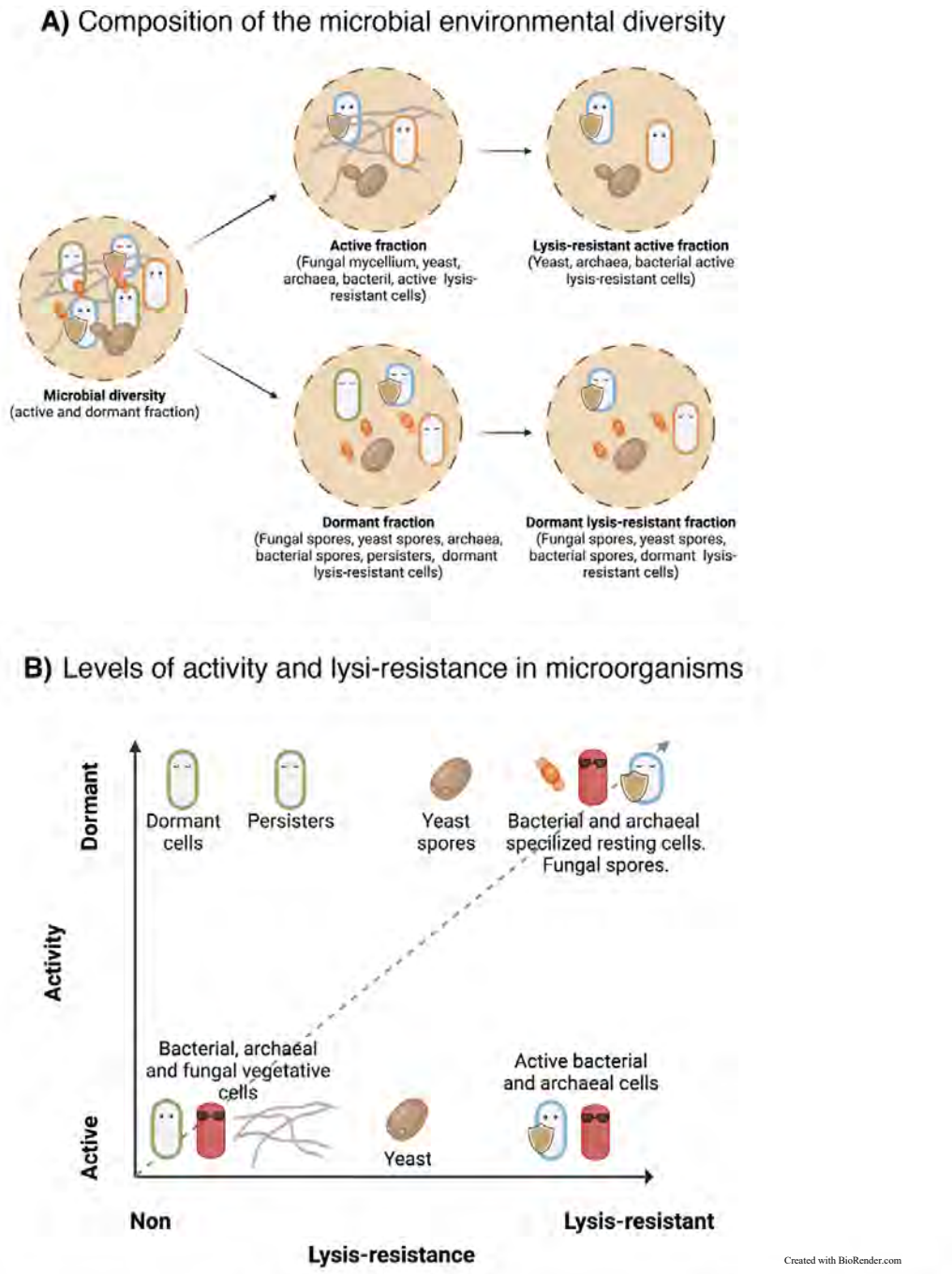

Figure 4. A) Graphical representation of the composition of the environmental microbial community, composed of active and dormant cells which in turn are composed of lysis-resistant and non-lysis-resistant cells. **B)** Graphical representation of the levels of microbial activity and lysis-resistance found in the environment. Little is known about dormancy and the production of specialized cells in archaea, but some evidence has been found that hints to their ability to enter dormancy.

In the next sections 4.2.1 to 4.2.3, the techniques tested for the identification of the "spore marker" and further development of the sorting platform are presented, considering their advantages and disadvantages.

4.2.1. Atomic Force Microscopy

AFM was explored as a possible way of identifying and assessing bacterial spores in environmental samples. This technique, as its name suggests, uses sub-atomic force to image a surface with atomic resolution. AFM are constituted of a flexible cantilever that acts like a spring (i.e., moving up and down) and moves over the sample surface applying sub-atomic force. The deflection of the cantilever is registered by a laser beam and a photodetector to profile the topography of the sample (Binnig et al., 1986) (Fig. 5A). This technology is able to produce surface images of high resolution, being capable of characterizing nm surfaces and provides three-dimension images of biological structures (Rugar & P Hansma, 1990). An important setback that was detected in the first preliminary tests was, that the AFM required a relatively long analytical time to assess one cell. As the end goal was to identify and isolate bacterial spores from a mix sample, the throughput offered by AFM was an important constrain. Additionally, the need to develop a system in which a stream of cells could be analyzed and sorted out was another constrain when working with AFM. Thus, the Raman-activated microbial cell sorting (RACS) platform developed by Lee *et al.* 2019 was selected.

4.2.2. Raman spectroscopy

Since the discovery of Raman scattering by Krishna and Raman in 1928 (Ferraro, 2003; Ramdas, 1928). Raman spectroscopy has been applied in all areas of research, such as chemistry (McCreery, 2005), biomedicine (Matousek & Morris, 2010), as well as in the food industry (He et al., 2013; Rösch et al., 2005), microbiology (Maquelin et al., 2002; Morrow et al., 2012; Yizhi et al., 2017), airport security (Hargreaves et al., 2008), and to analyze soft materials (Amer, 2009). Raman spectroscopy is a non-destructive, non-invasive and label free chemical analysis technique, that uses the interaction of light with the chemical bonds within a material to provide detailed information about the chemical composition, polymorphy, crystallinity and molecular interaction of it. A laser beam is emitted to the sample, that is then scattered by the molecules forming the sample. The majority of the scattered light has the same wavelength as the laser beam used (called the Rayleigh scattering), but a small part of the light is scattered at different wavelength depending on the chemical structure of the sample, this is the Raman scatter. This highly versatile tool has been used to

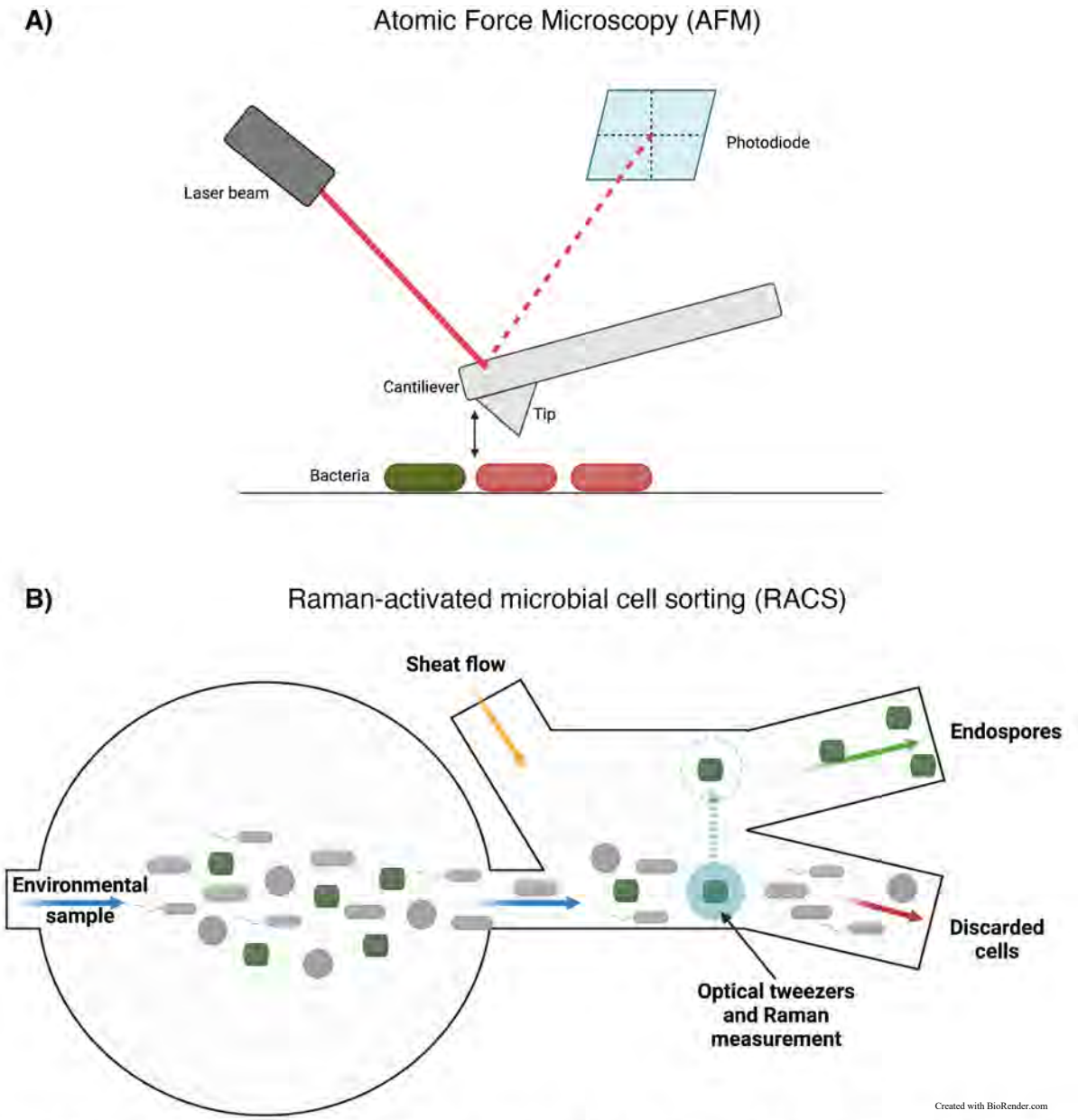

Figure 5. Methods evaluated for the development of a bacterial spore sorting platform. A) Atomic Force Microscopy (AFM), a flexible cantilever acts like spring and moves over the sample surface applying sub-atomic force, then the deflection of the cantilever is registered by a laser beam and a photodetector to profile the topography of the sample. **B)** Raman-activated microbial cell sorting (RACS), uses microfluidics to capture and move the cells before the Raman measurement and to sort the cells after the Raman measurement. The cells are moved to the analysis region by sheath flow and once there, individual cells are randomly captured by the optical tweezers and their Raman spectra is measured. After the spectra is measured, the sorting will take place using a reference spectrum previously established (in this case, the "spore-spectra") to determine if the cell would go to the collection outlet or to the waste outlet.

assess cells at the single-cell level, giving us the ability to explore bacterial communities not only looking at the population, but to focus also at individual members of the community (Guicheteau et al., 2008; Schuster et al., 2000; Wagner, 2009). For these reasons, we decided to use Raman spectroscopy to develop a method to identify

and isolate bacterial spores in environmental samples. Furthermore, Raman spectroscopy has been previously used in combination with optical tweezers to identify and discriminate between diverse bacterial species at the single cells level (Chan et al., 2004; Xie et al., 2005) as well as to isolate specific members of a bacterial community (Lee et al., 2019, 2020; Wang et al., 2020).

The Raman-activated microbial cell sorting (RACS) developed by Lee *et al.* 2019 is a fully automated sorting platform which can analyze up to 500 cells per hour and can be used for function-based cell culturing, mini-metagenomics or from single-cell metagenomics (Lee et al., 2019). The RACS platform (Fig. 5B) uses microfluidics to capture and move the cells before the Raman measurement and to sort the cells after the Raman measurement. The cells are moved to the analysis region by sheath flow and once there, individual cells are randomly captured by the optical tweezers and their Raman spectra is measured. After the spectra is measured, the sorting will take place using a reference spectrum previously established (in this case, the "spore-spectra") to determine if the cell would go to the collection outlet or to the waste outlet. A constrain of the RACS platform is the relatively small throughput for assessing multiple environmental samples and the inaccessibility of the equipment. Although learning to operate the RACS platform is relatively easy, the Raman equipment and microscope are expensive and require expert knowledge to set up.

4.2.3. Cryo-Electron Microscopy

In the 1930's, Ernst Ruska demonstrated the use of the electron microscope (Marton, 1934) but still its application in biological samples was not possible. A few year later, as a strategy to reduce the water evaporation and to protect the biological material from radiation-induced damage, the technique of analyzing the samples at cryogenic temperatures was developed by Taylor and Glaeser (Taylor & Glaeser, 1974, 1976). Since then, Cryo-Electron Microscopy (Cryo-EM) has been widely used in biology to analyze whole cells (Zuber et al., 2008), viruses (Luque & Castón, 2020) and even single particles such as proteins (Nakane et al., 2020). Many techniques and methods have been developed for the analysis of different biological samples with Cryo-EM, but they are all based on the same principle, which is, imaging radiation-sensitive specimens in a transmission electron microscope under cryogenic conditions. Electron microscopy, which is the core of Cryo-EM, works under the same principles of optical

microscopy, but instead of light refraction, electron microscopy uses electrons. The electrons are emitted from the source under high vacuum and once the electron have passed through the sample, the scattered electrons are focused by the electromagnetic lenses of the microscope creating the image. The main difference and advantage of using electron microscopy instead of optical microscopy is the high resolution that can be obtained with it. The resolution possible with visible light (wavelengths of approx. 4000–7000 Å ;1 Å=10^{-10} m) is much lower than the one that can be achieved with a typical electron microscope operating at 300kV (wavelength of approximately 0.02 Å) (Milne et al., 2013). Thus, by using electron microscopy, small changes in the characteristics of single bacterial cells can be assessed. In this dissertation, Cryo-EM was used to assess the morphological differences among vegetative cells and bacterial spores, as well as the similarities among all bacterial spores. Although Cryo-EM provides high resolution of the morphological characteristics of a cell, further studies of this cell are not possible as the cell is incased in the grid. Nonetheless, Cryo-EM can be paired with other technics to obtain more information about the morphology of a cell or types of cells as an important part of demonstrating the existence of spore-like cells are morphological data.

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Chapter 2 — Diversity of Lysis-Resistant Bacteria and Archaea in the Polyextreme Environment of Salar de Huasco

Foreword:

This chapter was published as part of “Microbial ecology and ecosystems from a Southern perspective” in the Journal Frontiers in Microbiology. Here, the lysis-resistant enrichment method was applied to sediments and microbial mats, to assess the environmental diversity of lysis-resistant bacterial and archaeal cells. My personal contribution as first author was sampling, sample processing, data analysis and writing of the manuscript.

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Diversity of Lysis-Resistant Bacteria and Archaea in the Polyextreme Environment of Salar de Huasco

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The production of specialized resting cells is a remarkable strategy developed by several organisms to survive unfavorable environmental conditions. Spores are specialized resting cells that are characterized by low to absent metabolic activity and higher resistance. Spore-like cells are known from multiple groups of bacteria, which can form spores under suboptimal growth conditions (e.g., starvation). In contrast, little is known about the production of specialized resting cells in archaea. In this study, we applied a culture-independent method that uses physical and chemical lysis, to assess the diversity of lysis-resistant bacteria and archaea and compare it to the overall prokaryotic diversity (direct DNA extraction). The diversity of lysis-resistant cells was studied in the polyextreme environment of the Salar de Huasco. The Salar de Huasco is a high-altitude athalassohaline wetland in the Chilean Altiplano. Previous studies have shown a high diversity of bacteria and archaea in the Salar de Huasco, but the diversity of lysis-resistant microorganisms has never been investigated. The underlying hypothesis was that the combination of extreme abiotic conditions might favor the production of specialized resting cells. Samples were collected from sediment cores along a saline gradient and microbial mats were collected in small surrounding ponds. A significantly different diversity and composition were found in the sediment cores or microbial mats. Furthermore, our results show a high diversity of lysis-resistant cells not only in bacteria but also in archaea. The bacterial lysis-resistant fraction was distinct in comparison to the overall community. Also, the ability to survive the lysis-resistant treatment was restricted to a few groups, including known spore-forming phyla such as Firmicutes and Actinobacteria. In contrast to bacteria, lysis resistance was widely spread in archaea, hinting at a generalized resistance to lysis, which is at least comparable to the resistance of dormant cells in bacteria. The enrichment of *Natrinema* and *Halarchaeum* in the lysis-resistant fraction could hint at the production of cyst-like cells or other resistant cells. These results can guide future studies aiming to isolate and broaden the characterization of lysis-resistant archaea.

Keywords: lysis-resistant, lake sediment, microbial mat, extreme environment, archaea, bacteria

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INTRODUCTION

Natural environments can experience fluctuation in biotic and abiotic factors, which impacts the growth and survival of the organisms living within them. In response, organisms must develop strategies to survive under environmental conditions that are suboptimal for their growth and reproduction (Yang et al., 2016; Gray et al., 2019). One common response is dormancy (Logan et al., 2000; Wood et al., 2013), which consists of the decrease in the metabolic activity of the organism and it can be achieved through different mechanisms (Jones and Lennon, 2010; Lennon and Jones, 2011; Hice et al., 2018). One of them is the production of specialized cells (Cáceres and Tessier, 2003). Single-cell or multicellular organisms can create such types of specialized structures for survival. Parasitic and free-living protists that form cysts (highly resistant structures) are examples (Aguilar-Díaz et al., 2011; Lambrecht et al., 2015). Furthermore, bacteria are known to form one of the most resistant dormant specialized structures, called endospores, which can resist physical and chemical stressors (Nicholson et al., 2000; Setlow, 2014).

Since the discovery of bacterial spores in the last quarter of the 19th century (Koch, 1876), microbiologists have uncovered many elements of the sporulation process, such as the triggers for entering sporulation and cell re-activation, levels of resistance, and structural properties of spores, among others. This has been done mainly for spores and spore-like cells from five bacterial groups: Firmicutes (Nicholson et al., 2000; Higgins and Dworkin, 2012), Actinobacteria (Chater et al., 2010; Sexton and Tocheva, 2020), Cyanobacteria (Herdman, 1988; Kaplan-Levy et al., 2010), and in the δ-proteobacterial orders *Myxococcales* (Kottel et al., 1975; Julien et al., 2000) and Azotobacteraceae (Socolofsky and Wyss, 1961; Kramer and Socolofsky, 1970). All these spores and spore-like cells share the characteristic of being more resistant to lysis than the vegetative cells and of displaying a reduced metabolic activity. The ability of an organism to produce spores is judged based on morphological and genetic comparisons with these well-studied model organisms forming spores or spore-like cells. However, even though almost all bacteria can enter a dormant state (Rittershaus et al., 2013; Wood et al., 2013), the majority of them do not form spores (asporogenic). Nonetheless, there is evidence of alternative survival structures in asporogenic bacteria (Tanaka et al., 2001; Mulyukin et al., 2003; Suzina et al., 2004, 2006; Lysak and Lapygina, 2018; Filippidou et al., 2019), which are often referred to as "cyst-like" cells as they share some morphological and physiological similarities with cysts (Socolofsky and Wyss, 1961; Sudo and Dworkin, 1973) and show higher resistance than the vegetative cellular state.

Although dormancy and the production of specialized cells is a common strategy in many microorganisms, little information is available about the formation of specialized dormant cells in archaea. Archaea are classified as asporogenic (Moissl-Eichinger et al., 2018) and no evidence of specialized resistant cells has been found so far. Archaea were for a long time thought to be synonyms of extreme environments, since many archaeal isolates were obtained from extreme habitats (Dassarma et al., 2017; Belilla et al., 2019; Medina Chávez et al., 2019). However, this has changed in the last 30 years thanks to the use of culture-dependent and culture-independent techniques, which demonstrated that these microorganisms are also common in non-extreme environments (Francis et al., 2005; Gleeson et al., 2010; Xu et al., 2020). It has been hypothesized that the higher resistance of archaea compared to bacteria originates in the composition of their membrane, which is composed of glycerol-ether lipids that are more chemically resistant than those found in the bacterial membrane (De Rosa et al., 1986; Albers et al., 2000; Konings et al., 2002; Koga and Morii, 2007). More recently, it has been suggested that archaea could also be able to form spores (Fendrihan et al., 2012). Even though there is no direct evidence for spores, the formation of persister cells has been found in *Haloferax volcanii* as a response to stress (Megaw and Gilmore, 2017). Likewise, the induction of dormancy in archaea by viruses has also been observed (Bautista et al., 2015; Gulbudak and Weitz, 2016). Lastly, genes encoding programmed cell death and dormancy in bacteria have been found in archaea (Makarova et al., 2012). Despite all the above-cited evidence, their ability to form specialized resistant cells as a mechanism to survive harsh environmental conditions is not widely recognized. One reason for this could be the inability to recreate environmental conditions that might trigger the formation of specialized resistant cells in archaea. Therefore, investigating specialized resistant cells requires a functional proxy for their formation in the environment.

Dormant microorganisms play an important role in the maintenance of microbial diversity. Their influence in population evolution and microbial processes has been previously highlighted (Jones and Lennon, 2010; Joergensen and Wichern, 2018; Shoemaker and Lennon, 2018). Now that our planet is facing a major ecological challenge in the shape of climate change, the resistant dormant microbial community might represent a seed bank from which new communities can emerge (Jansson and Taş, 2014; Garcia et al., 2020; Wahid et al., 2020). Thus, the ability to assess the lysis-resistant bacterial and archaeal diversity in the environment might help us to better understand the microbial response to environmental change.

The formation of dormant cells has been shown to affect the fitness and ecological distribution of bacteria (Mutlu et al., 2020). Hence, polyextreme environments might be more selective for microorganisms able to produce dormant cells (Filippidou et al., 2016). Polyextreme environments might harbor higher biodiversity of dormant cells than more stable environments where the formation of dormant cells might carry a higher fitness cost. The Salar de Huasco is a high-altitude athalassohaline wetland in the Chilean Altiplano. This salt flat is located at an altitude of 3,800 m.a.s.l. and its salinity ranges from freshwater to saturation (Dorador et al., 2008b). The Salar is known for its polyextreme conditions, with daily temperature ranging from −10 to +25°C, high solar radiation <1,100 W/m^2, and a negative water balance (Dorador et al., 2010; Cortés-Albayay et al., 2019). All these factors make the Salar de Huasco an environment where microorganisms would benefit from the formation of specialized structures to survive the constantly changing conditions. Furthermore, it has been shown that in this salt flat, there is high biodiversity of endemic bacteria and archaea (Demergasso et al., 2004; Dorador et al., 2008a,b). In this study, we assessed the environmental diversity of lysis-resistant cells in

bacteria and archaea by applying a method developed to explore the diversity of endospores in the environment (Wunderlin et al., 2016). This method was extensively validated for its use in bacteria using pure cultures of non-spore-forming Gram-negative and spore-forming Gram-positive bacteria (Wunderlin et al., 2014). To break less-resistant vegetative cells, the method includes chemical and physical lysis prior to DNA extraction of the lysis-resistant fraction. The application of this method enabled the enrichment of endospores and revealed the large biodiversity of other spore formers in environmental samples. In addition, bacterial species that are supposed to be asporogenic were enriched as well (Filippidou et al., 2016; Paul et al., 2018). The unexpected biodiversity observed after the endospore enrichment method, suggests higher biodiversity of highly lysis-resistant bacteria. To assess the microbial diversity of lysis-resistant prokaryotic cells in the Salar de Huasco, we applied the spore separation method (hereafter indicated as "lysis-resistant enrichment") to samples from sediments and microbial mats.

MATERIALS AND METHODS

Sampling

The sampling took place in September 2019. The samples were collected within 24 h from the main saline lake and the small ponds surrounding it. Seven sediment core samples (approximately 7 cm diameter and approximately 4 cm depth) were collected from the main lake, following a saline gradient, with salinity ranging from 53 to 0.706 PSU (**Figure 1**). The cores were cut on-site to 1 cm subsamples and stored in sterile plastic Petri dishes. Twenty microbial mats samples were collected from eighteen small ponds surrounding the main saline lake. Approximately 40 ml of microbial mats were collected in sterile 50 ml Falcon tubes. At every sampling point salinity, pH, conductivity, water temperature, and dissolved oxygen (%OD) were measured using a Multiparameter Water Quality Meter-HI98194, Hanna Instruments (Woonsocket, Rhode Island, United States), and the coordinates and altitude were also recorded (**Supplementary Table 1**). During the transport of the samples back to Switzerland, they were stored at room temperature (approximately 20–25°C) for 24 h. After arrival at the lab, the samples were stored at 5°C until processing a week later.

Biomass Extraction for Indirect DNA Extraction

Attachment to inorganic particles such as sediment can bias the separation of the lysis-resistant fraction by protecting cells from lysis or from enzymatic degradation of DNA (a key step to degrade released DNA from easy to lyse cells during the "lysis-resistant enrichment method"). Therefore, indirect DNA extraction was applied. For each sample, 3 g of material were weighted in ULTRA-TURRAX® disperser tubes (IKA-Werke, Staufen, Germany). Then, 15 ml of Na-Hexametaphosphate (1%) were added and the mixture was homogenized two times at 3,000 rpm for 1 min with the ULTRA-TURRAX® tube disperser workstation system. The sample was left to precipitate for 10 min

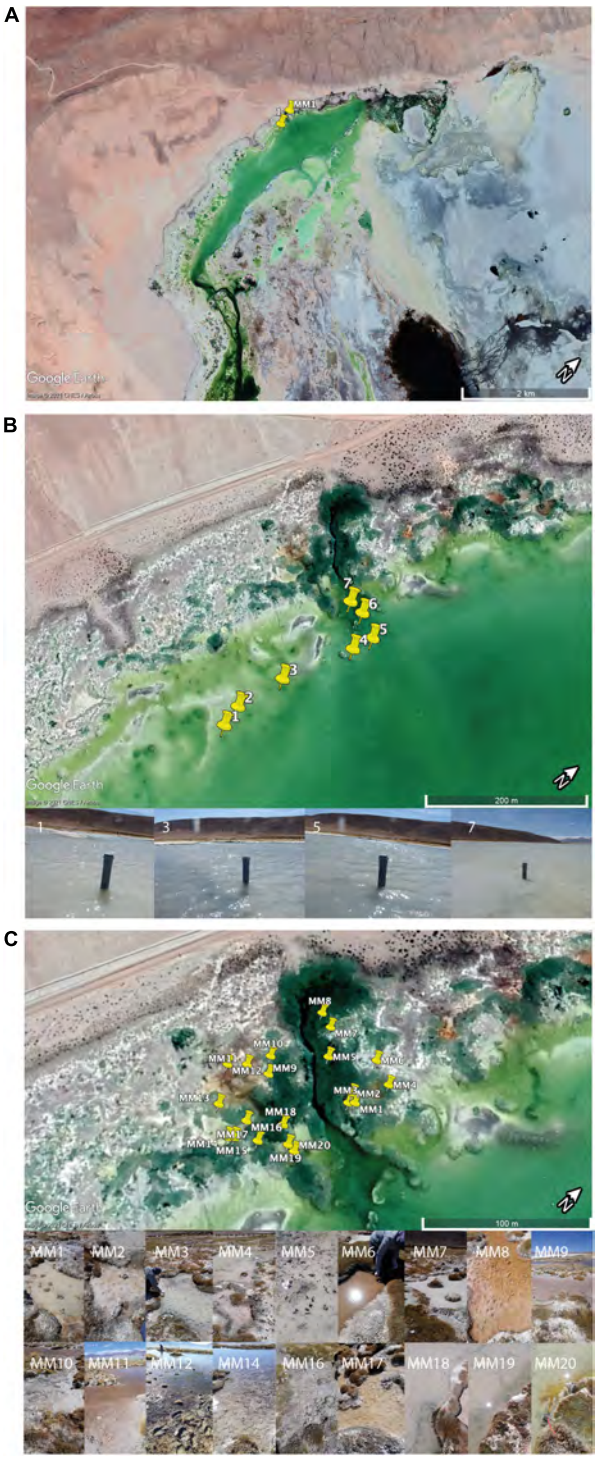

FIGURE 1 | Sampling area. **(A)** An overview of the sampling area indicating the region in which samples for this study were collected. The sampling area was selected based on a previous study (Dorador et al., 2008b) that showed a significant salinity gradient and a large diversity of microbial mats in a limited geographical area. **(B)** Close-up of the area of sampling within the saline lake showing the location of the seven sediment cores. **(C)** Close-up of the area of sampling within ponds containing microbial mats. The images of the 18 ponds are presented to illustrate the diversity of mats studied.

and the supernatant was collected. This initial step was repeated two times. The two 15 ml supernatants were pooled, centrifuged at 20 × *g* for 1 min to precipitate large particles, and the remaining supernatant was filtrated through a 0.2 μm sterile nitrocellulose filter. For each sample, the filter was cut in half. One half was used to assess the total microbial community by directly extracting DNA and the second half was subjected to the lysis-resistant enrichment method to assess the lysis-resistant fraction. This way the same filter was used to assess both communities (**Supplementary Figure 1**).

Lysis-Resistant Enrichment Method and DNA Extraction

To the cellular material on one half of the filter, 900 μl of 1× TE (Tris-EDTA) buffer were added and incubated at 65°C for 10 min shaking at 80 rpm. Afterward, 100 μl of 20 mg/ml lysozyme were added and the sample was incubated at 37°C for 60 min shaking at 80 rpm. Later, 250 μl of 3 N sodium hydroxide (NaOH) and 250 μl of 6% sodium dodecyl sulfate (SDS) solutions were added and the sample was incubated at room temperature for 60 min shaking at 80 rpm. Next, the sample was filtrated in a 0.2 μm sterile nitrocellulose filter, which was then washed two times with 2 ml of sterile physiological water to remove any residue of the solutions used. Once the filter was dry, 450 μl of sterile water, 50 μl of DNase reaction buffer (1×) and 0.5 μl DNase enzyme were added and let stand for 15 min on the filter. The addition of the DNase enzyme and DNase reaction buffer is a vital step to degrade the DNA released by the easy-to-lyse cells prior to the DNA extraction from the lysis-resistant fraction. After 15 min, the solution was filtrated, and once more, the residue was washed with 1 ml of sterile physiological water that was filtrated to wash away the degraded DNA as well as the DNAase solution. Finally, the filters (containing the lysis resistant fraction only) were stored at −20°C until the DNA extraction was performed (Wunderlin et al., 2016). The same DNA extraction method was used in both fractions (**Supplementary Figure 1**). The FastDNA® SPIN kit for soil (MP Biomedicals, Irvine, CA, United States) was used with a modified protocol that included three successive bead-beating rounds in the first step. The final DNA extracts from the successive bead-beating steps were pooled together (Wunderlin et al., 2013). The DNA was then precipitated with ethanol and resuspended in PCR-grade water. DNA quantification was performed using Qubit® dsDNA HS Assay Kit on a Qubit® 2.0 Fluorometer (Invitrogen, Carlsbad, CA, United States).

Sequencing and Statistical Analysis

The purified DNA extracts were sent to Fasteris (Geneva, Switzerland) for bacterial and archaeal 16S rDNA amplicon sequencing using an Illumina MiSeq platform (Illumina, San Diego, CA, United States), generating 300 bp paired-end reads. For the bacterial 16S rDNA, the V3–V4 region was amplified using the universal primers Bakt_341F (5′-CCT ACG GGN GGC WGC AG-3′) and Bakt_805R (5′-GAC TACHVG GGT ATCTAA TCC-3′) (Herlemann et al., 2011). For the archaeal 16S rDNA, the V3–V4 region was amplified using the universal primers 340F (5′-CCC TAY GGG GYG CAS CAG-3′) and 806rB (5′-GGA CTA CNV GGG TWT CTA AT-3′) (Bahram et al., 2019). Demultiplexed and trimmed sequence reads provided by Fasteris were processed using QIIME2 (Bolyen et al., 2019) with dada2 (Callahan et al., 2016) for the denoising step. Read lengths were truncated to optimized total nucleotide lengths (based on q-scores), 480 and 484 bases for the 16S and archaea datasets, respectively. These truncated sequences allowed the joining of denoised paired-end reads by at least 12 identical bases to obtain full denoised sequences. Sequences were grouped on amplicon sequence variants (ASVs). The ASVs then obtained were afterward taxonomically classified using QIIME2's VSEARCH-based consensus taxonomy classifier (Rognes et al., 2016) with the SILVA database (Quast et al., 2013), release 132, for 16S rDNA of bacteria and archaea. The number of ASVs obtained for bacteria was 153,000 and for archaea 2,128. All statistical analyses were performed with RStudio version 1.3.1093 (RStudio Team, 2020), the community and multivariate analyses were performed with the phyloseq (McMurdie and Holmes, 2012) and vegan (Oksanen et al., 2020) packages. The Venn diagram was calculated with the package Venn.Diagram (Chen and Boutros, 2011). The relative abundance was calculated using the Total-Sum Scaling (TSS) normalization. The principal coordinate analysis (PCoA) was calculated based on the weighted UniFrac distances. For the Venn diagram, PCoA, and enrichment analysis, the relative abundance was used. For the statistical test of the alpha diversity and the ANOVA, the raw abundance data were used. The proportion used in the distribution plots was calculated by phylum, the relative abundance of each ASV was divided by the sum of all relative abundance to obtain a proportion, the log base 10 of the proportion was used for the graphical representation. The enrichment proportion was calculated by first adding the relative abundance per genus once in the total fraction and once in the lysis-resistant fraction. The proportion was then calculated by subtracting the lysis-resistant abundance from the total abundance and dividing this by the sum of the lysis-resistant fraction and the total fraction.

RESULTS

In this study, we analyzed two types of environments: sediment cores from the main saline lake and microbial mats from the small ponds surrounding it. An ordination of the communities using a principal coordinate analysis (PCoA) showed that both the lysis-resistant (i.e., the fraction of the community which persisted after the lysis-resistant enrichment method) and a total fraction (i.e., obtained from the direct DNA extraction) grouped mostly by the type of environment (**Figures 2A,B**). Although the partial overlap of the communities originating from the two different environments appeared to exist (in particular for the total fraction), a Venn diagram to evaluate the overlap in community composition of both the community type (lysis-resistant and total fraction) and the environment (microbial mat and lake sediment), showed this is not the case. The number of ASVs shared between the same type of environment was higher than between samples from the same community (**Figures 2C,D**),

showing a stronger influence of the type of ecosystem than of the treatment in the overall community composition. This effect was observed for both bacteria and archaea. As the aim of this study was to determine the diversity of lysis-resistant communities, to establish those organisms enriched in this fraction, we compared them to the respective total community of the same environment. Therefore, herein, the two types of environments were analyzed independently, as their biotic characteristics and abiotic conditions were also different.

Lysis-Resistance in Lake Sediments

Seven sediment core samples were collected along the salinity gradient ranging from 53 to 0.706 PSU in the main saline lake. The pH in the lake ranged between 8.22 and 8.43 (not following a specific gradient). From these samples, sample 5 could not be analyzed as there was not enough sediment to perform the analysis. From the sequencing, we were not able to generate any products for the archaea sample 6 (lysis-resistant only). Although the community analysis was performed in all the samples, for the comparison, only samples that could be paired total vs. lysis-resistant were retained.

Bacterial Diversity in Lake Sediments

The PCoA of the saline lake core samples showed that the bacterial communities were clustered into two groups, the lysis-resistant fraction, and the total community (**Figure 3A**). In the PCoA the lysis-resistant community samples were more dispersed than the total community. Furthermore, there was no statistical difference (p-value = 0.18) between the Shannon alpha diversity of the lysis-resistant and the total fractions (**Supplementary Figure 2A**). Also, there was no statistically significant correlation between the abiotic parameters (pH, dissolved oxygen, and salinity) and the alpha diversity (data not shown).

We first analyzed the composition of the bacterial community per sediment core by looking at the top 50 ASVs (**Figure 3B**). The lysis-resistant fraction and the total fraction differed at the phylum level. Both the lysis-resistant and the total fractions included ASVs assigned to Actinobacteria, Bacteroidetes, Chloroflexi, Cyanobacteria, Deinococcus-Thermus, and Proteobacteria, whereas ASVs assigned to Firmicutes and Halanaerobiaeota were only present in the lysis-resistant fraction. In contrast, Cloacimonetes and Epsilonbacteraeota were only present in the total fraction. Moreover, the sediment core from sampling point 3, was significantly different from the other 5 sampling points. The top 50 ASVs in this sample were primarily dominated by Firmicutes in the lysis-resistant fraction and by Epsilonbacteraeota in the total fraction. The enrichment effect of the lysis-resistant method in all detected phyla can also be observed using a distribution plot comparing the relative abundance of given phyla in the two fractions (**Figure 3C**). As the same filter was used in the assessment of the total and the lysis-resistant community, any cell present in the total fraction capable of withstanding the physical and chemical lysis will show enrichment in the lysis-resistant fraction. When comparing **Figures 3B,C**, the limitations of only assessing the most abundant ASVs are highlighted. From the 39 different phyla observed in **Figure 3C**, only 10 were present among the most abundant ASVs, thus overlooking a large part of the bacterial diversity in the lake sediments. The distribution plot also allows the assessment of less abundant phyla and demonstrates the effect that the lysis-resistant method had on them. **Figure 3C** shows the enrichment of the spore-former phyla Firmicutes and Actinobacteria in the lysis-resistant fraction, as well as the enrichment of non-spore former phyla such as Aegiribacteria, Chlamydiae, Halanaerobiaeota, and Tenericutes in the same fraction. Phyla such as *Candidatus* Eremiobacterota and Elusimicrobia were only present in the lysis-resistant fraction, whereas *Candidatus* Omnitrophicaeota, Nitrospirae, Chrysiogenetes, Caldiserica, and BRC1 only appeared in the total fraction. The phyla Chloroflexi, Proteobacteria, and Bacteroidetes, were among the most abundant phyla in both lysis-resistant and total fractions, and although they were not enriched in the lysis-resistant fraction, they showed a similar distribution in both fractions.

Based on the pattern of enrichment described above, representatives of the phyla Firmicutes, Actinobacteria, Proteobacteria, and Cyanobacteria were analyzed in more detail. When assessing the relative abundance of genera within Firmicutes in the total and lysis-resistant fraction (**Figure 4A** only a subset, full in **Supplementary Figure 3**), most were clearly enriched in the lysis-resistant fraction. Only 10 genera had a higher abundance in the total fraction. This enrichment in the lysis-resistant fraction was not restricted to genera known to contain spore-forming species, but some asporogenic genera such as *Gracilibacter* and *Chungangia* were also enriched in this fraction (**Figure 4A**). For Actinobacteria (**Figure 4B** only a subset, full in **Supplementary Figure 4**) genera containing known spore former species such as *Streptomyces* and *Micromonospora*, were enriched in the lysis-resistant fraction, but, as in the case of Firmicutes, the lysis-enriched fraction also included non-spore formers genera such as *Nitriliruptor* and *Microbacterium* (**Supplementary Figure 4**). For both, Proteobacteria and Cyanobacteria, although the mean relative abundance of the lysis-resistant fraction was not higher than the total abundance (**Figure 3C**), a small fraction of the genera showed a higher abundance in the lysis-resistant fraction (**Figures 4C,D**; full **Figure 4C** in **Supplementary Figure 5**). The enriched fraction included, among others, representatives of the genera *Thioalkalimicrobium*, *Aquabacterium*, and *Spiribacter* in the Proteobacteria and *Nostoc* PCC-7524, *Anabaena* BECID22, and *Geitlerinema* PCC-7105 in Cyanobacteria. A summary of the groups enriched in the bacterial lysis-resistant fraction for other phyla can be found in **Supplementary Table 2**.

Archaeal Diversity in Lake Sediments

The Shannon alpha diversity of the archaea samples did not show a statistical difference (p-value = 0.52) between the samples after the lysis treatment and the samples from the direct DNA extraction (**Supplementary Figure 2B**). Furthermore, in the PCoA no clear clustering could be observed (**Figure 5A**). The distribution of the lysis-resistant fraction and the total community overlap in the plot. However, in the sample, each lysis-resistant fraction could be distinguished from the same total community. The communities were largely dominated by two phyla: Euryarchaeota and Thaumarchaeota (data not shown).

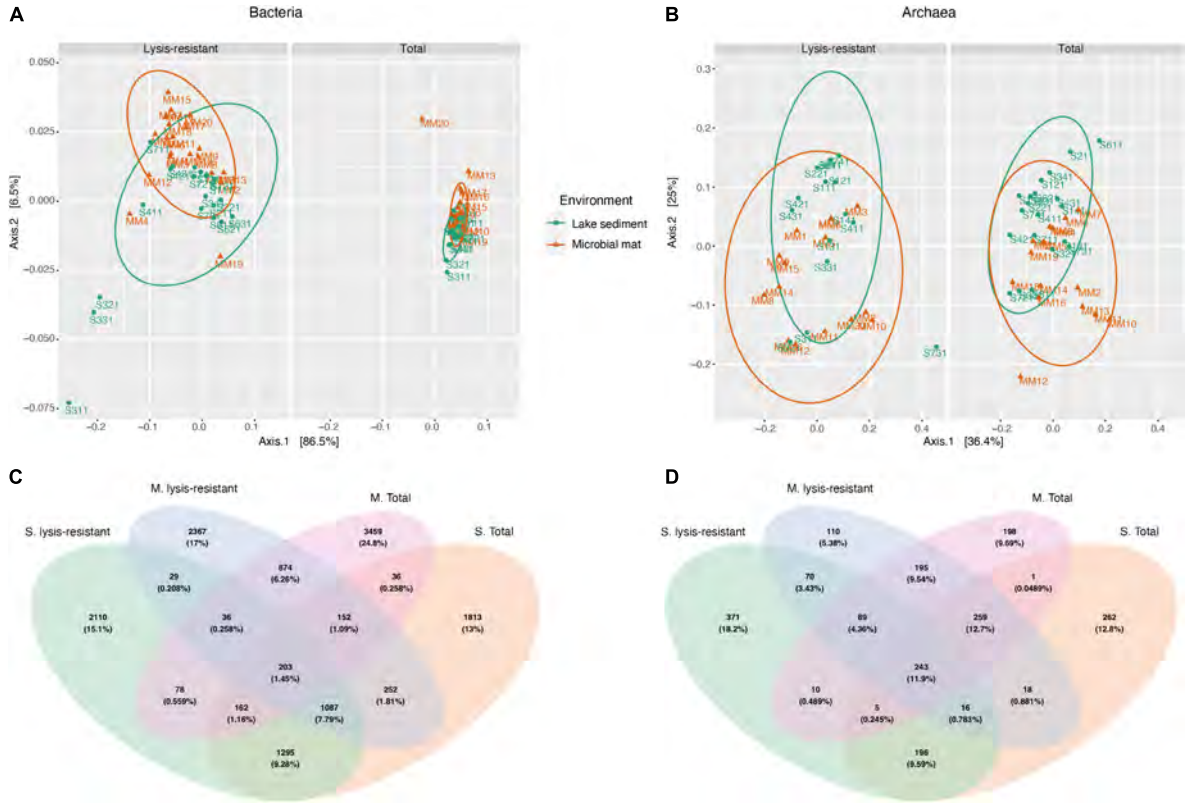

FIGURE 2 | Principal coordinate analysis (PCoA) and Venn diagram of the bacteria and archaea community. **(A)** Weighted UniFrac PCoA of the bacterial community comparing microbial mats (in orange) and lake sediments (in green) in the lysis-resistant (left) and total fractions (right). The ellipses show a 95% confidence level per group. **(B)** PCoA of the archaeal community comparing microbial mats (in orange) and lake sediments (in green) in the lysis-resistant (left) and total fractions (right). The ellipses show a 95% confidence level per group. **(C)** Venn diagram of the bacterial community (top 5,000 of 15,300 ASVs) comparing both environments (M, microbial mats; S, lake sediments) and both community fractions (lysis-resistant and total community). **(D)** Venn diagram of the archaeal community (top 1,000 of 2,128 ASVs) comparing both environments (M, microbial mats; S, lake sediments) and both community fractions (lysis-resistant and total community).

For archaea, the picture offered by the most abundant ASVs leaves out four phyla, as compared to the phyla observed in the distribution plot, thus underestimating the archaeal diversity. Nonetheless, to further understand the composition of this highly abundant phyla, we went to the genus level for the 50 most abundant ASVs (**Figure 5B**). The genera that were present both in the total and in the lysis-resistant were *Candidatus* Nitrosocosmicus, *Halohasta*, *Halorubrum*, and *Natronorubrum*, while the genera *Halomicroarcula* (halophilic archaea) and *Methanospirillum* (anaerobic methanogenic) were only present in two and one cores in the lysis-resistant fraction, respectively. In the total community, the presence of *Methanolobus* and *Methanosaeta* was observed.

To assess the enrichment of different archaeal phyla in the lysis-resistant community, we plotted the proportion of each community for each phylum (**Figure 5C**). The lake sediments sampled from the main Salar showed the presence of 6 phyla: Thaumarchaeota, Nanoarchaeaeota, Euryarchaeota, Diapherotrites, Crenarchaeota, and in lower abundance Asgardaeota. From these phyla, only Thaumarchaeota showed enrichment in the lysis-resistant fraction, while Euryarchaeota had similar means in both fractions. Moreover, it can also be observed that Thaumarchaeota and Crenarchaeota showed more than one distribution in the data set, indicating that there was also a set of less abundant ASVs with high distribution in both types of community fractions. For Thaumarchaeota, there were two enriched genera in the lysis-resistant fraction, these were *Nitrososphaera* and *Candidatus* Nitrosocosmicus, while unidentified genera were enriched in the total fraction (data not shown). Both *Nitrososphaera* and *Candidatus* Nitrosocosmicus are soil ammonia oxidizers from which no resistant cell form is known. Although the Euryarchaeota phyla did not show significant enrichment in the lysis-resistant fraction (**Figure 5D**, only a subset full in **Supplementary Figure 6**), we observed that many genera were enriched in the lysis-resistant fraction (**Figure 5D**). Those included *Natrinema*, *Halonotius*, and *Halarchaeum*. These genera belong to the Halobacteria class, known to be halophilic archaea found mainly in water-saturated or nearly saturated with salt, such as the Salar de Huasco.

Lysis-Resistance in Microbial Mats

Twenty samples of microbial mats were collected from 18 small ponds surrounding the main saline lake. The samples varied in color, texture, and location. The pH in these

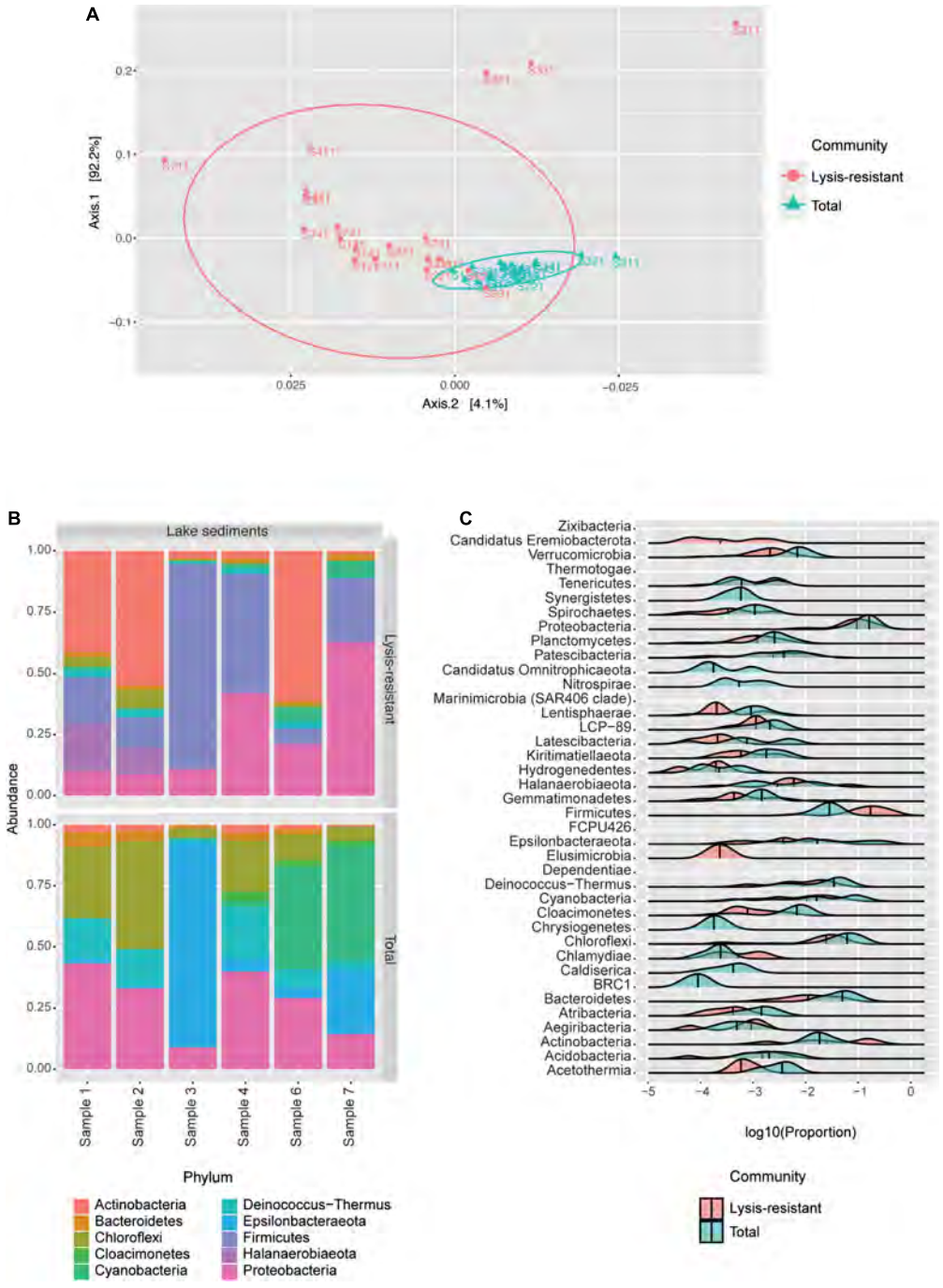

FIGURE 3 | The bacterial community in the lake sediments. **(A)** Weighted UniFrac PCoA of the bacterial community, belonging to the lysis-resistant fraction (red) or the total fraction (blue). The ellipses show a 95% confidence level per group. **(B)** Top 50 bacterial ASVs relative abundance per lake sediment core at the phylum level. **(C)** This plot shows the abundance distribution of the different phyla, for either the lysis-resistant (red) and the total fraction (blue) in the lake sediments, the abundance is shown in log10 of the relative abundance.

ponds ranged between 7.67 and 9.74, and the salinity from 0.41 to 4.22 PSU. Sequencing data were collected for all the samples for bacteria. However, for archaea, we were not able to generate sequencing products for all the samples. The samples missing data were samples 12, 15, 16, and 17 (in the lysis-resistant fraction), and samples 8, 15, and 18 (total fraction). Although the community analysis was performed in all the samples, for the comparison only samples that could be paired, total vs. lysis-resistant were retained.

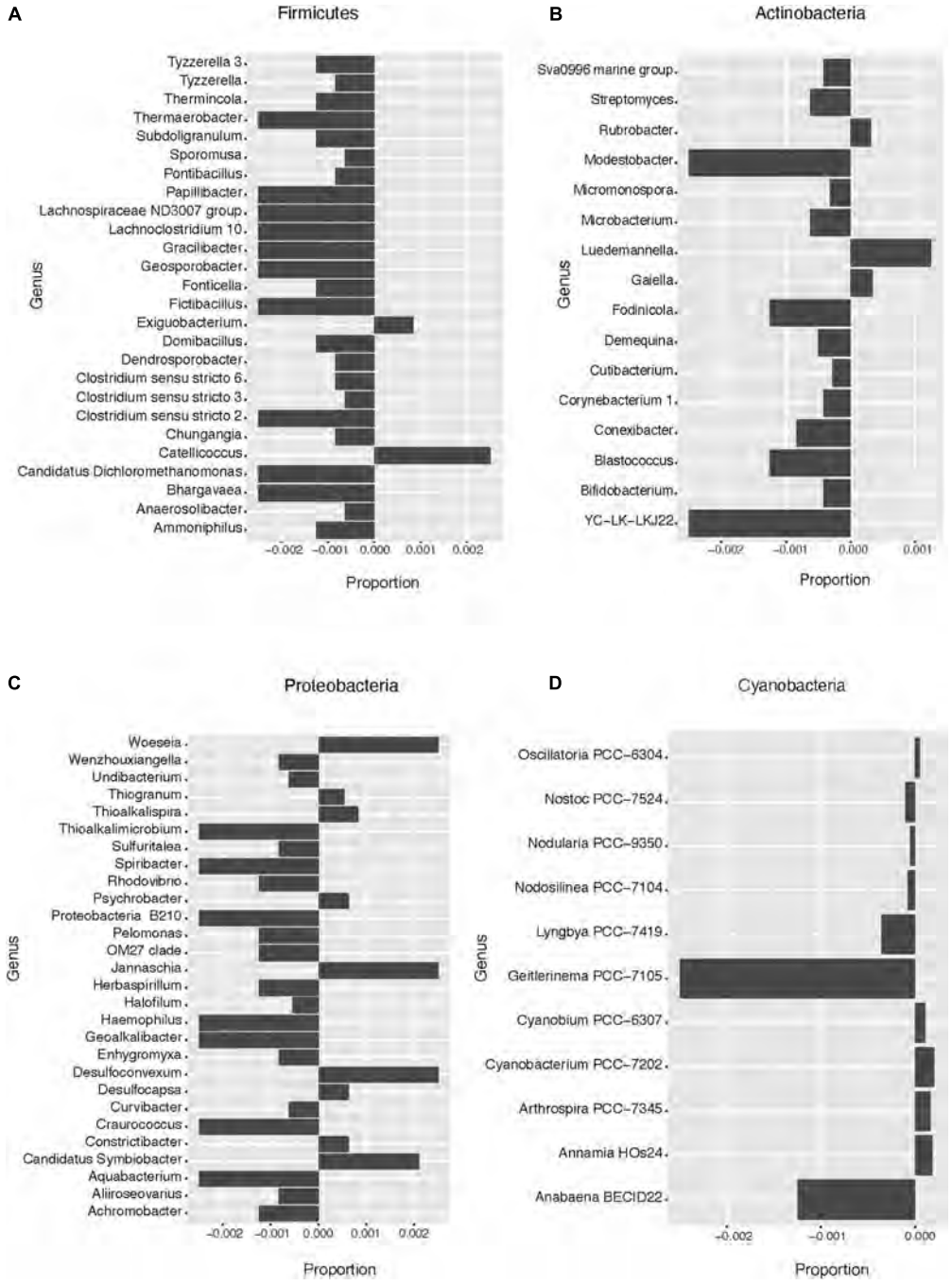

FIGURE 4 | Enrichment of different bacterial genera in the lysis-resistant or total fraction in the lake sediments. Negative values show enrichment in the lysis-resistant fraction, while positive values show enrichment in the total fraction for each genus. **(A)** Firmicutes genus. Proportion threshold at ≤0.0005 and >0.0005. **(B)** Actinobacteria. Proportion threshold at ≤0.00025 and >0.00025. **(C)** Proteobacteria. Proportion threshold at ≤0.0005 and >0.0005. **(D)** Cyanobacteria. Full graph.

Bacterial Diversity in Microbial Mats

The PCoA of the microbial mat samples showed the same clustering as the one observed in the lake sediments (**Supplementary Figure 7A**). The total community was tightly clustered except for one sample, MM20, which was clustered with the lysis-resistant fraction. On the other hand, the lysis-resistant fraction had a broader distribution. The difference in alpha diversity between the two fractions was significant in the case of bacteria (**Supplementary Figure 7B**). There was no significant statistical correlation between the abiotic

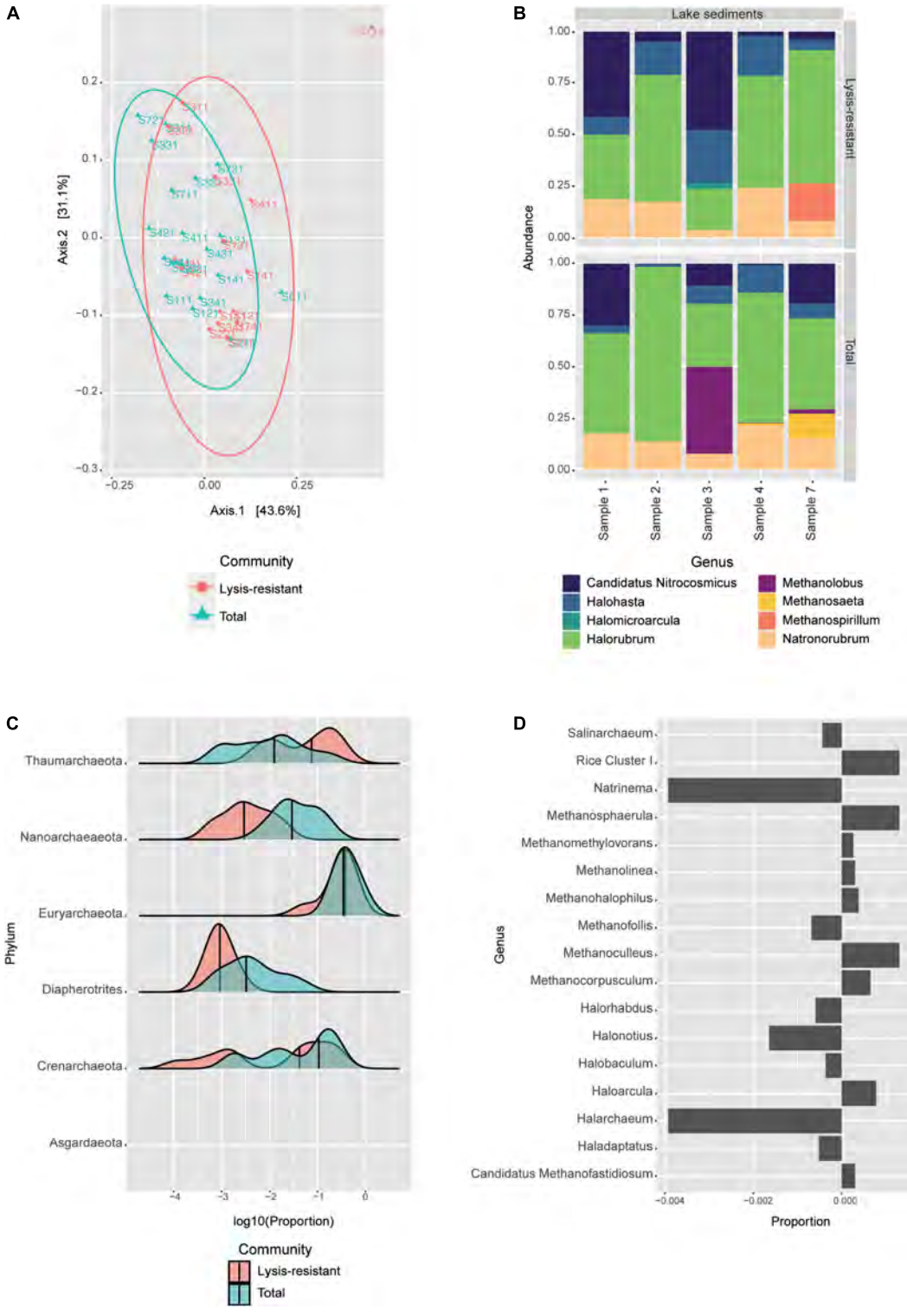

FIGURE 5 | Archaeal community in the lake sediments. **(A)** Weighted UniFrac PCoA of the archaeal community, belonging to the lysis-resistant fraction (red) or the total fraction (blue). The ellipses show a 95% confidence level per group. **(B)** Top 50 archaeal ASVs relative abundance per lake sediment core at the genus level. **(C)** This plot shows the abundance distribution of the different phyla, for either the lysis-resistant (red) and the total fraction (blue) in the lake sediments, the abundance is shown in log10 of the relative abundance. **(D)** Enrichment of Euryarchaeota in the lysis-resistant or total fraction. Negative values show enrichment in the lysis-resistant fraction, while positive values show enrichment in the total fraction. Proportion threshold at $\leq$0.00025 and $>$0.00025.

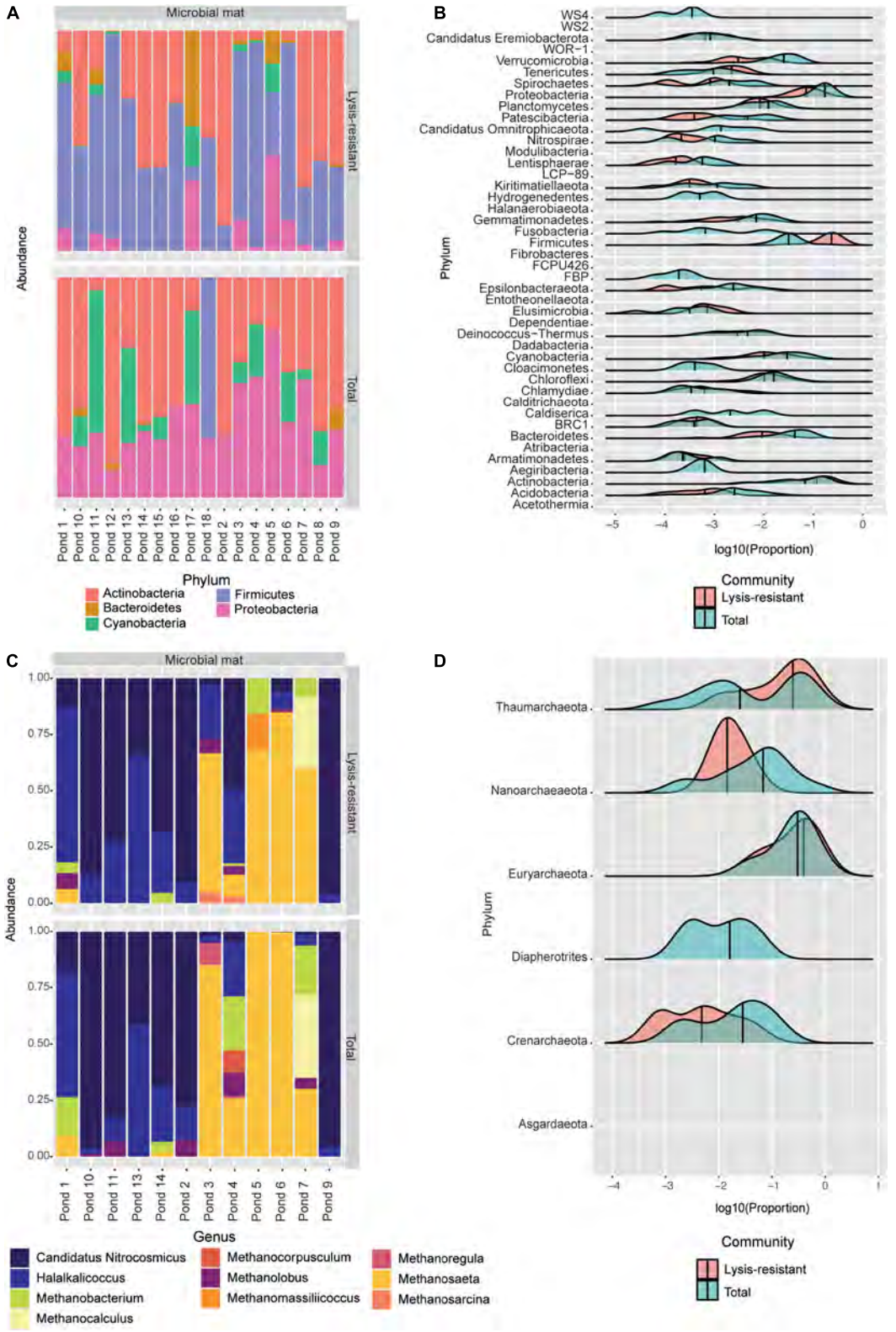

FIGURE 6 | (A) Top 50 bacterial ASVs relative abundance per pond at the phylum level. **(B)** This plot shows the abundance distribution of the different bacterial phyla, for either the lysis-resistant (red) or the total fraction (blue) in the microbial mats. The abundance is shown in log10 of the relative abundance. **(C)** Top 50 archaeal ASVs relative abundance per pond at the genus level. **(D)** This plot shows the abundance distribution of the different archaeal genus, for either the lysis-resistant (red) or the total fraction (blue) in the microbial mats. The abundance is shown in log10 of the relative abundance.

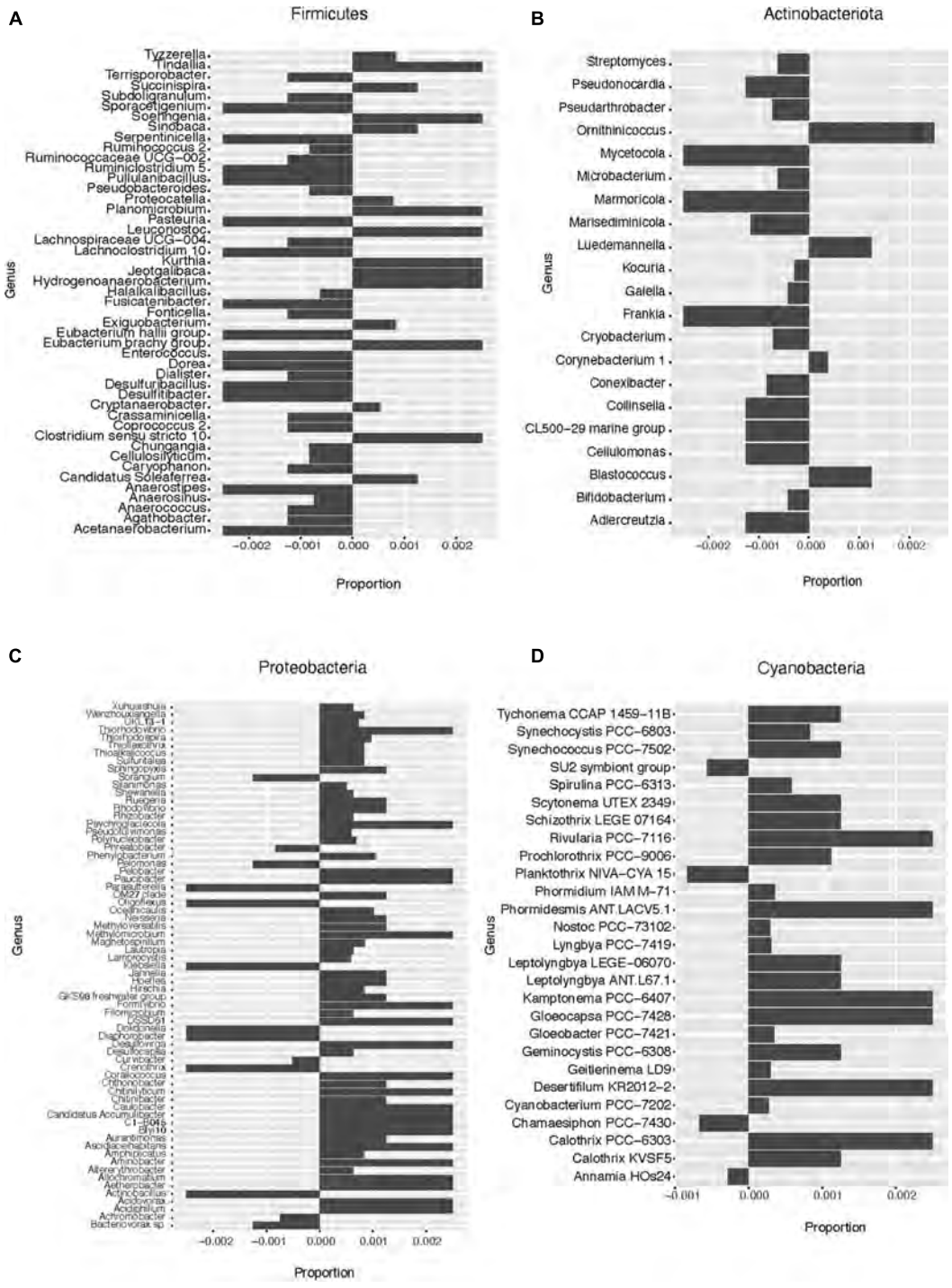

FIGURE 7 | Enrichment of different bacterial genera in the lysis-resistant or total fraction in the microbial mats. Negative values show enrichment in the lysis-resistant fraction, while positive values show enrichment in the total fraction for each genus. **(A)** Firmicutes genus. Proportion threshold at ≤0.0005 and >0.0005. **(B)** Actinobacteria. Proportion threshold at ≤0.00025 and >0.00025. **(C)** Proteobacteria. Proportion threshold at ≤0.0005 and >0.0005. **(D)** Cyanobacteria. Proportion threshold at ≤0.00025 and >0.00025.

parameters and alpha diversity. The most abundant ASVs in the microbial mat and the lake sediments belonged to the same five main phyla: Actinobacteria, Bacteroidetes, Cyanobacteria, Firmicutes, and Proteobacteria (**Figure 6A**). However, the abundance of these phyla is significantly different between the two communities (lysis-resistant and total). As it can be

observed, in the microbial mats, the total fraction was mainly composed of Actinobacteria, Proteobacteria, and Cyanobacteria, while Firmicutes only appeared in two of the sampling points (pond 4 and pond 18). However, in the lysis-resistant fraction, the most abundant phyla were Firmicutes and Actinobacteria; Proteobacteria were present in almost all samples but in lower relative abundance and Cyanobacteria were present in only seven ponds.

To obtain a general idea of the bacterial diversity and the enrichment of the different phyla in the microbial mat community, we plotted the proportion of each community for each phylum (**Figure 6B**). Here, 44 phyla were identified while in the lake sediments only 39 phyla were identified. Furthermore, these two environments shared only 1.45% of their bacterial ASVs (**Figure 2C**). When comparing the enrichment of specific phyla in the lysis-resistant community in microbial mats (**Figure 6B**) vs. lake sediments (**Figure 3C**), Firmicutes, Proteobacteria, Actinobacteria, Cyanobacteria, Chlamydiae, Chloroflexi, *Candidatus* Omnitrophicaeota, and Caldiserica showed the same distribution. On the other hand, the phyla *Candidatus* Eremiobacterota, and Elusimicrobia were only present in the lysis-resistant fraction. Nitrospirae and BRC1 were only present in the total fraction in the lake sediments, while in the microbial mat they are present in both fractions. The opposite was true for the phyla Hydrogenedentes, Cloacimonetes, and Aegiribacteria, which were only present in the total fraction in the microbial mats but were present in both fractions in the lake sediments. A more detailed assessment of the enriched phyla of the microbial mats is shown in **Figure 7** (only a subset, full in **Supplementary Figures 8–11**). The majority of the genus in Firmicutes (**Figure 7A**) showed a higher abundance in the lysis-resistant fraction as compared to the total fraction. *Clostridium*, *Bacillus*, *Paenibacillus*, and *Sporosarcina* are known spore formers found among those genera enriched. In addition, genera not known to form spores but with higher abundance in the lysis-resistant fraction, such as *Enterococcus* and *Dorea*, were also detected. In the case of Actinobacteria (**Figure 7B**), only six of 36 genera present showed higher abundance in the total fraction in comparison to the lysis-resistant fraction (**Supplementary Figure 9**). Not only known spore formers such as *Streptomyces*, *Micromonospora*, and *Frankia* showed higher abundance in the lysis-resistant fraction, but also asporogenic genera such as *Mycetocola*, *Conexibacter*, and *Adlercreutzia*. In the other two phyla with known spore formers, Proteobacteria, and Cyanobacteria, most of the genera had higher abundance in the total fraction than in the lysis-resistant fraction (**Figures 7C,D**). The Cyanobacteria genera enriched in the lysis-resistant fraction were SU2 symbiont group, *Planktothrix* NIVA-CYA-15, *Nostoc* PCC7524, *Chamaesiphon* PCC7430, and *Annamia* HoS24, from which only *Nostoc* PCC7524 is known to produce akinetes. For Proteobacteria the most enriched genera in the lysis-resistant fraction were *Parasutterella*, *Oligoflexus*, *Klebsiella*, *Dokdonella*, and *Actinobacillus*, all of which are not known to form spores.

A comparison of the genera of Firmicutes, Actinobacteria, Proteobacteria, and Cyanobacteria shared between the microbial mats and lake sediments showed that only 3.63, 1.9, 1.1, and 0.98% ASVs were shared, respectively (**Figures 8A–D**). When looking at the shared ASVs per fraction (lysis-resistant and total) the maximum number shared is 0.737%, for Cyanobacteria in the lysis-resistant fraction, and 0.246% in the total fraction. This confirms that although most of the enriched phyla were shared between the two environments, the ASVs from those phyla were distinct.

Archaeal Diversity in Microbial Mats

As observed in the lake sediments, the archaeal PCoA did not show any defined clustering either for the lysis-resistant or for the total community (**Supplementary Figure 7C**). Also, there was no statistical difference in the alpha diversity of these two communities (**Supplementary Figure 7D**). At the phylum level, only Euryarchaeota and Thaumarchaeota were present. The community composition of the top 50 ASVs was assessed per sampled pond at the genus level (**Figure 6C**). Most genera could be found in both the lysis-resistant and the total fraction. On the other hand, the genus *Methanocorpusculum* was only present in the total fraction and the genera *Methanomassiliicoccus* and *Methanosarcina* were only present in the lysis-resistant fraction. Additionally, these genera were only present in a few samples. The genera *Methanocorpusculum* and *Methanomassiliicoccus* were only present in one pond each (pond 4 and pond 5, respectively). The two microbial mats collected from pond 4 were highly structured, pink submerged mats with a salinity of 0.738 PSU and a pH of 7.89, while the sample from pond 5 was a brown submerged mat presenting the same salinity and pH as pond 4. The genus *Methanosarcina* was only found in ponds 3 (black-green submerged mat, salinity of 0.412 PSU, and pH of 7.9) and 4. Meanwhile, the top 50 ASVs of ponds 5 and 6 (whitish submerged mat, salinity of 0.738 PSU, and pH of 7.89) were dominated by the genus *Methanosaeta* in the total fraction, whereas the lysis-resistant fraction of the same ponds also presented the genera, *Candidatus* Nitrosocosmicus, *Halalkalicoccus*, *Methanobacterium*, and *Methanocalculus*. A larger archaeal diversity was revealed when assessing the enrichment of each phylum (**Figure 6D**). Here it can be observed that as for the lake sediments, only the Thaumarchaeota was enriched in the lysis-resistant fraction. All other phyla were enriched in the total fraction, while Diapherotrites were only present in the total fraction.

A closer inspection of the enriched phyla showed that in the Thaumarchaeota phylum there were only two genera identified *Candidatus* Nitrosocosmicus and Nitrososphaera from which only the latter showed enrichment in the lysis-resistant fraction (data not shown). In the case of the Euryarchaeota phylum, many genera showed no enrichment in either fraction, but the same relative abundance in both (**Figure 9A**, only a subset, full in **Supplementary Figure 12**). *Methanobrevibacter*, *Mathanocalculus*, *Methanoculleus*, and *Methanomassiliicoccus* were genera enriched in the lysis-resistant fraction, with *Methanobrevibacter* showing the highest enrichment in this fraction. These four genera are all known to contain methanogenic archaea, and the formation of resistant modified structures is not yet known. In the case of the main genera enriched in the total fraction, *Methanocorpusculum*, *Methanolinea*, and *Candidatus* Methanofastidiosum, all three

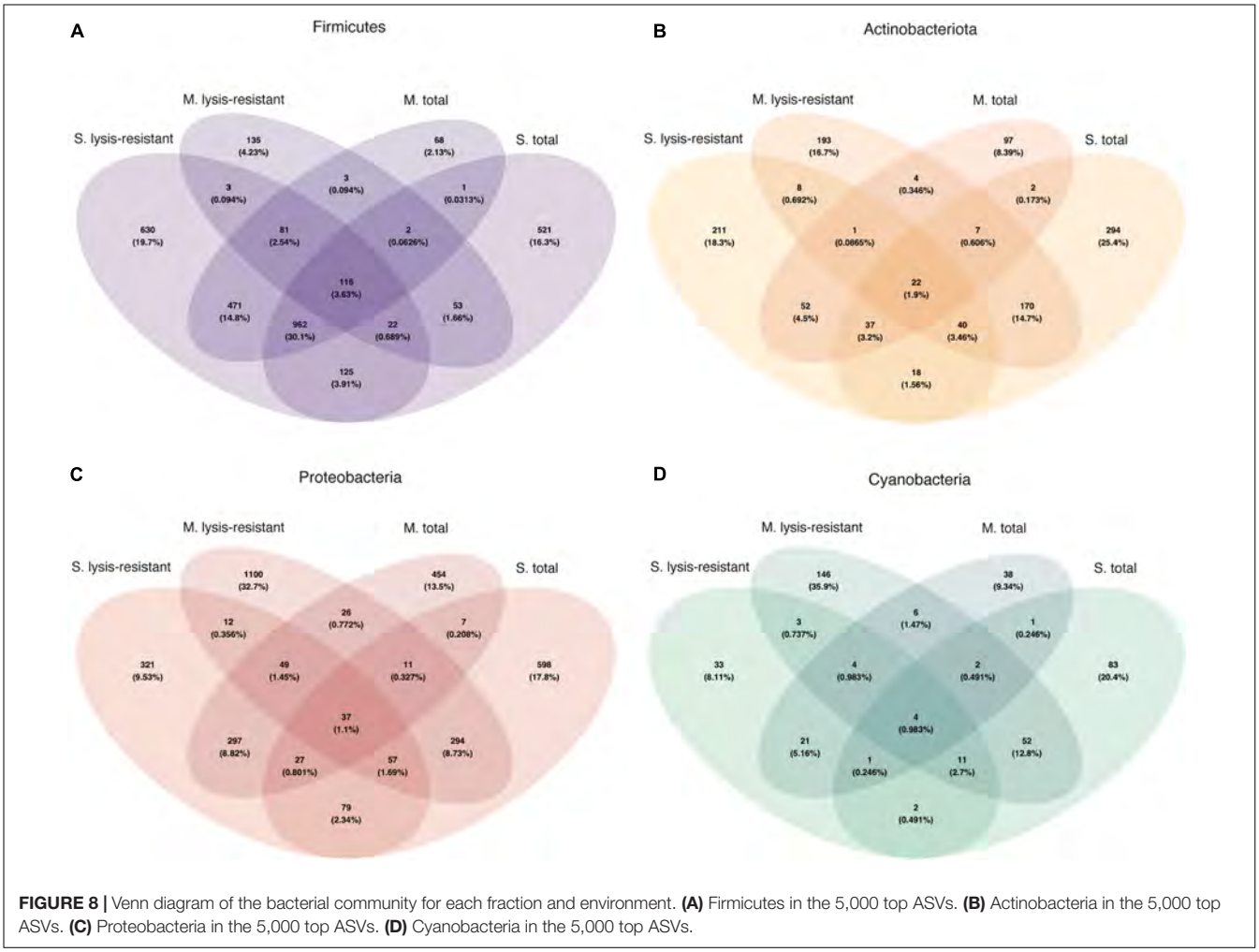

FIGURE 8 | Venn diagram of the bacterial community for each fraction and environment. **(A)** Firmicutes in the 5,000 top ASVs. **(B)** Actinobacteria in the 5,000 top ASVs. **(C)** Proteobacteria in the 5,000 top ASVs. **(D)** Cyanobacteria in the 5,000 top ASVs.

are methanogenic anaerobic archaea. Additionally, the Venn diagram of Euryarchaeota (**Figure 9B**), showed that the total fractions of the microbial mats and the lake sediments share more ASVs (4.73%) than each of the fractions from the same environment (lake sediment 1.25% and microbial mat 0.28% ASVs). In the case of Thaumarchaeota and Diapherotrites, the diversity in each environment and fraction appears to be unique (**Figures 9C,D**).

DISCUSSION

The overall diversity of the bacterial and archaeal communities in the two types of samples analyzed in the Salar de Huasco agrees with previous studies in this site and similar environments. The high relative abundance of Proteobacteria and Actinobacteria in both the lake sediments and microbial mats (lysis-resistant and total fraction) is in agreement with previous studies assessing the bacterial diversity in the site (Aguilar et al., 2016; Molina et al., 2018a; Castro-Severyn et al., 2021), as well as in other saline lakes around the world (Qin et al., 2019; Mehta et al., 2021). Furthermore, the high relative abundance of Chloroflexi in the lake sediments and of Cyanobacteria in the microbial mats is also in agreement with previous studies (Dorador et al., 2008b; Rasuk et al., 2016; Prieto-Barajas et al., 2018; Castro-Severyn et al., 2021). Moreover, although the most abundant phyla (Proteobacteria, Cyanobacteria, Actinobacteria, and Bacteroidetes) seem to be present in both environments (lake sediments and microbial mats), the number of shared ASVs between them was low. Such differences in the community composition of each environment have been previously observed at different sampling stations in Salar de Huasco (Dorador et al., 2020). Thaumarchaeota and Euryarchaeota were the dominant archaea phyla in the Salar de Huasco. These phyla have been previously reported to be highly abundant in sediments and water (Dorador et al., 2010; Molina et al., 2018b). On the other hand, our results contrast to those found by Molina et al. (2018b), where they found that the archaeal phylum *Candidatus* Parvarchaeota was the most abundant in both microbial mats and sediments, whereas in our community analysis this phylum was not found. Genera containing methanogenic archaea such as *Methanobacterium*, *Methanocorpusculum*, *Methanoregula*, *Methanosaeta*, and *Methanospirillum*, were among the most abundant in the microbial mats as reported in previous studies

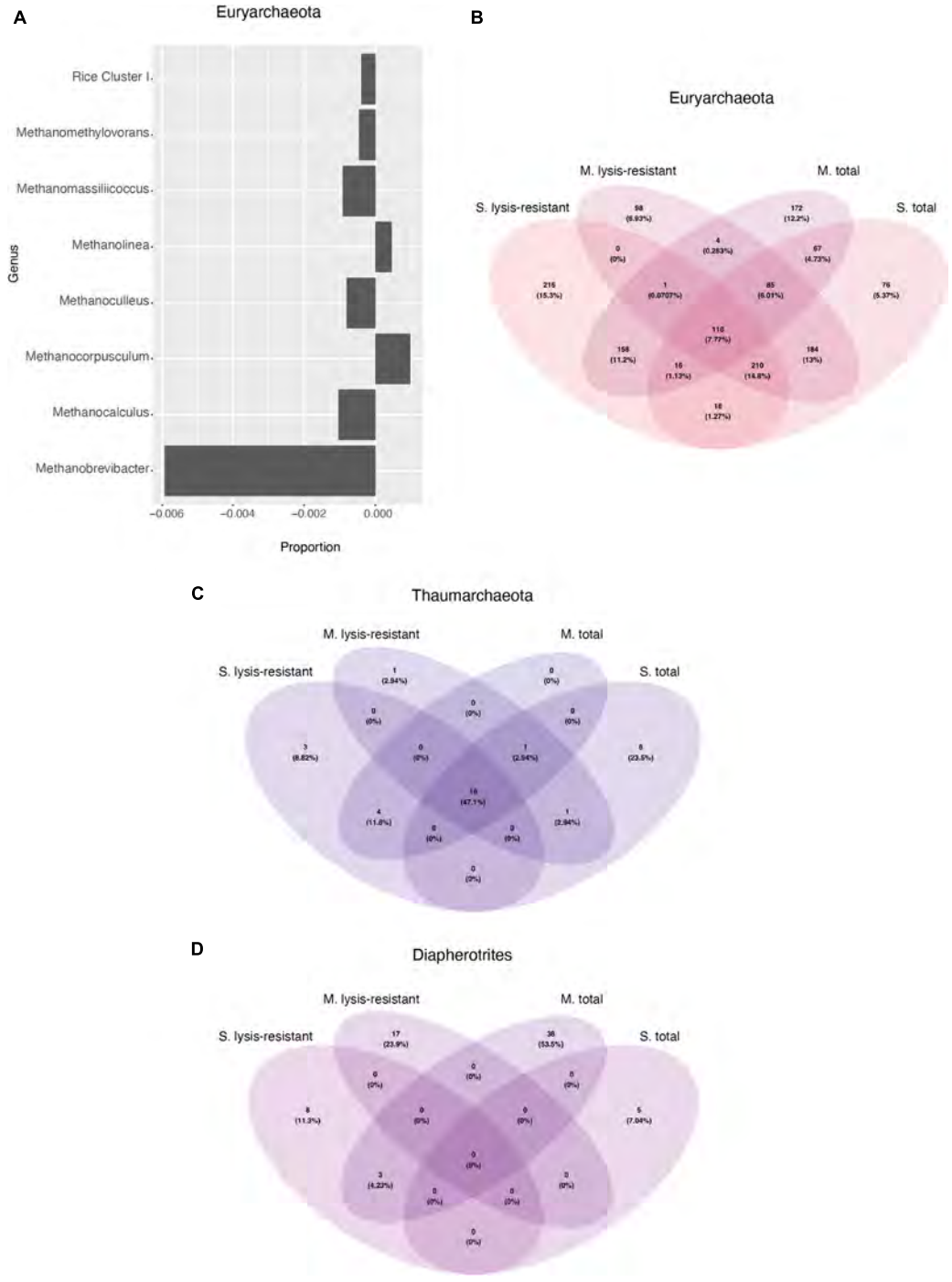

FIGURE 9 | (A) Enrichment of different Euryarchaeota genus in the lysis-resistant or total fraction in the microbial mats. Negative values show enrichment in the lysis-resistant fraction, while positive values show enrichment in the total fraction for each genus. Threshold at ≤0.00025 and >0.00025. **(B)** Venn diagram of the Euryarchaeota in the 5,000 top ASVs for each fraction and environment. **(C)** Venn diagram of the Thaumarchaeota in the 5,000 top ASVs for each fraction and environment. **(D)** Venn diagram of the Diapherotrites in the 5,000 top ASVs for each fraction and environment.

(Dorador et al., 2010; Molina et al., 2018b). On the other hand, halophilic archaea, such as *Halalkalicoccus*, *Halohasta*, *Halomicroarcula*, and *Halorubrum*, were abundant in lake sediments, something that was observed previously in samplings held during the Austral winter season as in our case (Dorador et al., 2013). A high number of uncultured and/or unidentified ASVs belonging to both bacteria and archaea was also detected. This is not only characteristic of the Salar de Huasco but many

extreme environments (Bull et al., 2016; Dorador et al., 2020; Pascoal et al., 2021).

This study used, for the first time, an experimental approach developed originally for the enrichment of endospores to evaluate the diversity of potentially dormant microorganisms in this polyextreme environment. The method used here was initially validated by testing the survival of endospore-formers and non-endospore formers to chemical and physical lysis. The final treatment was shown to destroy vegetative cells, but not highly resistant endospores, which are only found in Firmicutes. In addition to the enrichment of Firmicutes, other taxa were also detected and therefore the enriched fraction was considered to contain diverse lysis-resistant cells as, for many of the enriched taxa, endospore formation (or other forms of sporulation) was unproven (Wunderlin et al., 2014). The results presented here for the Salar de Huasco confirmed that lysis-resistance is a specialized mechanism found only in a fraction of the bacterial population, resulting in the enrichment of phyla in which species are known to form spores, such as Firmicutes and Actinobacteria. The high abundance of Firmicutes and Actinobacteria in the lysis-resistant fraction is in agreement with Kearney et al. (2018), who also found these two phyla highly enriched in human fecal samples after the application of a modified version of the same method. Furthermore, not only known spore formers were enriched in the lysis-resistant fraction, but also genus not yet known to form resistant specialized structures were enriched in this fraction. This includes, for instance, *Gracilibacter* (Lee et al., 2006) and *Enterococcus* (Schleifer and Kilpper-Balz, 1984; Byappanahalli et al., 2012) in Firmicutes, and *Nitriliruptor* (Sorokin et al., 2017) and *Microbacterium* (Gneiding et al., 2008; Fidalgo et al., 2016) in Actinobacteria. Their enrichment in the lysis-resistant fraction, suggests that a highly resistant cell form might be responsible for enhancing their survival in the environment.

In Proteobacteria, the formation of resistant specialized structures is known in the *Azotobacter* genus (Høidal et al., 2000; Rejman and Kozubek, 2004) and in the *Myxococcales* order (Kottel et al., 1975; Julien et al., 2000; Garcia et al., 2009; Huntley et al., 2011; Mohr et al., 2012). A member of the latter, the genus *Sorangium*, was enriched in the spore fraction of the microbial mats, while a second one (*Phaselicystis*) was enriched in the lake sediment samples. The formation of exospores (a type of spore usually produced by members of the Actinobacteria phyla) has been described in *Thermosporothrix hazakensis* (Yabe et al., 2010a,b) and *Thermogemmatispora onikobensis* (Yabe et al., 2011), two species within the class Ktedonobacter in Chloroflexi. Although these two genera were not present in our results, the enrichment of *Nitrolancea* (Sorokin et al., 2012), *Candidatus* Chloroploca (Bryantseva et al., 2021), and *Chloronema* (Chiriac et al., 2017) in the lysis-resistant fraction, could indicate a larger distribution of exospore-like cells within Chloroflexi.

The enrichment in the lysis-resistant fraction of other taxa that are composed so far of exclusively culturable asporogenic species such as Tenericutes, Chlamydiae, Aegiribacteria, Halanaerobiaeota, *Candidatus* Eremiobacterota (formerly known as WPS-2), and Elusimicrobia is noteworthy. Although for none of them spore-like cells are known, the extent to which culturable type strains are a good representation of their environmental counterparts is still unclear. It has been shown that the "domestication" of bacterial isolates can affect their metabolism, morphology, fitness, sporulation, pathogenesis, and resistance (Fux et al., 2005; Sastalla and Leppla, 2012; Eydallin et al., 2014; Norris et al., 2020). In the case of representatives within Tenericutes and Chlamydiae, their lifestyle might provide a mechanism to explain their enrichment in the lysis-resistant fraction. In the case of Tenericutes, *Mollicutes* is the only class described to date. Members of this class, are characterized by the lack of a cell wall and by the establishment of close association with animal and plant hosts (Skennerton et al., 2016). Likewise, some species within the phylum Chlamydiae, although also asporogenic, are known for the production of elementary bodies, which are non-replicating infectious particles. The elementary bodies are released from infected cells and can survive in the environment until they encounter a new host (Elwell et al., 2016). Thus, the fact that Tenericutes and Chlamydiae are obligated intracellular pathogens might explain their enrichment in the lysis-resistant fraction, as it would be necessary to first break the host cell to attain their DNA. In addition, the enrichment of Chlamydiae has been previously observed after the application of the lysis-resistant method in sediments from the Rhone delta in Lake Geneva (Madueño et al., 2018) and in permafrost from Fairbanks, Alaska (Burkert et al., 2019).

In comparison to bacteria, in archaea, lysis resistance seems to be widely spread as there was no clear grouping of the lysis-resistant fraction relative to the total fraction in the PCoA. Also, there was no statistical difference in the alpha diversity between these two communities. The widespread enrichment of archaeal ASVs in the lysis-resistant fraction might be due to the high tolerance of archaea to lysis. The assessment of lysis resistance of archaeal cells faces a few limitations. One of them is the large fraction of unassigned ASVs found in our data set. Another one is that while the method to enrich lysis-resistant cells was extensively validated to enrich lysis-resistant bacterial cells, until now it has not been validated for archaea, and thus the results obtained in the case of archaea should be interpreted with caution. Nevertheless, the same filter was used for the assessment of the bacterial and archaeal community, and it is unlikely that archaeal DNA released during the physical and chemical lysis has a higher resistance to the DNase digestion than the bacterial DNA (Miettinen et al., 2019; Sorensen et al., 2021). Moreover, although some genes encoding programmed cell death and dormancy in bacteria have been found in archaea (Makarova et al., 2012), the lack of markers such as the regulator protein Spo0A (Molle et al., 2003), and calcium dipicolinic acid (Ca-DPA) (Fichtel et al., 2007) in Firmicutes or the protein SsgA in *Streptomyces* (Van Wezel et al., 2000), makes the identification and characterization of specialized resistant cells in archaea more difficult. Even though all these limitations make the assessment of the archaeal results challenging, the enrichment of two genera, *Natrinema* and *Halarchaeum*, is worth mentioning. Interestingly, cyst-like cells have been produced for *Natrinema pallidum* (Suzina et al., 2006). Also, cells belonging to the genus *Halarchaeum* have been isolated from 100 to up to 300 million years old salt deposits (Gruber et al., 2004;

Schubert et al., 2010). These two genera were the most enriched of Euryarchaeota detected in the lysis-resistant fraction of the lake sediments, suggesting that the enrichment might reflect the existence of cyst-like or other types of resistant archaeal cells in the environment.

In summary, the application of the lysis-resistant method in the samples collected at the Salar de Huasco revealed a high diversity of lysis-resistant bacteria and potentially in archaea. Some bacteria are known to form resistant specialized structures, but the results shown here suggest a broader diversity of lysis-resistant bacteria. The culture-independent method used in this study also shows the presence of archaea that display similar resistance to lysis as lysis-resistant bacteria, based on their enrichment in the lysis-resistant fraction. Moreover, to our knowledge, this is the first study investigating lysis-resistance diversity in environmental archaea. To better understand both bacterial and archaeal production of lysis-resistant cells in the environment, new methods that enable the identification of lysis-resistant cells without the limitations of culturing and that also allow further analysis of the cells (e.g., microscopy, resistant tests) are still needed. Furthermore, in the face of a future in which the planet has an even more unpredictable and changing climate, understanding the diversity of microorganisms with the ability to survive highly fluctuating environmental conditions might help us predict the response of microbial communities to climate change.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: NCBI GenBank – PRJNA786740.

AUTHOR CONTRIBUTIONS

AC, PJ, GC, MF, and CD made major contributions to the acquisition of the data. AC and PJ made major contributions to the analysis or interpretation of the data. All authors proofread and approved the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2022.826117/full#supplementary-material

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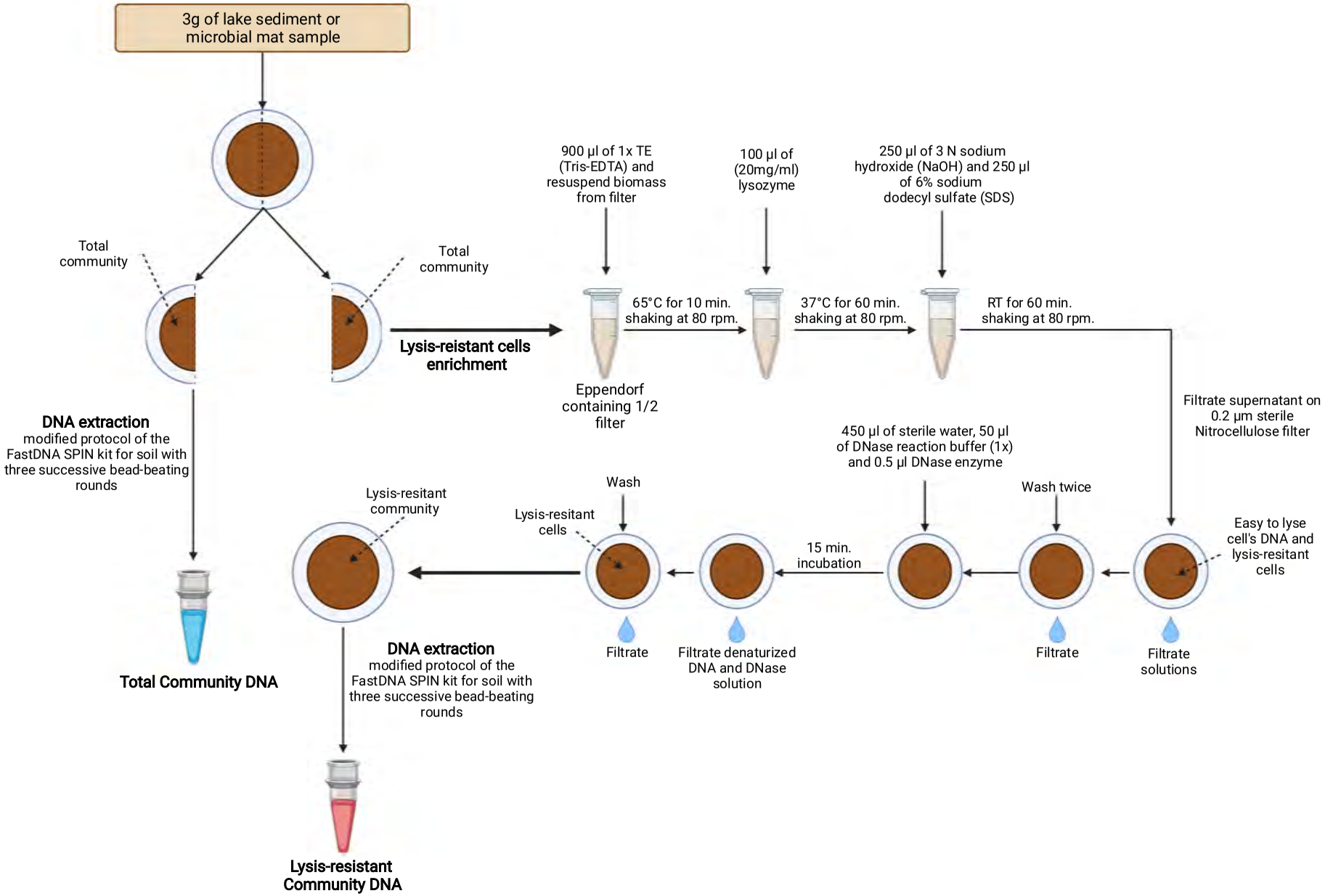
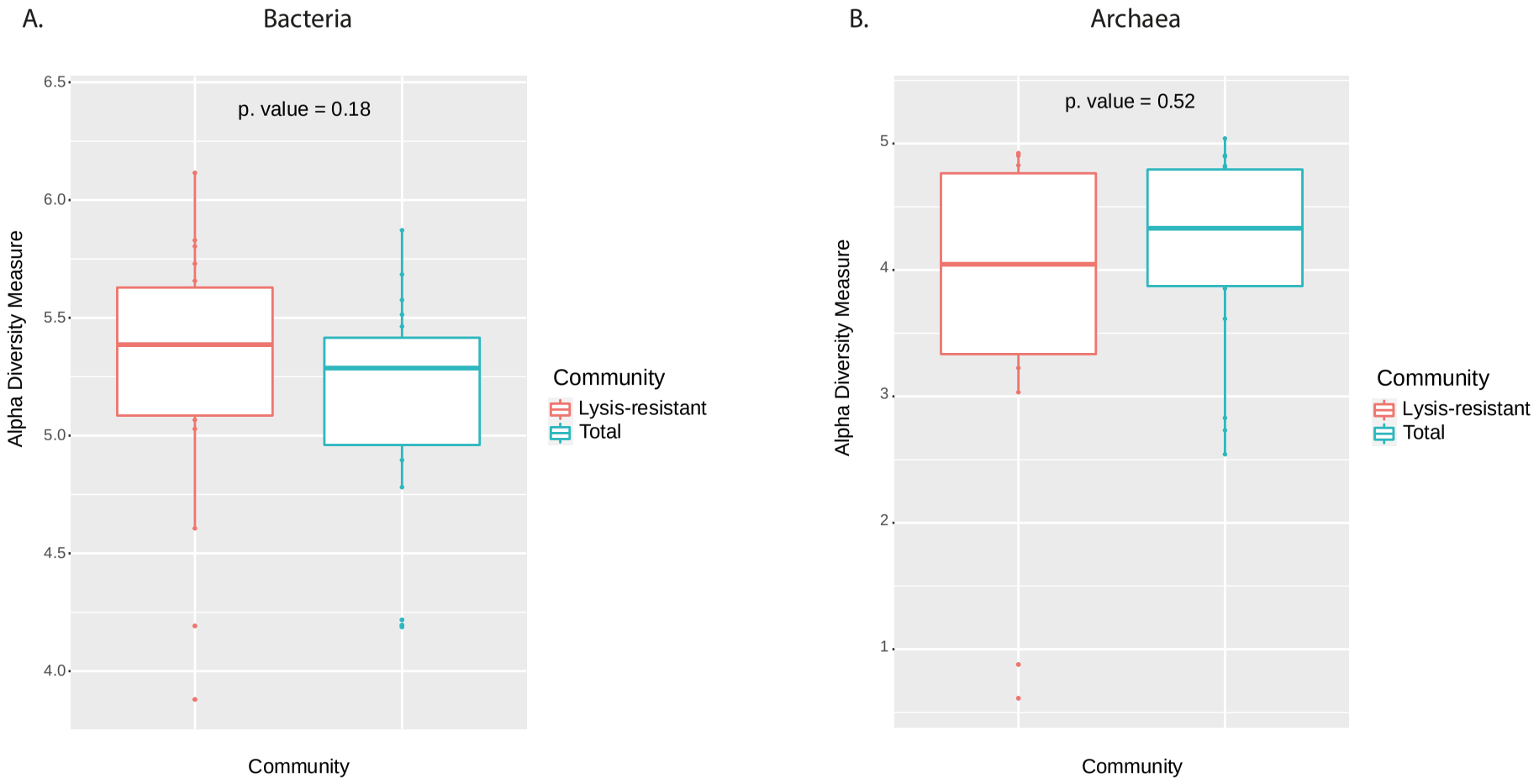
Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

2.1 Supplementary Data

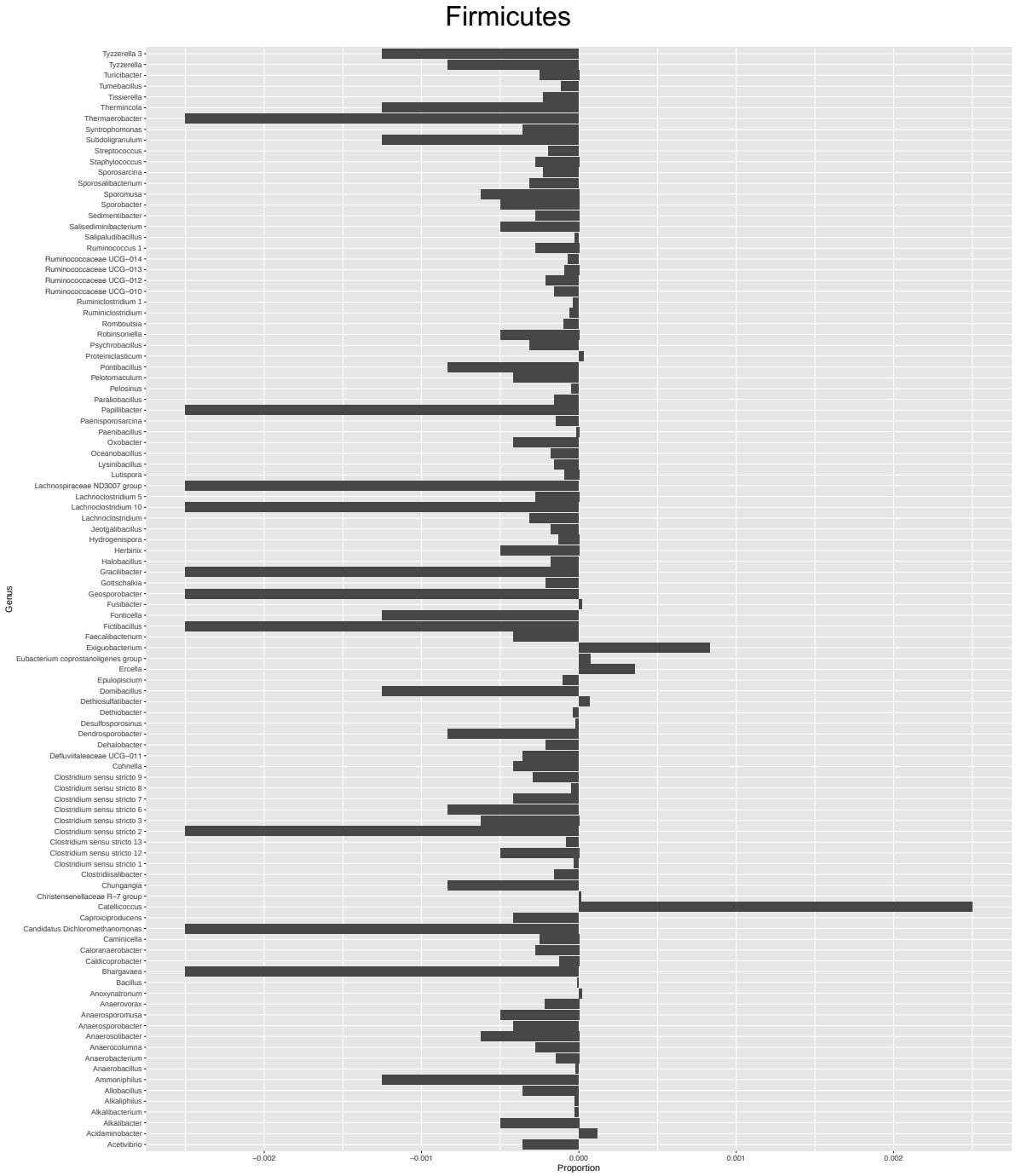


Supplementary Figure 1 . Overview of the method used to obtain the DNA from the total and the lysis-resistant community from lake sediment and microbial mat samples. Created with BioRender.com

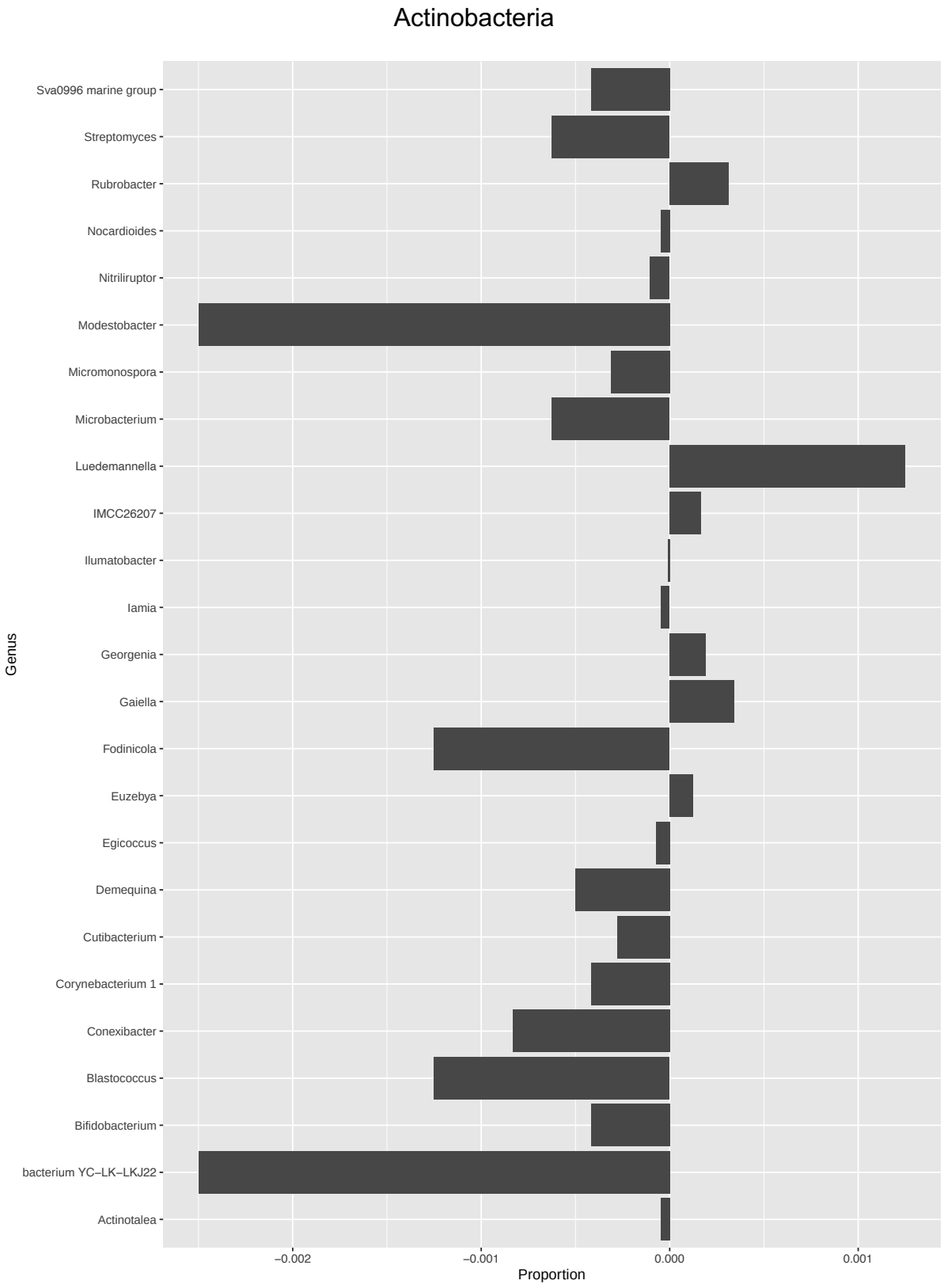
Lake sediments



Supplementary Figure 2. Alpha diversity of the lake sediments **A**. Bacteria. **B.** Archaea

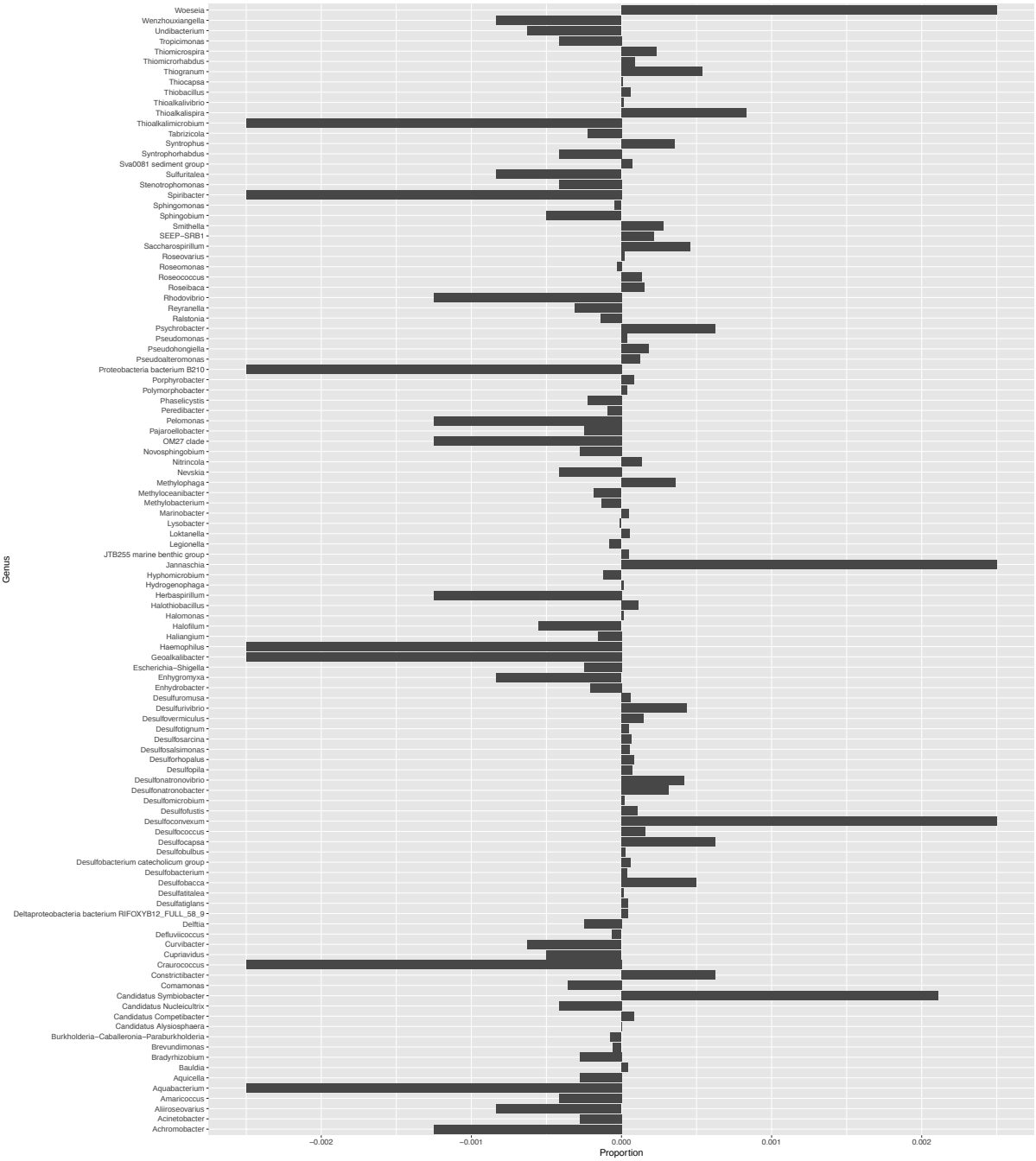


Supplementary Figure 3. Enrichment in the lake sediment samples of genus belonging to the phylum Firmicutes. Negative values show enrichment in the lysis-resistant fraction, while positive values show enrichment in the total fraction for each genus.

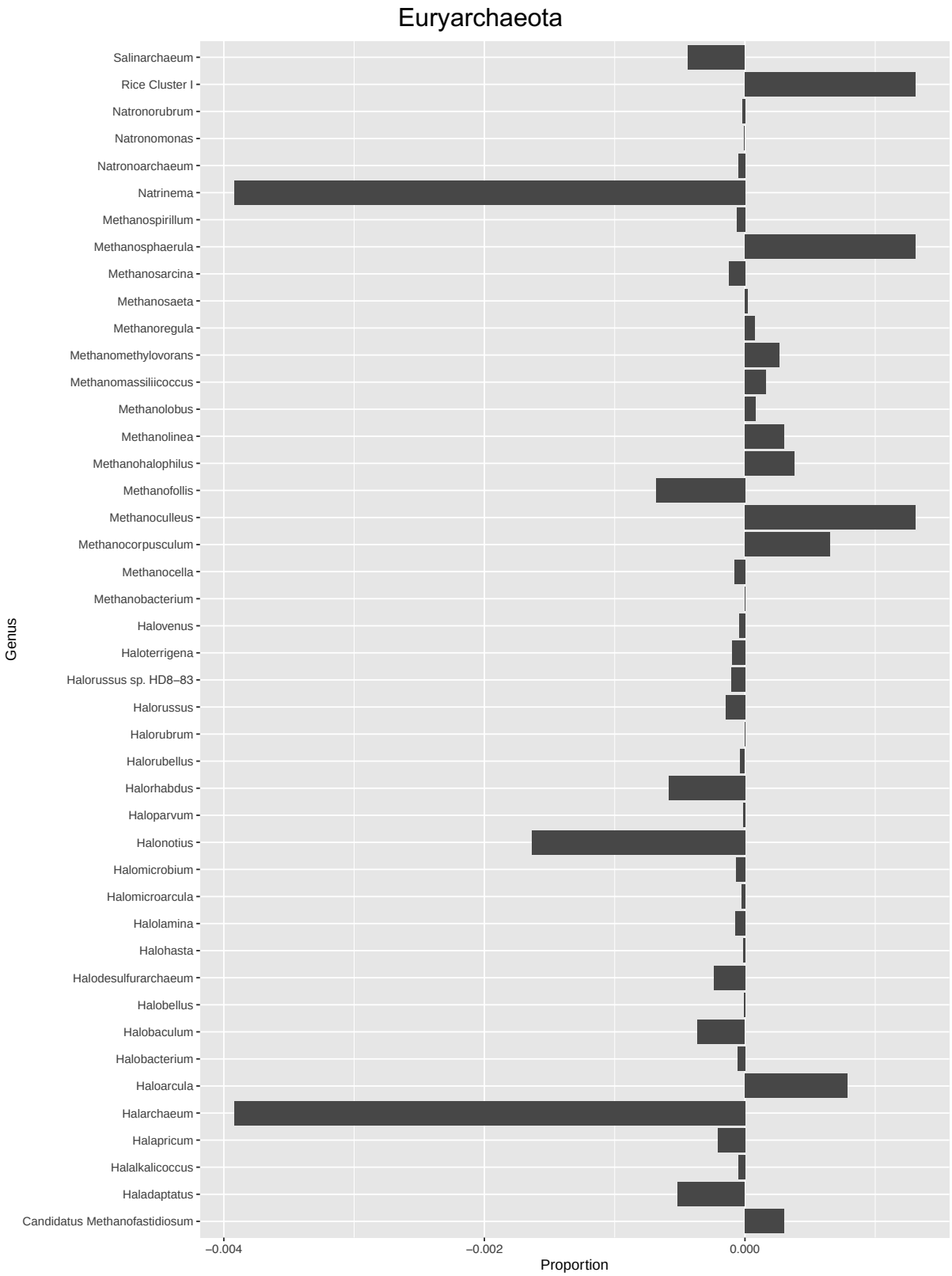


Supplementary Figure 4. Enrichment in the lake sediment samples of genus belonging to the phylum Actinobacteria. Negative values show enrichment in the lysis-resistant fraction, while positive values show enrichment in the total fraction for each genus.

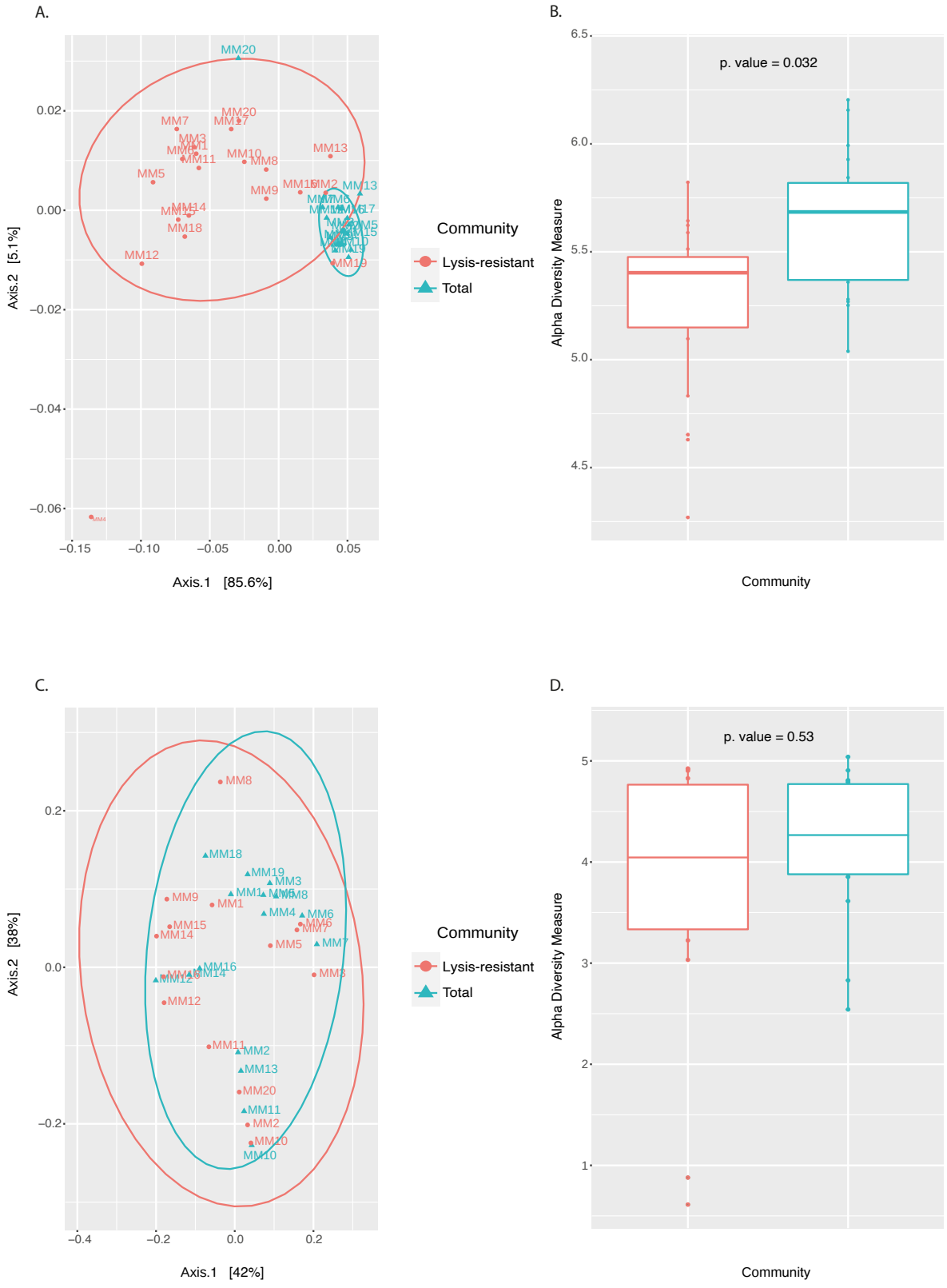
Proteobacteria



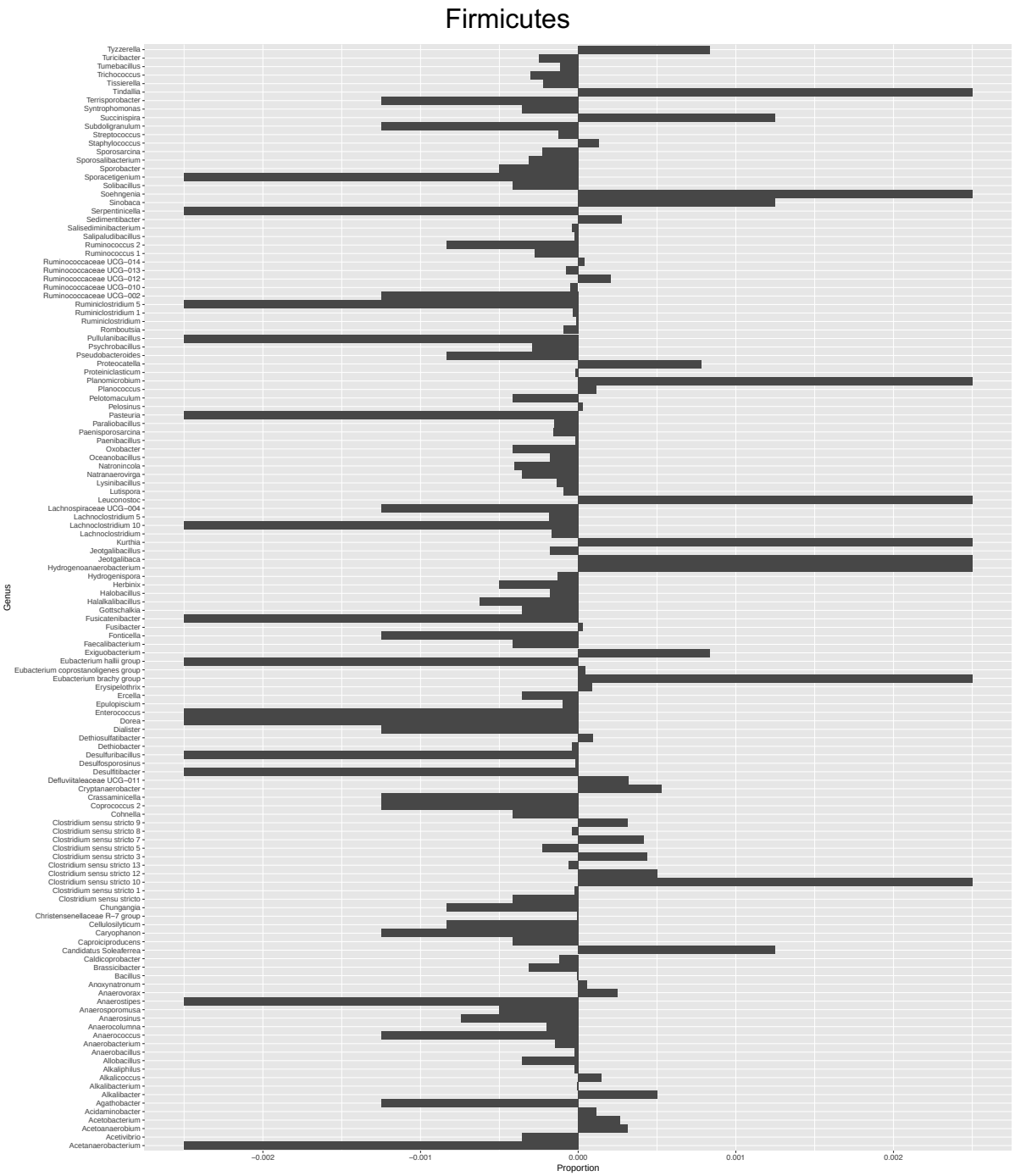
Supplementary Figure 5. Enrichment in the lake sediment samples of genus belonging to the phylum Proteobacteria. Negative values show enrichment in the lysis-resistant fraction, while positive values show enrichment in the total fraction for each genus.



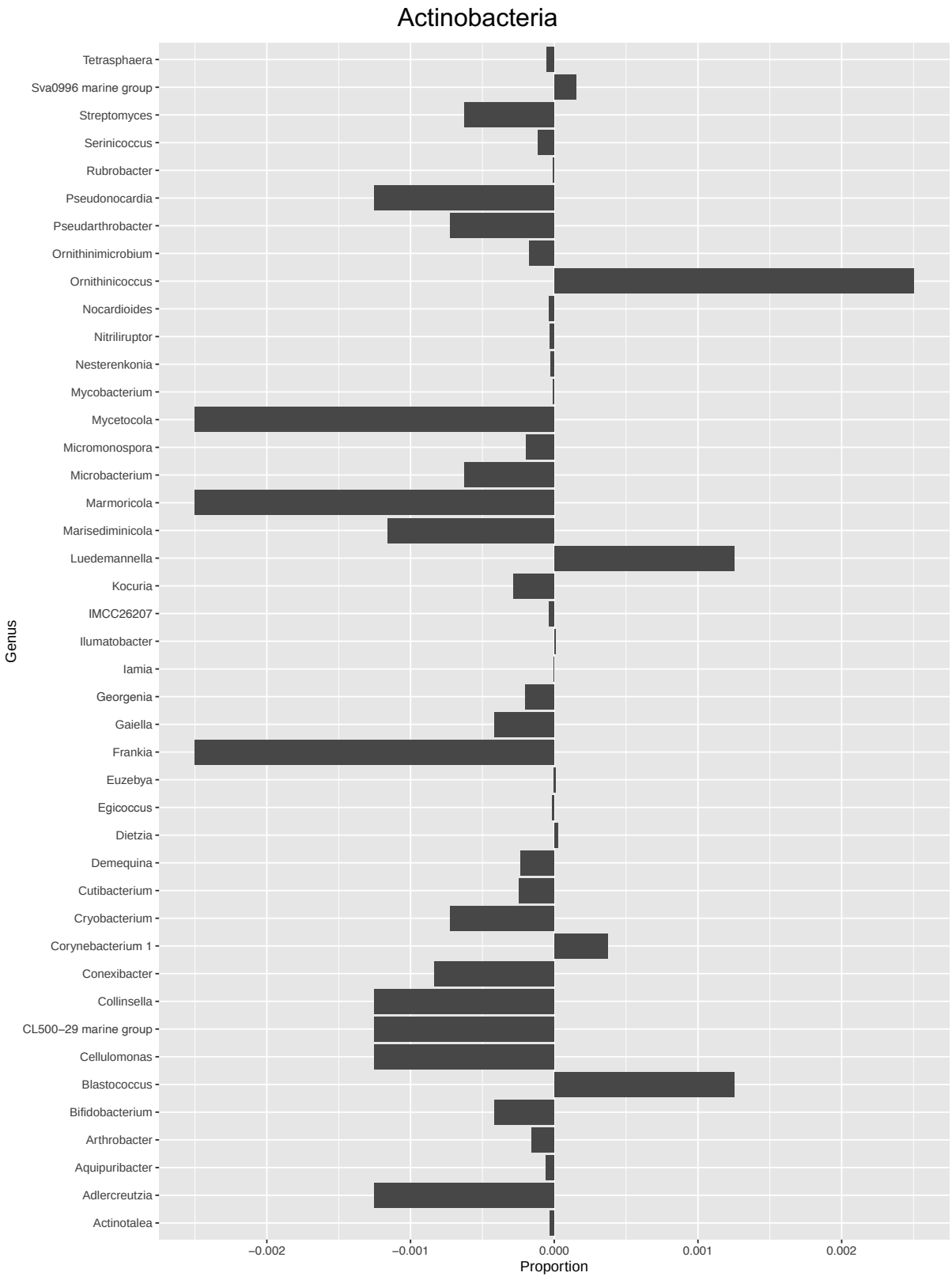
Supplementary Figure 6. Enrichment in the microbial mat samples of genus belonging to the phylum Euryarchaeota. Negative values show enrichment in the lysis-resistant fraction, while positive values show enrichment in the total fraction for each genus.



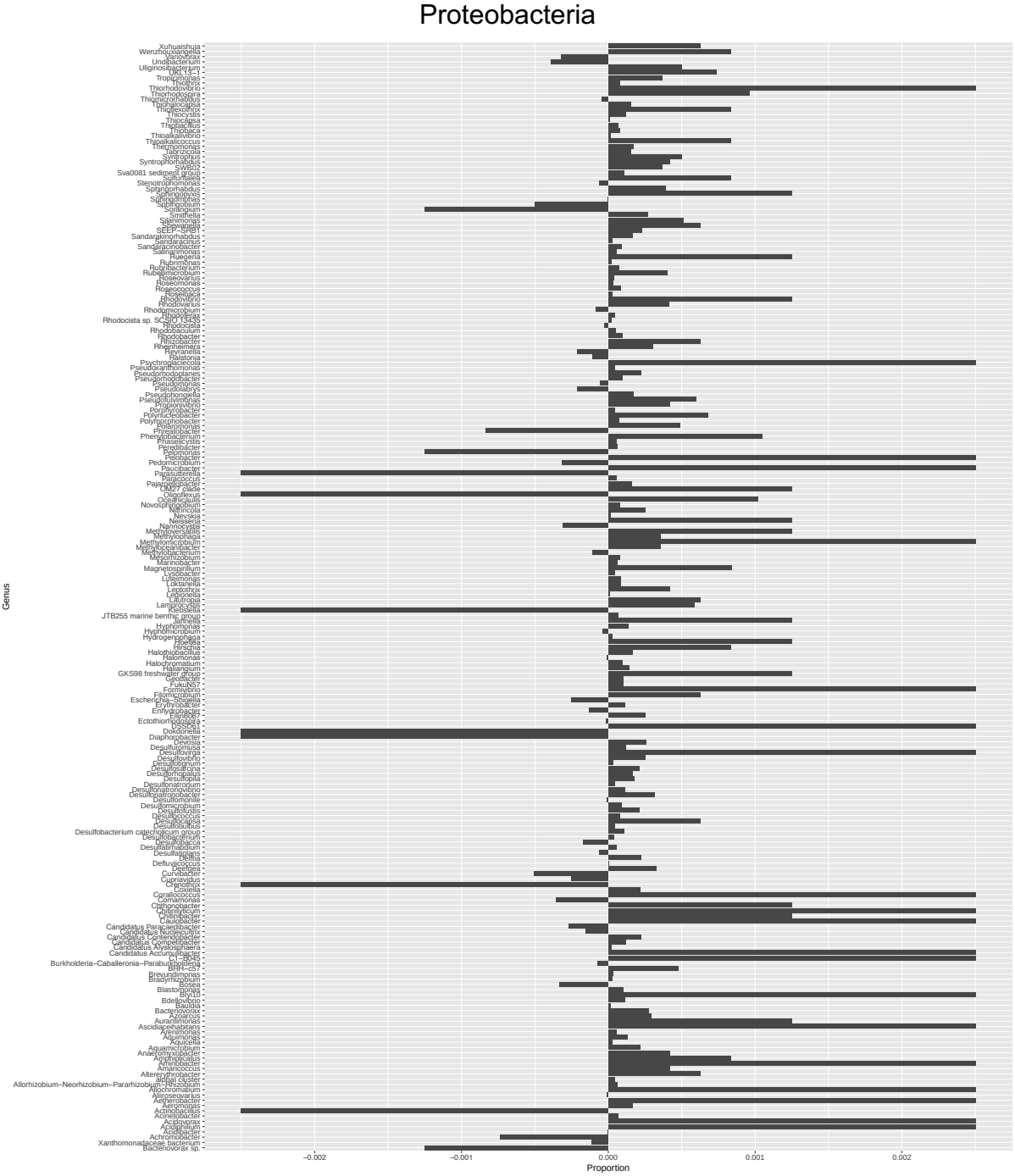
Supplementary Figure 7. Analysis of the microbial mat community **A.** PCoA of the bacterial community. **B.** Alpha diversity of the bacterial community. **C.** PCoA of the archaeal community. **D.** Alpha diversity of the archaeal commu-nity.



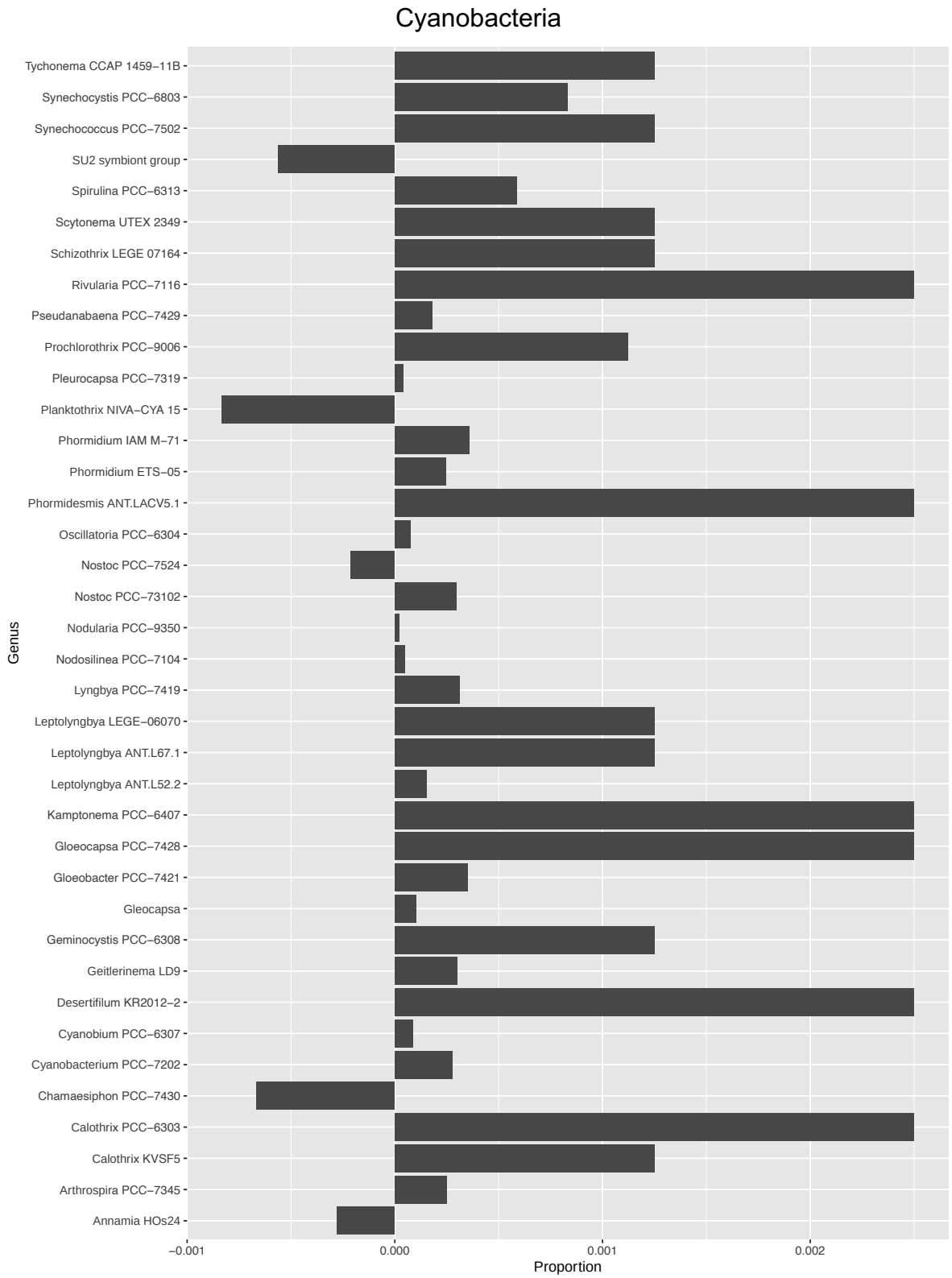
Supplementary Figure 8. Enrichment in the microbial mat samples of genus belonging to the phylum Firmicutes. Negative values show enrichment in the lysis-resistant fraction, while positive values show enrichment in the total fraction for each genus.



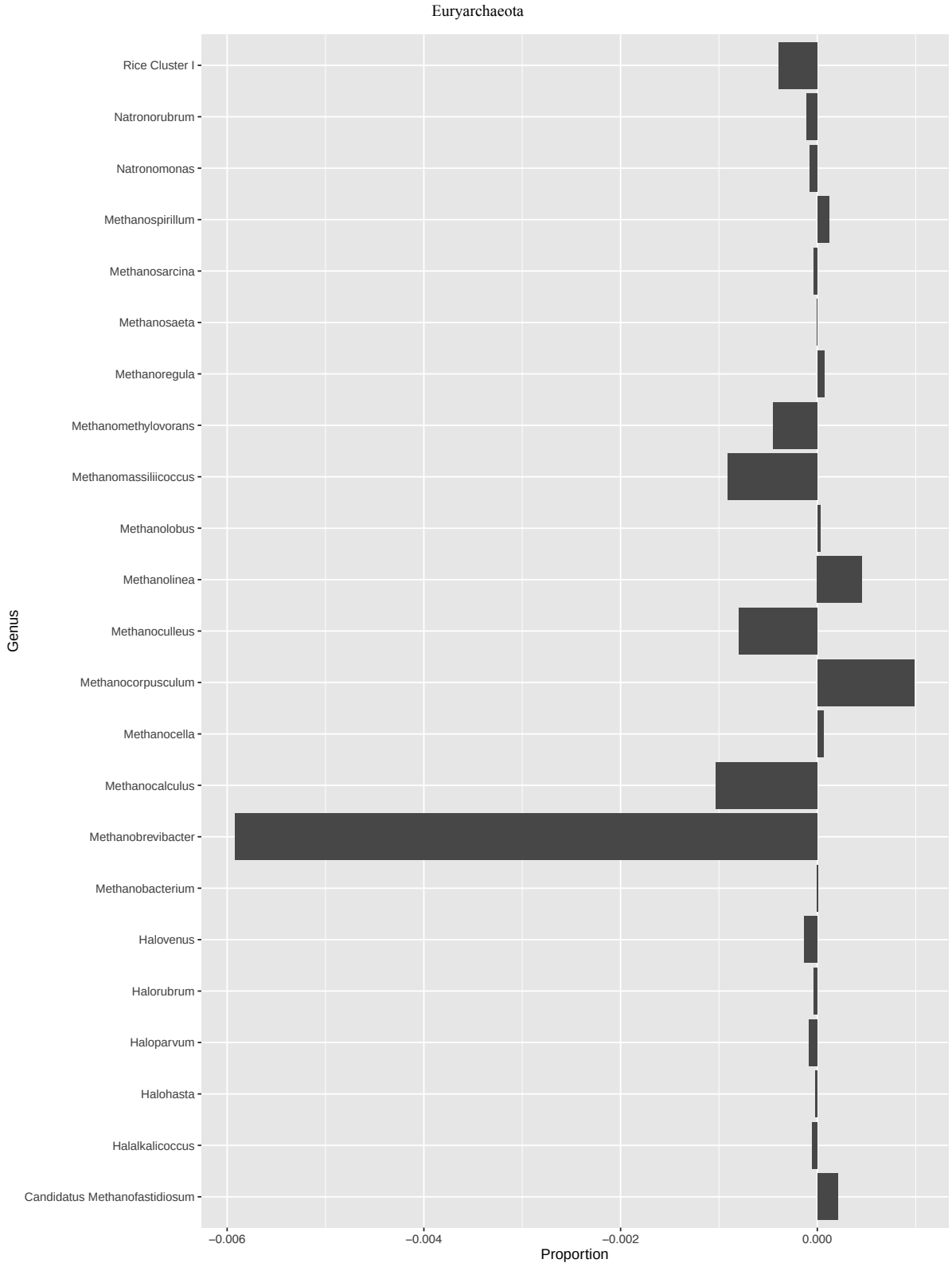
Supplementary Figure 9. Enrichment in the microbial mat samples of genus belonging to the phylum Actinobacteria. Negative values show enrichment in the lysis-resistant fraction, while positive values show enrichment in the total fraction for each genus.



Supplementary Figure 10. Enrichment in the microbial mat samples of genus belonging to the phylum Proteobacteria. Negative values show enrichment in the lysis-resistant fraction, while positive values show enrichment in the total fraction for each genus.



Supplementary Figure 11. Enrichment in the microbail mat samples of genus belonging to the phylum Cyanobacteria. Negative values show enrichment in the lysis-resistant fraction, while positive values show enrichment in the total fraction for each genus.



Supplementary Figure 12. Enrichment in the microbial mat samples of genus belonging to the phylum Euryarchaeota. Negative values show enrichment in the lysis-resistant fraction, while positive values show enrichment in the total fraction for each genus.

Supplementary table 1. Information on the abiotic parameters measured in situ for the different sampling locations.

Name	Pairs	Community	Type	Sampling_point	Description	Depth (cm from surface)	DNA sent to sequence (ng/ul)	Extracted DNA after precipitation (ng/ul)	Elevation (m.a.s.l)	Temperature (ºC)	Conductivity (us/cm)	Salinity (PSU)	OD%	pH	Coordinates
MM1-1	MM1	Total	Microbial mat	Pond_1	Submerged not structured	Surface	4	112	3797	16	1565	0.831	13.9	7.67	20.283139°S 68.888778°W
MM1-1S	MM1	Lysis-resistants	Microbial mat	Pond_1	Submerged not structured	Surface	0.374	0.374	3797	16	1565	0.831	13.9	7.67	20.283139°S 68.888778°W
MM10-1	MM10	Total	Microbial mat	Pond_9	White black mat. Almost fully dry	Surface	4	254	3791	16	3788	2.029	9.4	9.31	20.283306°S 68.888778°W
MM10-1S	MM10	Lysis-resistants	Microbial mat	Pond_9	White black mat. Almost fully dry	Surface	2.3	2.3	3791	16	3788	2.029	9.4	9.31	20.283306°S 68.888778°W
MM11-1	MM11	Total	Microbial mat	Pond_10	3rd region with vegetation only 3cm water	Surface	4	110	3791	16	3788	2.029	9.4	9.31	20.283444°S 68.888778°W
MM11-1S-QM	MM11	Lysis-resistants	Microbial mat	Pond_10	4th region with vegetation only 3cm water	Surface	4	9.08	3791	16	3788	2.029	9.4	9.31	20.283444°S 68.888778°W
MM12-1	MM12	Total	Microbial mat	Pond_11		Surface	4	158	3791	16	3788	2.029	9.4	9.31	20.283528°S 68.888778°W
MM12-1S	MM12	Lysis-resistants	Microbial mat	Pond_11		Surface	0.47	0.47	3791	16	3788	2.029	9.4	9.31	20.283528°S 68.888778°W
MM13-1	MM13	Total	Microbial mat	Pond_12		Surface	4	149	3792	16	3788	2.029	9.4	9.31	20.283722°S 68.888778°W
MM13-1S	MM13	Lysis-resistants	Microbial mat	Pond_12		Surface	0.112	0.112	3792	16	3788	2.029	9.4	9.31	20.283722°S 68.888778°W
MM14-1	MM14	Total	Microbial mat	Pond_13		Surface	4	35.4	3789	16	3788	2.029	9.4	9.31	20.283806°S 68.888778°W
MM14-1S	MM14	Lysis-resistants	Microbial mat	Pond_13		Surface	4	4.9	3789	16	3788	2.029	9.4	9.31	20.283806°S 68.888778°W
MM15-1	MM15	Total	Microbial mat	Pond_13		Surface	4	10.7	3791	16	3788	2.029	9.4	9.31	20.283778°S 68.888778°W
MM15-1S	MM15	Lysis-resistants	Microbial mat	Pond_13		Surface	2.5	2.5	3791	16	3788	2.029	9.4	9.31	20.283778°S 68.888778°W
MM16-1	MM16	Total	Microbial mat	Pond_14		Surface	4	140	3794	16	4395	2.365	21.5	9.51	20.283667°S 68.888778°W
MM16-1S	MM16	Lysis-resistants	Microbial mat	Pond_14		Surface	4	13.8	3794	16	4395	2.365	21.5	9.51	20.283667°S 68.888778°W
MM17-1	MM17	Total	Microbial mat	Pond_15		Surface	4	123		16.9	4395	2.365	21.5	9.51	20.283694°S 68.888778°W
MM17-1S	MM17	Lysis-resistants	Microbial mat	Pond_15		Surface	4	6.54		16.9	4395	2.365	21.5	9.51	20.283694°S 68.888778°W
MM18-1	MM18	Total	Microbial mat	Pond_16		Surface	4	308	3796	15.42	4395	2.365	21.5	9.51	20.283528°S 68.888778°W
MM18-1S	MM18	Lysis-resistants	Microbial mat	Pond_16		Surface	4	4.34	3796	15.42	4395	2.365	21.5	9.51	20.283528°S 68.888778°W
MM19-1	MM19	Total	Microbial mat	Pond_17		Surface	4	38.8	3794	19.4	4395	2.365	21.5	9.51	20.283583°S 68.888778°W
MM19-1S	MM19	Lysis-resistants	Microbial mat	Pond_17		Surface	0.226	0.226	3794	19.4	4395	2.365	21.5	9.51	20.283583°S 68.888778°W
MM2-1	MM2	Total	Microbial mat	Pond_2	Dry samples from the surface. Green mat	Surface	4	346	3791	16	1565	0.831	13.9	7.65	20.283111°S 68.888778°W
MM2-1S	MM2	Lysis-resistants	Microbial mat	Pond_2	Dry samples from the surface. Green mat	Surface	4	19.3	3791	16	1565	0.831	13.9	7.65	20.283111°S 68.888778°W
MM20-1	MM20	Total	Microbial mat	Pond_18		Surface	4	114	3788	16	1588	0.846	16.5	8.73	20.283583°S 68.888778°W
MM20-1S	MM20	Lysis-resistants	Microbial mat	Pond_18		Surface	4	4.9	3788	16	1588	0.846	16.5	8.73	20.283583°S 68.888778°W
MM3-1	MM3	Total	Microbial mat	Pond_3	Submerged. Black/green. High content of EPS	Surface	4	278		16	747	0.412	8.06	7.9	20.283167°S 68.888778°W
MM3-1S	MM3	Lysis-resistants	Microbial mat	Pond_3	Submerged. Black/green. High content of EPS	Surface	0.616	0.616		16	747	0.412	8.06	7.9	20.283167°S 68.888778°W
MM4-1	MM4	Total	Microbial mat	Pond_4	Highly structured. Submerged. Pink area	Surface	4	110	3795	16	747	0.412	8.06	7.9	20.282917°S 68.888778°W
MM4-1S	MM4	Lysis-resistants	Microbial mat	Pond_4	Highly structured. Submerged. Pink area	Surface	1.56	1.56	3795	16	747	0.412	8.06	7.9	20.282917°S 68.888778°W
MM5-1	MM5	Total	Microbial mat	Pond_4	Highly structured. Submerged. Pink area	Surface	4	168	3796	16	1386	0.738	9.4	7.89	20.283056°S 68.888778°W
MM5-1S	MM5	Lysis-resistants	Microbial mat	Pond_4	Highly structured. Submerged. Pink area	Surface	0.706	0.706	3796	16	1544	0.738	9.4	7.89	20.283056°S 68.888778°W
MM6-1	MM6	Total	Microbial mat	Pond_5	Submerged. Brown	Surface	4	114	3803	16	1459	0.738	9.4	7.89	20.282861°S 68.888778°W
MM6-1S	MM6	Lysis-resistants	Microbial mat	Pond_5	Submerged. Brown	Surface	0.2	0.2	3803	16	1459	0.738	9.4	7.89	20.282861°S 68.888778°W
MM7-1	MM7	Total	Microbial mat	Pond_6	Submerged	Surface	4	110	3789	16	1142	0.738	9.4	7.89	20.282917°S 68.888778°W
MM7-1S	MM7	Lysis-resistants	Microbial mat	Pond_6	Submerged	Surface	1.54	1.54	3789	16	1142	0.738	9.4	7.89	20.282917°S 68.888778°W
MM8-1	MM8	Total	Microbial mat	Pond_7	Highly structured. Several layers	Surface	4	195	3799	16	8322	4.229	11.3	9.74	20.282889°S 68.888778°W
MM8-1S	MM8	Lysis-resistants	Microbial mat	Pond_7	Highly structured. Several layers	Surface	4	6.18	3799	16	8322	4.229	11.3	9.74	20.282889°S 68.888778°W
MM9-1	MM9	Total	Microbial mat	Pond_8	Reddish mat not highly structured. Submerged	Surface	4	17.5	3790	16	3788	2.029	21.3	9.31	20.283389°S 68.888778°W
MM9-1S	MM9	Lysis-resistants	Microbial mat	Pond_8	Reddish mat not highly structured. Submerged	Surface	4	4.18	3790	16	3788	2.029	21.3	9.31	20.283389°S 68.888778°W
Salar1-1-1	S111	Total	Lake sediment	Sample_1	Gray and liquid	1	4	112	3791	2	78.04	53.69	17.1	8.32	20.285444°S 68.888778°W
Salar1-1-1S	S111	Lysis-resistants	Lake sediment	Sample_1	Gray and liquid	1	1.04	1.04	3791	2	78.04	53.69	17.1	8.32	20.285444°S 68.888778°W
Salar1-2-1	S121	Total	Lake sediment	Sample_1	Gray and liquid	2	4	118	3791	2	78.04	53.69	17.1	8.32	20.285444°S 68.888778°W
Salar1-2-1S	S121	Lysis-resistants	Lake sediment	Sample_1	Gray and liquid	2	1.34	1.34	3791	2	78.04	53.69	17.1	8.32	20.285444°S 68.888778°W
Salar1-3-1	S131	Total	Lake sediment	Sample_1	Gray and liquid	3	4	102	3791	2	78.04	53.69	17.1	8.32	20.285444°S 68.888778°W
Salar1-3-1S	S131	Lysis-resistants	Lake sediment	Sample_1	Gray and liquid	3	0.648	0.648	3791	2	78.04	53.69	17.1	8.32	20.285444°S 68.888778°W
Salar1-4-1	S141	Total	Lake sediment	Sample_1	Gray and liquid	4	4	51.4	3791	2	78.04	53.69	17.1	8.32	20.285444°S 68.888778°W
Salar1-4-1S	S141	Lysis-resistants	Lake sediment	Sample_1	Gray and liquid	4	0.918	0.918	3791	2	78.04	53.69	17.1	8.32	20.285444°S 68.888778°W
Salar2-1-1	S211	Total	Lake sediment	Sample_2	Dark gray	1	4	212	3787	5	78.04	53.69	17.1	8.32	20.285194°S 68.888778°W
Salar2-1-1S	S211	Lysis-resistants	Lake sediment	Sample_2	Dark gray	1	1.88	1.88	3787	5	78.04	53.69	17.1	8.32	20.285194°S 68.888778°W
Salar2-2-1	S221	Total	Lake sediment	Sample_2	Dark gray	2	4	136	3787	5	78.04	53.69	17.1	8.32	20.285194°S 68.888778°W
Salar2-2-1S	S221	Lysis-resistants	Lake sediment	Sample_2	Dark gray	2	2.18	2.18	3787	5	78.04	53.69	17.1	8.32	20.285194°S 68.888778°W
Salar3-1-1	S311	Total	Lake sediment	Sample_3	4m from delta	4	4	74	3786	4	78.04	53.69	17.1	8.32	20.284611°S 68.888778°W
Salar3-1-1S	S311	Lysis-resistants	Lake sediment	Sample_3	4m from delta	4	0.678	0.678	3786	4	78.04	53.69	17.1	8.32	20.284611°S 68.888778°W
Salar3-2-1	S321	Total	Lake sediment	Sample_3	4m from delta	3	4	27.8	3786	4	78.04	53.69	17.1	8.32	20.284611°S 68.888778°W
Salar3-2-1S	S321	Lysis-resistants	Lake sediment	Sample_3	4m from delta	3	0.776	0.776	3786	4	78.04	53.69	17.1	8.32	20.284611°S 68.888778°W
Salar3-3-1	S331	Total	Lake sediment	Sample_3	4m from delta	2	4	118	3786	4	78.04	53.69	17.1	8.32	20.284611°S 68.888778°W
Salar3-3-1S	S331	Lysis-resistants	Lake sediment	Sample_3	4m from delta	2	0.772	0.772	3786	4	78.04	53.69	17.1	8.32	20.284611°S 68.888778°W
Salar3-4-1	S341	Total	Lake sediment	Sample_3	4m from delta	1	4	91.2	3786	4	78.04	53.69	17.1	8.32	20.284611°S 68.888778°W
Salar3-4-1S	S341	Lysis-resistants	Lake sediment	Sample_3	4m from delta	1	1.94	1.94	3786	4	78.04	53.69	17.1	8.32	20.284611°S 68.888778°W
Salar4-1-1	S411	Total	Lake sediment	Sample_4	Foul odour	1	4	85.6	3780	10	52450	34.19	12	8.43	20.283778°S 68.888778°W
Salar4-1-1S	S411	Lysis-resistants	Lake sediment	Sample_4	Foul odour	1	0.274	0.274	3780	10	52450	34.19	12	8.43	20.283778°S 68.888778°W
Salar4-2-1	S421	Total	Lake sediment	Sample_4	Foul odour	2	4	102	3780	10	52450	34.19	12	8.43	20.283778°S 68.888778°W
Salar4-2-1S	S421	Lysis-resistants	Lake sediment	Sample_4	Foul odour	2	0.458	0.458	3780	10	52450	34.19	12	8.43	20.283778°S 68.888778°W
Salar4-3-1	S431	Total	Lake sediment	Sample_4	Foul odour	3	4	100	3780	10	52450	34.19	12	8.43	20.283778°S 68.888778°W
Salar4-3-1S	S431	Lysis-resistants	Lake sediment	Sample_4	Foul odour	3	0.88	0.88	3780	10	52450	34.19	12	8.43	20.283778°S 68.888778°W
Salar5	S4	/	Lake sediment	Sample_5	No sediment could be collected	/	/	/	3789	13	52.48	34.17	7.3	8.43	20.283528°S 68.888778°W
Salar6-1-1	S611	Total	Lake sediment	Sample_6	Strong foul odour	1	4	94.8	3791	15	50490	32.77	7.9	8.43	20.283444°S 68.888778°W
Salar6-1-1S	S611	Lysis-resistants	Lake sediment	Sample_6	Strong foul odour	1	0.198	0.198	3791	15	50490	32.77	7.9	8.43	20.283444°S 68.888778°W
Salar6-2-1	S621	Total	Lake sediment	Sample_6	Strong foul odour	2	4	70	3791	15	50490	32.77	7.9	8.43	20.283444°S 68.888778°W
Salar6-2-1S	S621	Lysis-resistants	Lake sediment	Sample_6	Strong foul odour	2	0.606	0.606	3791	15	50490	32.77	7.9	8.43	20.283444°S 68.888778°W
Salar6-3-1	S631	Total	Lake sediment	Sample_6	Strong foul odour	3	4	108	3791	15	50490	32.77	7.9	8.43	20.283444°S 68.888778°W
Salar6-3-1S	S631	Lysis-resistants	Lake sediment	Sample_6	Strong foul odour	3	0.134	0.134	3791	15	50490	32.77	7.9	8.43	20.283444°S 68.888778°W
Salar7-1-1	S711	Total	Lake sediment	Sample_7		4	4	114	3777	16	132	0.706	12	8.22	20.283472°S 68.888778°W
Salar7-1-1S	S711	Lysis-resistants	Lake sediment	Sample_7		4	0.432	0.432	3777	16	132	0.706	12	8.22	20.283472°S 68.888778°W
Salar7-2-1	S721	Total	Lake sediment	Sample_7		3	4	102	3777	16	132	0.706	12	8.22	20.283472°S 68.888778°W
Salar7-2-1S	S721	Lysis-resistants	Lake sediment	Sample_7		3	0.36	0.36	3777	16	132	0.706	12	8.22	20.283472°S 68.888778°W
Salar7-3-1	S731	Total	Lake sediment	Sample_7		2	4	63.6	3777	16	132	0.706	12	8.22	20.283472°S 68.888778°W
Salar7-3-1S	S731	Lysis-resistants	Lake sediment	Sample_7		2	0.364	0.364	3777	16	132	0.706	12	8.22	20.283472°S 68.888778°W
Salar7-4-1	S741	Total	Lake sediment	Sample_7		1	4	112	3777	16	132	0.706	12	8.22	20.283472°S 68.888778°W
Salar7-4-1S	S741	Lysis-resistants	Lake sediment	Sample_7		1	0.658	0.658	3777	16	132	0.706	12	8.22	20.283472°S 68.888778°W

Supplementary Table 2. Summary of the bacterial genera belonging to the enriched phyla in the lysis-resistant fraction.

Phylum	Genus	Enriched in Salar	Enriched in Mat	Species	Sporeformer	Genome	Description
	Tyzzerella 3	Spore	0	Uncultured	Unknown	Only DNA info	Gut microbiom
	Tyzzerella	Spore	Total	Uncultured	Some	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=1965545	Gut microbiom (related to diseased patients). Gram-positive, obligately anaerobic rods 0.3–1.5μm×1.5–20μm. Some of its members are non-motileand non-spore-forming and have higher G+C contents, from40% inC. nexileto 46.8% inC. colinum. Strains are mesophilic orthermophilic (temperature range from 20°C to 63°C) and grow in neutral to alkaline pH (some up to pH 11). Chemoorganotrophs. Oxidase and catalase are not pro-duced. Some mono- and disaccharides can be fermented;acetate is produced as a major end product. Sulfate is notreduced
	Turicibacter	Spore	Spore	Uncultured Turicibacter sp. S37_09_1	one species	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1712675&lvl=3&lin=f&keep=1&srchmode=1&unlock	Gut microbiota. Gram-positive non-spore-forming irregularly shaped rods.
	Tumebacillus	Spore	Spore	Uncultured Unclassified	Yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1214604&lvl=3&lin=f&keep=1&srchmode=1&unlock	Tumebacillus is a genus of Gram-positive, rod-shaped, spore-forming bacteria. Members of the genus can be motile or non-motile, and form white or yellow colonies on R2A agar. Isolated from arctic permafrost, soil samples, cassava wastewater, decomposing algal scum, river water, and the gut of a vulture
	Trichococcus	0	Spore	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=82802&lvl=3&lin=f&keep=1&srchmode=1&unlock	Mesophilic and psychrotolerant genus. Activated sludge, duck pond, penguin guano in Chilean Patagonia, high-elevation wetland
	Tissierella	Spore	Spore	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1123404&lvl=3&lin=f&keep=1&srchmode=1&unlock	Gram-positive, non-spore-forming bacterium, anaerobic, gut microbiota
	Tindallia	0	Total	Unclassified	some	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=159292&lvl=3&lin=f&keep=1&srchmode=1&unlock	Strain Z-7934, an alkaliphilic, obligately anaerobic, fermentative, asporogenous bacterium with Gram-positive cell wall structure, was isolated from soda deposits in Lake Magadi, Kenya. Tindallia californiensissp. isolated from sediments of the athalassic, meromictic,alkaline Mono Lake in California. The Gram-positive,spore-forming, slightly curved rods with sizes 0.55–0.7·1.7–3.0lm were motile by a single laterally at-tached flagellum. Strain APO was mesophilic (range10–48°C, optimum of 37°C); halophilic (NaCl range1–20% (w/v) with optimum of 3–5% (w/v), and alka-liphilic (pH range 8.0–10.5, optimum 9.5). The novelisolate required sodium ions in the medium.
	Thermincola	Spore	0	Uncultured	Some	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=281456&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cells of strain Z-0001 were straight, Gram-positive rods, slowly motile. Strain Z-0001 was found to be an obligate anaerobe. It grew in the temperature range from 45 to 70 degrees C with an optimum at 57-60 degrees C, in a pH range from 5.9 to 8.0 with an optimum at 7.0-7.2, and in NaCl concentration range 0-3.5% with an optimum at 0%.
	Thermaerobacter	Spore	0	uncultured compost bacterium	some	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1294259&lvl=3&lin=f&keep=1&srchmode=1&unlock	The variably Gram-stained cells were motile rods with flagella, did not form spores and proliferated at 52-78 degrees C (optimum, 70 degrees C), pH 5-8 (optimum, pH 7) and 0-4.5 % NaCl (optimum, 1.0 %). The novel isolate was a strictly aerobic
						https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=867903&lvl=3&lin=f&keep=1&srchmode=1&unlock	A strictly aerobic, thermophilic, gram-positive, spore-producing, rod-shaped bacterium (2.0-10.0 x 0.3 microm), designated isolate C21T, was isolated from a sample collected from an open drain run-off channel of a bore in the Great Artesian Basin of Australia (New Lorne Bore, registered number 17263). Isolate C21T grew optimally at 70 degrees C (temperature range for growth was 55-80 degrees C) and pH 8.5 (pH range for growth was 6.0-10.5), with a generation time of 90 min.
	Terrisporobacter	0	Spore	Uncultured	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1121315&lvl=3&lin=f&keep=1&srchmode=1&unlock	A Gram-staining-positive, spore-forming, strictly anaerobic bacterium, designated strain LAM0A37T, was isolated from enrichment samples collected from a petroleum reservoir in Shengli oilfield. Cells of strain LAM0A37T were rod-shaped and motile by peritrichous flagella. The optimal temperature and pH for growth were 40 °C and 7.0–7.5, respectively.
						https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1577792&lvl=3&lin=f&keep=1&srchmode=1&unlock	
	Syntrophomonas	Spore	Spore	Uncultured	Yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=378794&lvl=3&lin=f&keep=1&srchmode=1&unlock	The strains formed spores when cocultured with methanogens on butyrate, or when grew on butyrate plus dimethyl sulfoxide (DMSO) in pure culture. The cells were slightly curved rods with Gram-negative cell wall structure, and contained small amount of poly beta-hydroxyalkanoate. The growth of the new strains occurred in the range of pH 5.5-8.4, and of temperature 20-48 degrees C, and at NaCl concentration of 0-700 mM. The G+C content of the genomic DNA of strain 5-3-Z(T) was 40.6mol%.
	Succinispira	0	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1069080&lvl=3&lin=f&keep=1&srchmode=1&unlock	Longer cells can be spirals of up to 3 or 4 turns with an amplitude of 1.5–2.0 μm, and a wavelength of 3–4 μm. Gram-stain-negative. Aminopeptidase-negative. Cell-wall structure appears to be of a Gram-negative type with an outer membrane. Highly motile with laterally inserted flagella. Cytochromes are not formed. No endospores. Strict anaerobe;
	Subdoligranulum	Spore	Spore	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=411471&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cells are coccoid in shape but may show some pleomorphism. Gram-stain-negative. Nonmotile and nonspore-forming. Strictly anaerobic; no growth occurs in 2% oxygen. Cells do not survive heating at 80°C for 10 min. Catalase-negative. Glucose and some other carbohydrates are fermented.
	Streptococcus	Spore	Spore	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1326&lvl=3&lin=f&keep=1&srchmode=1&unlock	Streptococci are coccoid bacterial cells microscopically, and stain purple (Gram-positive) when Gram staining technique is applied. They are nonmotile and non-spore forming. These cocci measure between 0.5 and 2 μm in diameter. As cellular division of Streptococcus spp.

Pseudobacteroides	0	0	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=398512&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cells are Gram-stain-negative, thermotolerant, non-spore-forming, nonmotile, and strictly anaerobic rods (0.8–6 μm), occurring singly, in pairs, or occasionally in chains of up to eight cells. Cells grow at 20–45°C, with an optimum at 40°C and pH 6.0–8.0, with an optimum at 7.0. Cells tolerate the heat treatment at 75°C for 10 min.
Proteocatella	0	Total	Unclassified	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1123009&lvl=3&lin=f&keep=1&srchmode=1&unlock	Gram-stain-positive, spore-forming, straight rods (0.7–0.8×3.0–5.0 μm) that were motile by means of peritrichous flagella. Growth was observed at pH 6.7–9.7 (optimum pH 8.3) and 2–37 °C (optimum 29 °C). Growth was observed between 0 and 4 % (w/v) NaCl with optimum growth at 0.5 % (w/v).
Proteiniclasticum	Total	Spore	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1410668&lvl=3&lin=f&keep=1&srchmode=1&unlock	The strains were Gram-stain negative and non-spore-forming with cell sizes of 0.5-0.8 x 0.6-2.0 mum. The temperature range for growth was 24-46 degrees C (optimum 38-39 degrees C) and the pH range was between 5.6 and 8.7 (optimum 7.0-7.3).
Pontibacillus	Spore	Total	Unclassified	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1385513&lvl=3&lin=f&keep=1&srchmode=1&unlock	Gram-positive, spore-forming and strictly aerobic genus of bacteria from the family of Bacillaceae. designated strain JSM 076056(T), was isolated from a sea urchin collected from the South China Sea.
Planomicrobium	0	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=244&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cells are coccoid or short rods, 0?861?0 mm. Gram-positive and non-spore-forming. Motile by polar flagella. Strictly aerobic. Colonies are smooth, circular, low-convex and yellow to orange in colour when cultivated on MA. Growth occurs at 10–45 uC; optimum growth at 30–35 uC and no growth above 46 uC.
Planococcus	0	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1185653&lvl=3&lin=f&keep=1&srchmode=1&unlock	is a genus of Gram-Positive or Gram-variable, cocci or short rod-shaped bacteria in the family Caryophanaceae from the order Caryophanales.
Pelotomaculum	Spore	Spore	Uncultured	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=258475&lvl=3&lin=f&keep=1&srchmode=1&unlock	Pelotomaculum thermopropionicum(Ishiiet al., 2005).The cells are Gram-positive. Spores are spherical and central.Spore formation was observed during the late-exponentialgrowth phase.
Pelosinus	Spore	Total	Uncultured	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1149860&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cells are 2–6 μm long and 0.6 μm in diameter. Cells have up to 6 peritrichous flagella. Oval endospores are formed terminally. Optimal growth occurs at pH 7.0; no growth occurs at initial pH values lower than 5.5 or higher than 8.0. Growth is most rapid at 22–30 °C; no growth occurs at temperatures below 4 °C or above 36 °C.
Pasteuria	0	Spore	Uncultured	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=238030&lvl=3&lin=f&keep=1&srchmode=1&unlock	Pasteuriaappears to be a well-defined group ofGram-positive, 'mycelial', endospore-forming, endoparasi-tic prokaryotes with worldwide distribution, which arefound in many different groups of soil-inhabiting nema-todes and freshwater cladocerans (Prestonet al., 2003).
Paraliobacillus	Spore	Spore	Unclassified	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1158996&lvl=3&lin=f&keep=1&srchmode=1&unlock	A Gram-positive, moderately halophilic, endospore-forming, catalase- and oxidase-positive, obligately aerobic bacterium, designated strain YIM-C158T, was isolated from sediment of a salt lake in the Qaidam Basin, north-west China. Other salin soils.
Sedimentibacter	Spore	Total	Uncultured	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=304766&lvl=3&lin=f&keep=1&srchmode=1&unlock	cells were strict anaerobic, motile, Gram-negative, sporulating, straight, or slightly curved rods.
Salisediminibacterium	Spore	Spore	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1464123&lvl=3&lin=f&keep=1&srchmode=1&unlock	Gram stain-positive, rod-shaped, non-motile, orange-pigmented and non-endospore-forming novel bacterial strain 10nlg(T) was isolated from Lonar soda lake in India.
Salipaludibacillus	Spore	Spore	Unclassified	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1601833&lvl=3&lin=f&keep=1&srchmode=1&unlock	Two novel (S9T and S12) Gram-stain-positive, rod shaped, non-motile and endospore forming bacteria were isolated from Narayan Sarovar lake, in India.
Ruminococcus 1 &2	Spore	Spore	Uncultured	/	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1263&lvl=3&lin=f&keep=1&srchmode=1&unlock	unidentified Gram-positive, anaerobic, non-sporulating coccobacillus-shaped bacterium isolated from human faeces. The novel organisms were catalase-negative, indole-negative and produced acetate and succinate as end products of metabolism. Little info https://doi.org/10.1099/ijs.0.65208-0
Ruminococcaceae	Spore	Spore/Mat	Unclassified	/		Ruminococcaceae are obligate anaerobes. the family have diverse shapes, with some rod-shaped and others cocci.[8]
Ruminiclostridium 5	0	Spore	Unclassified			
Ruminiclostridium 1	Spore	Spore	Uncultured			
Ruminiclostridium	Spore	Spore	Uncultured	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=394503&lvl=3&lin=f&keep=1&srchmode=1&unlock	Species of the genus Ruminiclostridium are strictly anaerobic, spore forming, straight or slightly curved rods, and motile. Optimum growth normally occurs under mesophilic temperatures (28–45°C) and around neutral pH.
Romboutsia	Spore	Spore	Uncultured	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1121325&lvl=3&lin=f&keep=1&srchmode=1&unlock	A Gram-stain-positive, spore-forming, obligately anaerobic bacterium, designated LAM201(T), was isolated from sediment samples from an alkaline-saline lake located in Daqing oilfield, Daqing City, PR China.
Robinsoniella	Spore	0	Unclassified	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=180332&lvl=3&lin=f&keep=1&srchmode=1&unlock	A polyphasic taxonomic study was performed on six strains of an unknown Gram-positive, non-motile, spore-forming, short oval to rod-shaped bacterium isolated from a swine-manure storage pit.
Pullulanibacillus	0	Spore	Unclassified	yes		Two Gram-positive-staining, rod-shaped, endospore-forming isolates (UG-2(T) and UG-3), with an optimum growth temperature of around 37 °C and an optimum pH for growth of about 4, were recovered from an acidic effluent of the uranium mill tailing at Urgeiriça in Central Portugal.
Psychrobacillus	Spore	Spore	Unclassifird	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=2283160&lvl=3&lin=f&keep=1&srchmode=1&unlock	aerobic, psychrotolerant, Gram-stain-positive, endospore-forming strain, NHI-2(T), was isolated from oil-contaminated soil near a gas station in Mongolia. This strain was characterized by motile rods and grew over a wide range of temperatures (-2 to 40 °C) with optimal growth at 28-30 °C.

Staphylococcus	Spore	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1418406&lvl=3&lin=f&keep=1&srchmode=1&unlock	Staphylococci are microbiologically characterized as gram-positive (in young cultures), non-spore-forming, nonmotile, facultative anaerobes (not requiring oxygen).
Sporosarcina	Spore	Spore	Unclassified	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=582850&lvl=3&lin=f&keep=1&srchmode=1&unlock	Sporosarcina ureae is a type of bacteria of the genus Sporosarcina, and is closely related to the genus Bacillus. S. ureae is an aerobic, motile, spore-forming, Gram-positive coccus
Sporosalibacterium	Spore	Spore	Uncultured	yes		Gram-positive and were motile, straight and spore-forming. Strain SOL3f37(T) had a typical Gram-positive-type cell-wall structure, unlike the thick, multilayered cell wall of its closest relative Clostridiisalibacter paucivorans
Sporomusa	Spore	0	Unclassified	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1123287&lvl=3&lin=f&keep=1&srchmode=1&unlock	A new genus of strictly anaerobic, gram-negative, banana-shaped bacteria is described. Cells formed spores and were motile by means of up to 15 laterally inserted flagella.
Sporobacter	Spore	Spore	Uncultured	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1123282&lvl=3&lin=f&keep=1&srchmode=1&unlock	This organism was a slightly curved spore-forming rod-shaped bacterium. It had a gram-positive-type cell wall and was obligately anaerobic.
Sporacetigenium	0	Spore	Unclassified	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1123280&lvl=3&lin=f&keep=1&srchmode=1&unlock	The strains were Gram-positive, spore-forming, motile rods (0.9-1.0 x 3.6-7.3 microm). Growth of the strains was observed at 20-42 degrees C and pH 6.0-9.5.
Solibacillus	0	Spore	Unclassified	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1224748&lvl=3&lin=f&keep=1&srchmode=1&unlock	Gram-stain-positive, rod-shaped, endospore-forming, aerobic bacterial strain,
Soehngenia	0	Total	Unclassified	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1123260&lvl=3&lin=f&keep=1&srchmode=1&unlock	Gram-stain-positive. In the early exponential phase of growth, cells are slightly motile by means of peritrichous flagella (Figure 2); older cells lose their motility. Colonies are rhizoid, resemble a snowflake, dark, and creamy. Rare terminal and subterminal spore formation. Mesophilic. Anaerobic, but aerotolerant. Anaerobic sludge
Sinobaca	0	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=342944&lvl=3&lin=f&keep=1&srchmode=1&unlock	A Gram-positive, non-spore-forming isolate, designated YIM 70212(T), was isolated from a hypersaline soil sample collected from Qinghai, north-west China. Cells of the isolate were orange-pigmented, motile cocci with multiple flagella. A polyphasic taxonomic investigation was carried out on the isolate. The organism grew at 10-45 degrees C and pH 7.5-11.0, with optimum growth at 28 degrees C and pH 8.0-9.5
Serpentinicella	0	Spore	Unclassified	yes		A novel anaerobic, alkaliphilic, Gram-stain-positive, spore-forming bacterium was isolated from a carbonaceous hydrothermal chimney in Prony Bay, New Caledonia. This bacterium, designated strain 3bT, grew at temperatures from 30 to 43 °C (optimum 37 °C) and at pH between 7.8 and 10.1 (optimum 9.5).
Papillibacter	Spore	0	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1122930&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cells are rod-shaped and stain Gram-positive. Nonmotile. Nonsporulating Strict anaerobic. Grows on a limited number of aromatic compounds and crotonate but not on carbohydrates, organic acids and alcohols.
Paenisporosarcina	Spore	Spore	Unclassified	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=417367&lvl=3&lin=f&keep=1&srchmode=1&unlock	A Gram-stain-positive, aerobic, spore-forming, rod-shaped bacterium, PN2(T), was isolated from a soil sample collected near the Pindari glacier.
Paenibacillus	Spore	Spore	Unclassified Paenibacillus ruminocola	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=417367&lvl=3&lin=f&keep=1&srchmode=1&unlock	The genus Paenibacillus is characterized as rod-shaped Gram-positive or Gram-variable endospore forming aerobic or facultatively anaerobic bacteria.
Oxobacter	Spore	Spore	Uncultured	yes	ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=36849&lvl=3&p=genome&lin=f&keep=1&srchmode=1&unlock	Cells are gram positive and rod shaped. Oval, subterminal to termi- nal spores are produced. Obligately anaerobic.
Oceanobacillus	0	Spore	Unclassified	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1229153&lvl=3&p=genome&lin=f&keep=1&srchmode=1&unlock	Gram-positive, spore-forming rods, motile by means ofperitrichous flagella. Ellipsoidal spores are sub-terminal orterminal within swollen sporangia. Obligately aerobic orfacultatively anaerobic, obligately or facultatively alkaliphi-lic and grow at 0–24 % (w/v) NaCl. Catalase and oxidasereactions are positive. Growth occurs at 5–48uC.
Natronicola	0	Spore	Unclassified	yes		Cells are rod-shaped, flexible, and motile by peritrichous flagella. The cell wall has a Gram-stain-positive structure. Obligately anaerobic. Chemo-organotrophic with fermentative metabolism.
Natranaerovirga	0	Spore	Unclassified Natranaerovirga pectinivora	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=680378&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cells are nonmotile rods with variable length, 0.5–0.6 × 2.5–15 µm. Gram-positive, forming round terminal endospores. Strictly anaerobic fermentative saccharolytic bacterium, utilizing only galacturonic and glucuronic acids, fructose and polygalacturonates.
Lysinibacillus	Spore	Spore	Uncultured	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=2498145&lvl=3&lin=f&keep=1&srchmode=1&unlock	A novel aerobic, alkaliphilic, Gram-staining-positive, endospore-forming bacterium, strain OMN17T, was isolated from a typical sandy loam soil under long-term OMN fertilization (half organic manure N plus half mineral N fertilizer) in northern China and was subjected to a polyphasic taxonomic study. The best growth was achieved at 30 °C and pH 8-10 in medium containing 0.5% (w/v) NaCl.
Lutispora	Spore	Spore	Uncultured	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1122184&lvl=3&lin=f&keep=1&srchmode=1&unlock	Lutispora is an anaerobic, spore-forming, rod-shaped and moderately thermophilic bacterial genus from the family of Clostridiaceae with one known species
Leuconostoc	0	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1229758&lvl=3&lin=f&keep=1&srchmode=1&unlock	Leuconostoc is a non-motile, non-spore forming catalase-negative coccus usually occurring in pairs or chains.

Firmicutes	Lachnospiraceae UCG−004	0	Spore	Unclassified	known in family		Lachnospiraceae are a family of anaerobic, spore-forming bacteria in the order Clostridiales that ferment diverse plant polysaccharides to short-chain fatty acids (butyrate, acetate) and alcohols (ethanol). These bacteria are among the most abundant taxa in the rumen and the human gut microbiota.
	Lachnospiraceae ND3007	Spore	0	Unclassified	known in family		gut microbiota
	Lachnoclostridium 5	Spore	Spore	Unclassified	known in family		Lachnoclostridium is a genus of Gram-positive, obligate anaerobic, spore-forming, motile bacteria. Organisms in this genus can grow in moderate 'mesophilic' as well as in extremely high 'thermophilic' temperatures, ranging from 20°C to 45°C and from 203°C to 633°C, respectively
	Lachnoclostridium 10	Spore	Spore	Uncultured	known in family		
	Lachnoclostridium	Spore	Spore	Uncultured	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1917870&lvl=3&lin=f&keep=1&srchmode=1&unlock	Gram-positive, motile, obligately anaerobic spore-formingrods 0.3–1.5μm×1.5–20μm. Strains are mesophilic orthermophilic (temperature range from 20°C to 63°C) andgrow in neutral to alkaline pH (some up to pH 11).
	Kurthia	0	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1033740&lvl=3&lin=f&keep=1&srchmode=1&unlock	Kurthia huakuii LAM0618T (ACCC 06121T = JCM 19187T), a Gram-positive, motile, non-spore-forming, short-rod-shaped, and facultative anaerobic bacterium isolated from biogas slurry (1), was found to be capable of degrading sulfonylurea herbicides (2), acetochlor, and alkanes and decolorizing triphenylmethane dyes, and it may provide new insights into bioremediation applications.
	Jeotgalibacillus	Spore	Total	Unclassified	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=135826&lvl=3&lin=f&keep=1&srchmode=1&unlock	A moderately halophilic, round-endospore-forming bacterium (strain YKJ-13T) was isolated from jeotgal, a traditional Korean fermented seafood, and studied by a polyphasic taxonomic approach.
	Jeotgalibaca	0	Total	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1868794&lvl=3&lin=f&keep=1&srchmode=1&unlock	novel, Gram-stain-positive, non-spore-forming, facultatively anaerobic bacterium, designated strain H21T32T, was isolated from the faeces of an Oriental stork, Ciconia boyciana
	Hydrogenoanaerobacterium	0	Total	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=474960&lvl=3&lin=f&keep=1&srchmode=1&unlock	anaerobic bacterial strains, designated SW512(T) and W72, were isolated from a laboratory-scale H(2)-producing up-flow anaerobic sludge blanket (UASB) reactor. Cells were Gram-stain-negative, non-motile and 0.3-0.4x2.0-14.5 mum; they did not form spores.
	Hydrogenispora	Spore	Spore	Uncultured	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1082276&lvl=3&lin=f&keep=1&srchmode=1&unlock	An anaerobic, spore-forming, ethanol-hydrogen-coproducing bacterium, designated LX-BT, was isolated from an anaerobic sludge treating herbicide wastewater. Cells of strain LX-BT were non-motile rods (0.3-0.5×3.0-18.0 μm). Spores were terminal with a bulged sporangium. Growth occurred at 20-50 °C (optimum 37-45 °C), pH 5.0-8.0 (optimum pH 6.0-7.7) and 0-2.5% (w/v) NaCl.
	Herbinix	Spore	Spore	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1564487&lvl=3&lin=f&keep=1&srchmode=1&unlock	Phenotypic and phylogenetic studies were performed on new isolates of a novel Gram-stain-positive, anaerobic, non-sporulating, rod-shaped bacterium isolated from a thermophilic biogas plant. The novel organisms were able to degrade crystalline cellulose.
	Halobacillus	Spore	Spore	Unclassified	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=240302&lvl=3&lin=f&keep=1&srchmode=1&unlock	Halobacillus dabanensis sp. nov. and Halobacillus aidingensis sp. nov., isolated from salt lakes in Xinjiang, China: Two moderately halophilic spore-forming bacteria were isolated from salt lakes in the Xinjiang region of China. The two strains, designated AD-6(T) and D-8(T), were aerobic, Gram-positive, rod-shaped and motile by means of peritrichous flagella.
	Halalkalibacillus	0	Spore	Halalkalibacillus halophilus Unclassified	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=2018042&lvl=3&lin=f&keep=1&srchmode=1&unlock	A Gram-stain-positive, rod-shaped (0.3-0.4×1.2-2.0 μm), strictly aerobic and beige-pigmented bacterium, designated B3227T, was isolated from the sediment of a sea cucumber culture pond in Rongcheng, China (122.2° E 36.9° N). Its biochemical characteristics analysis revealed that the cells of this bacterium were catalase-positive and oxidase-negative. Cell growth occurred at 15-45 °C (optimum, 37 °C), pH 6.5-9.0 (pH 7.5-8.0) and in the presence of 0.0-22.0 % (w/v) NaCl (6.0-9.0 % NaCl). Endospore forming
	Gracilibacter	Spore	0	Unclassified	non		A member of the low-G+C (about 43 mol%) Gram-positive subphylum Bacillus–Clostridium. Specific habitat unknown. Anaerobic chemo-organotrophs. No spores observed. The type species is Gracilibacter thermotolerans. Isolated from a sediment sample of a constructed wetland system receiving acid sulfate water(pH 1.6–3.0).
	Gottschalkia	Spore	Spore	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1128398&lvl=3&lin=f&keep=1&srchmode=1&unlock	Gram-stain-positive, obligately anaerobic, straight orslightly curved rods, 0.5–1.52.5–10 μm. Motile by meansof lateral flagella. Growth occurs from 18–19C and up to37–42C. The optimum temperature for growth is 30–37C. The pH range for growth is from 6.5 to 7.0 and up to9.0; the optimum pH for growth is between 7.5 and 8.1.Form spores that are round to oval and terminal or subter-minal.
	Geosporobacter	Spore	0	Unclassified	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1121919&lvl=3&lin=f&keep=1&srchmode=1&unlock	A novel, strictly anaerobic, chemo-organotrophic bacterium, designated strain VNs68(T), was isolated from a well that collected water from a deep aquifer at a depth of 800 m in the Paris Basin, France. Cells were thin, non-motile, Gram-positive rods forming terminal endospores (3.0-5.0 x 0.5 microm).
	Fusicatenibacter	0	Spore	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1150298&lvl=3&lin=f&keep=1&srchmode=1&unlock	Three Gram-stain-positive, obligately anaerobic, non-motile, non-spore-forming, spindle-shaped bacterial strains (HT03-11(T), KO-38 and TT-111), isolated from human faeces were characterized by phenotypic and molecular taxonomic methods.
	Fusibacter	Total	Total	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1150298&lvl=3&lin=f&keep=1&srchmode=1&unlock	Spindle-shaped rods with cells that are 1 × 2–5 μm, and occurring singly or in pairs. Motile by one to four peritrichous flagella. Spores are not formed. Gram-stain-positive. Chemoorganotrophic and obligately anaerobic. Requires yeast extract for growth. Oxidizes a few carbohydrates to butyrate, acetate, CO2, and H2. Uses thiosulfate and sulfur as electron acceptors during glucose fermentation with production of H2S. Mesophilic, neutrophilic, and halotolerant.

Fonticella	Spore	Spore	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1096341&lvl=3&lin=f&keep=1&srchmode=1&unlock	Gram-positive, non-motile, non-spore-forming, thermo-philic rods with fermentative and obligately anaerobicmetabolism. Fonticella tunisiensisgen. nov., sp. nov., isolatedfrom a hot spring.
Fictibacillus	Spore	0	Unclassified	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=2021314&lvl=3&lin=f&keep=1&srchmode=1&unlock	Thephenotypic characteristics corresponding to this genus high-light that these are Gram-stain-positive, motile, rod-shaped,endospore-forming bacteria.
Faecalibacterium	Spore	Spore	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=411483&lvl=3&lin=f&keep=1&srchmode=1&unlock	Faecalibacterium is a genus of bacteria. Its sole known species, Faecalibacterium prausnitzii is gram-positive, mesophilic, rod-shaped,anaerobic and is one of the most abundant and important commensal bacteria of the human gut microbiota. It is non-spore forming and non-motile.
Exiguobacterium	Total	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1255571&lvl=3&lin=f&keep=1&srchmode=1&unlock	Exiguobacterium are Gram-positive, rod-shaped, nonspore-forming, and motile bacteria. The cell morphology ranges from ovoid, rods, double rods, or chain of cells depending on species, strain, and environmental conditions (Vishnivetskaya et al., 2009). Colonies of the strain are yellow to orange pigmented, 1–1.5 mm in diameter, circular, and smooth
Eubacterium hallii group	0	Spore	Uncultured	/		
Eubacterium coprostanoligenes	Total	Total	Uncultured	non		Eu- bacterium coprostanoligenes (co. pro .stan. 01. i ' gen . es. L. adj . coprostanoligenes, producing coprostanol) . The cocco- bacilloid cells are 0.5 to 0.7 km in diameter and 0.7 to 1.0 pm long and occur singly and in pairs. The cells are nonmotile, gram positive, and non-spore forming
Eubacterium brachy	0	Total	Uncultured	non		Strains of this species are obligately anaerobic, nonmotile, non-sporeforming, short, gram-positive rods that oc- cur predominantly in long chains (Fig. 1B)
Erysipelothrix	0	Total	Uncultured	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1121873&lvl=3&lin=f&keep=1&srchmode=1&unlock	Erysipelothrix, including cell morphology, Gram-staining, motility, spore formation, growth ranges(temperature, pH, NaCl) and optimum culture condi-tions
Ercella	Total	Spore	Uncultured	non		A novel anaerobic succinate-producing bacterium, strain ZWB(T), was isolated from sludge collected from a biogas desulfurization bioreactor (Eerbeek, the Netherlands). Cells were non-spore-forming, motile, slightly curved rods (0.4-0.5 µm in diameter and 2-3 µm in length), and stained Gram-negative.
Epulopiscium	Spore	Spore	Uncultured	similar		Epulopiscium spp. are a group of Gram-positive bacteria that have a symbiotic relationship with surgeonfish. These bacteria are known for their unusually large size, many ranging from 200–700 µm in length. Until the discovery of Thiomargarita namibiensis in 1999, Epulopiscium spp. were thought to be the largest bacteria. They are still the largest known heterotrophic bacteria. The largest Epulopiscium morphologies exhibit a unique viviparous reproduction. This unusual and derived form of sporulation produces anywhere from one to twelve daughter cells that grow inside of the parent cell, until the parent eventually lyses, and dies.
Enterococcus	0	Spore	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1274384&lvl=3&lin=f&keep=1&srchmode=1&unlock	The core morphological and physiological features of all enterococci include being Gram-positive spherical or ovoid cells arranged in pairs or chains (Thiercelin, 1899). They are non-spore-forming facultative anaerobes and obligatory fermentative chemoorganotrophs. They typically have an optimum growth temperature of 35°C and a growth range from 10 to 45°C (Sherman, 1937). They are normal inhabitants of the intestinal tract of man and other animals and often are used in microbiology as indicators of fecal contamination; some species are pathogens. The ability of enterococci to grow in the presence of salt (6.5% NaCl) is one of the distinguishing characteristics of the genus
Dorea	0	Spore	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1211818&lvl=3&lin=f&keep=1&srchmode=1&unlock	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1211818&lvl=3&lin=f&keep=1&srchmode=1&unlock
Domibacillus	0	0	Unclassified	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=29332&lvl=3&lin=f&keep=1&srchmode=1&unlock	Members of theDomibacillusare Gram-stain-positive, spore-forming and strictly aerobicrods. Isolated from pharmaceutical clean room, marinesediments and soil samples
Dialister	0	Spore	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=888062&lvl=3&lin=f&keep=1&srchmode=1&unlock	Dialister is the genus classified within Veillonellaceae family in Firmicutes phylum. Although it is in Gram positive phylum, it has Gram negative cell wall. It is nonmotile, nonspore forming, nonfermentative small coccobacilli shaped cells. Dialister are obligatory anaerobic or microaerophilic bacteria.
Dethiosulfatibacter	Total	Total	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1121476&lvl=3&lin=f&keep=1&srchmode=1&unlock	A sulfate-reducing enrichment culture originating from coastal marine sediment of the eutrophicTokyo Bay, Japan, was successfully established with Casamino acids as a substrate. A thiosulfatereducer, strain C/G2T, was isolated from the enrichment culture after further enrichment withglutamate. Cells of strain C/G2Twere non-motile rods (0.6–0.8mm¾2.2–4.8mm) and werefound singly or in pairs and sometimes in short chains. Spores were not formed. Cells of strainC/G2Tstained Gram-negatively, despite possessing Gram-positive cell walls.
Dethiobacter	Spore	Spore	Uncultured		https://www.ncbi.nlm.nih.gov/Tax	Dethiobacter alkaliphilussp. nov. Cells are Gram-positive terminal

Desulfuribacillus	0	Spore	Unclassified	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1390249&lvl=3&lin=f&keep=1&srchmode=1&unlock	An anaerobic enrichment culture inoculated with a sample of sediments from soda lakes of the Kulunda Steppe with elemental sulfur as electron acceptor and formate as electron donor at pH 10 and moderate salinity inoculated with sediments from soda lakes in Kulunda Steppe (Altai, Russia) resulted in the domination of a Gram-positive, spore-forming bacterium strain AHT28.
Desulfosporosinus	Spore	Spore	Uncultured	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=476652&lvl=3&lin=f&keep=1&srchmode=1&unlock	Three strains of sulfate-reducing bacteria (M1(T), D, and E) were isolated from acidic sediments (White river and Tinto river) and characterized phylogenetically and physiologically. All three strains were obligately anaerobic, mesophilic, spore-forming straight rods, stained Gram-negative and displayed variable motility during active growth. The pH range for growth was 3.8-7.0, with an optimum at pH 5.5. The temperature range for growth was 15-40 °C, with an optimum at 30 °C.
Desulfitibacter	0	Spore	Uncultured	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1304884&lvl=3&lin=f&keep=1&srchmode=1&unlock	A novel alkalitolerant, anaerobic bacterium, designated strain sk.kt5(T), was isolated from a metal coupon retrieved from a corrosion-monitoring reactor of a Danish district heating plant (Skanderborg, Jutland). The cells of strain sk.kt5(T) were motile, rod-shaped (0.4-0.6 x 2.5-9.6 microm), stained Gram-positive and formed endospores. Strain sk.kt5(T) grew at pH 7.6-10.5 (with optimum growth at pH 8.0-9.5), at temperatures in the range 23-44 degrees C (with optimum growth at 35-37 degrees C), at NaCl concentrations in the range 0-5 % (w/v) (with optimum growth at 0-0.5 %) and required yeast extract for growth.
Dendrosporobacter	Spore	0	Uncultured	yes		Cells are straight, motile rods with a Gram-negativecell wall. Endospores are formed. `Lipid F ', withocta- and nonaprenologues (ratio approx. 1 : 1) as thepredominant isoprenologues, is present, but no cyto-chromes.
Dehalobacter	Spore	0	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=871738&lvl=3&lin=f&keep=1&srchmode=1&unlock	Dehalobacter restrictus(sp. nov.) re.stric'tus. L.adj. re-strictus, limited, confined (referring to the limited sub-strate range utilized). Rod-shaped, motile cells with ta-pered ends, 0.3–0.5 ×2–3 µm. Cells appear singly or inpairs. No spores formed. Cells stain gram-negative.
Defluviitaleaceae UCG–011	Spore	Total	Uncultured	/		
Cryptanaerobacter	0	Total	Unclassified	non		Gram-positive, anaerobic bacteria in the form of shortrods; electron-
Crassaminicella	0	Spore	Unclassified	yes		Crassaminicella profunda gen. nov., sp. nov.. A novel, anaerobic,
Coprococcus 2	0	Spore	Unclassified	unknown		human microbiota
Cohnella	Spore	Spore	Unclassified	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=2507935&lvl=3&lin=f&keep=1&srchmode=1&unlock	A strictly aerobic, motile, endospore-forming, rod-shaped bacterium, designated HS21T, was isolated from rhizospheric soil of the Korean fir tree (Abies koreana) from Halla mountain on Jeju island, Korea. Growth of strain HS21T was observed at pH 6.0–8.0 (optimum: pH 7.0), 0–2% (w/v) NaCl and 4–30°C (optimum: 25°C).
Clostridium sensu stricto 9	Spore	Total	Uncultured			The species of Clostridium comprise a very heterogeneous assemblage of bacteria that do not form a phylogenetically coherent group.
Clostridium sensu stricto 8	Spore	Spore	Uncultured			
Clostridium sensu stricto 7	Spore	Total	Unclassified			
Clostridium sensu stricto 6	Spore	0	Unclassified			
Clostridium sensu stricto 5	0	Spore	Unclassified			
Clostridium sensu stricto 3	Spore	Total	Unclassified			
Clostridium sensu stricto 2	Spore	0	Unclassified			
Clostridium sensu stricto 13	Spore	Spore	Uncultured			
Clostridium sensu stricto 12	Spore	Total	Unclassified			
Clostridium sensu stricto 10	0	Total	Unclassified			
Clostridium sensu stricto 1	Spore	Spore	Uncultured			
Clostridium sensu stricto	0	Spore	Unclassified			
Clostridiisalibacter	Spore	0	Unclassified	yes	https://www.ncbi.nlm.nih.gov/Tax	A moderately halophilic, strictly anaerobic bacterium, designated
Chungangia	Spore	Spore	Unclassified	non		A Gram-staining-positive, strictly aerobic, non-spore-forming, rod-
Christensenellaceae R–7 group	Total	Spore	Unclassified	/		
Cellulosilyticum	0	Spore	Unclassified	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1125704&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cellulosilyticum lentocellum DSM 5427 is an anaerobic, endospore-forming member of the Firmicutes. We describe the complete genome sequence of this cellulose-degrading bacterium, which was originally isolated from estuarine sediment of a river that received both domestic and paper mill waste. Comparative genomics of cellulolytic clostridia will provide insight into factors that influence degradation rates.
Catellicoccus	Total	0	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1234409&lvl=3&lin=f&keep=1&srchmode=1&unlock	Catellicoccusgen. nov. Cells are Gram-positive, non-spore-forming and coccoid inshape. Cells are arranged in pairs or chains. Cells are non-motile, facultatively anaerobic and catalase-negative.
Caryophanon	0	Spore	Unclassified		https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=33977&lvl=3&lin=f&keep=1&srchmode=1&unlock	
Caproiciproducens	Spore	Spore	Uncultured	non		Caproiciproducens galactitolivorans BS-1T, a non-spore-forming, anaerobic, Gram-positive bacterium, was isolated from an anaerobic digestion reactor (1). Strain BS-1T is able to produce acetic, butyric, and caproic acid using, e.g., the sugar alcohol d-galactitol as the substrate (2).

Candidatus Soleaferrea	0	Total	Uncultured	/	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1211843&lvl=3&lin=f&keep=1&srchmode=1&unlock	
Candidatus Dichloromethanomonas	Spore	0	Unclassified	spore-like		Candidatus Dichloromethanomonas elyunquensis. Utilizes DCM under anoxic conditions as sole energy source and produces acetate, chloride and biomass. Carbon sources are DCM and CO2. DCM is not reductively dechlorinated to CM and CM does not support growth. No growth occurred with hydrogen and CO2 indicating the organism cannot grow via H2/CO2 reductive acetogenesis. The Wood–Ljungdahl pathway is probably used for CO2 fixation. Cells are rod shaped (4 µm × 0.4 µm) and have spore-like inclusions.
Caminicella	Spore	0	Uncultured	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1121266&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cells are rod-shaped (3±10lm long˙0±5±0±7lmwide), motile by means of peritrichous Øagella, andexhibit a Gram-negative cell wall ultrastructure. Spor-ulation is observed in the late stationary phase ofgrowth. Growth occurs at 45±65∞C (optimum, 55±60∞C), at pH 4±5±8±0 (optimum, pH 7±5±8±0), and atsea salt concentrations of 20±60 g l–"(optimum, 25±30 g sea salt l–").
Caloranaerobacter	Spore	0	Uncultured			
Caldicoprobacter	Spore	Spore	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1170224&lvl=3&lin=f&keep=1&srchmode=1&unlock	hyperthermophilic anaerobic bacterium, designated D2C22(T), was isolated from the hydrothermal hot spring of Guelma in north-east Algeria. The isolate was a Gram-stain-positive, non-sporulating, non-motile rod, appearing singly or in pairs (0.3-0.4 × 8.0-9.0 µm). Strain D2C22(T) grew anaerobically at 45-85 °C (optimum 65 °C), at pH 5-9 (optimum pH 6.8) and with 0-20 g NaCl l(-1).
Brassicibacter	0	Spore	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=745119&lvl=3&lin=f&keep=1&srchmode=1&unlock	A novel mesophilic, strictly anaerobic bacterium, strain BM(T), was isolated from food industry wastewater. The cells were motile, non-spore-forming rods and stained Gram-negative. Growth of strain BM(T) was observed at 16-44 °C (optimum 37 °C) and pH 6.0-9.0 (optimum pH 7.5).
Bhargavaea	Spore	0	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=426756&lvl=3&lin=f&keep=1&srchmode=1&unlock	cells are alkaligenous, Gram-positive-staining, strictlyaerobic, non-motile (non-flagellated), short rods to almostspherical in shape (1.0–1.560.5–0.8mm). Endospores arenot observed on LB agar supplemented with MnSO4.
Bacillus	Spore	Spore	Uncultured Bacillus sp. RS-33	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1053247&lvl=3&lin=f&keep=1&srchmode=1&unlock	Bacillus species are ubiquitous in nature, e.g. in soil. They can occur in extreme environments such as high pH (B. alcalophilus), high temperature (B. thermophilus), and high salt concentrations (B. halodurans). B. thuringiensis produces a toxin that can kill insects and thus has been used as insecticide.
Anoxynatronum	Total	Total	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=489973&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cells are non-motile rods with sharpened ends (0.4–0.72.0–6.5 µm). Multiplication is performed by binary fission. Gram-positive with an S-layer. Moderately aeroto-lerant, catalase-negative and oxidase-positive anaerobe.Obligate alkaliphilic, grows within the range of pH 7.4–11.0with an optimum at 9.6. Mesophilic.
Anaerovorax	Spore	Total	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1120998&lvl=3&lin=f&keep=1&srchmode=1&unlock	Anaerovorax is a Gram-positive, non-sporeforming, strictly anaerobic, chemoorganotrophic bacterial genus from the family of Eubacteriaceae with one known species (Anaerovorax odorimutans)
Anaerostipes	0	Spore	Uncultured	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=411490&lvl=3&lin=f&keep=1&srchmode=1&unlock	Gram-positive-staining, spore-forming, non-motile, anaer-obic long rods (5–15mm) that occur in filaments. OnM2GSC agar, colonies are white, circular, smooth, convex,shiny and 1.5–2.5 mm in diameter after 24 h at 41uC.
Anaerosporomusa	Spore	Spore	Uncultured	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1794912&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cells are slightly curved rods, Gram-stain-negative andmotile. Spore-forming. Mesophilic and neutrophilic.Anaerobic. Limited to growth by fermentation on shortchain carboxylic acids. Unable to reduce sulfate or Fe(III).The cellular fatty acid profile is primarily composed of saturated hydrocarbon chains.
Anaerosporobacter	Spore	0	Unclassified	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1120996&lvl=3&lin=f&keep=1&srchmode=1&unlock	A strictly anaerobic, Gram-positive, endospore-forming bacterium, strain HY-37-4T, was isolated from a forest-soil sample collected in Jeju, Republic of Korea. The cells were motile rods with peritrichous flagella. Strain HY-37-4T fermented various carbohydrates and the end products from glucose were formate, acetate and H2. The major cellular fatty acids were C16 : 0, C16 : 0 3-OH and iso-C17 : 1 I/anteiso B. The G+C content of the DNA was 41 mol%.
Anaerosolibacter	Spore	0	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1417629&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cells are anaerobic, straight rods, motile by means offlagella and Gram-staining-negative. No spores are pro-duced. The major cellular fatty acids are C14 : 0, iso-C15 : 0and C16 : 0. Isolated from contaminated soil Korea
Anaerosinus	0	Spore	Massilibacillus massiliensis	non		Anaerosinus is a Gram-negative, non-spore-forming,
Anaerocolumna	Spore	Spore	Uncultured	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1121297&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cells are Gram-stain-positive. Motile. Strictly anaerobic,fermentative metabolism. Slightly curved to straight rodsoccurring singly or in pairs. Produces terminal, sphericalspores from swollen cells. Optimum temperature forgrowth is 30–35C.
Anaerococcus	0	Spore	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=2742993&lvl=3&lin=f&keep=1&srchmode=1&unlock	An obligate anaerobic, Gram-stain-positive, non-spore forming, non-motile, catalase and oxidase-negative, coccoid-shaped bacterium designated AGMB00486T was isolated from swine faeces. The optimal growth of the isolate occurred at pH 8.0 and 37 °C.
Anaerobacterium	Spore	Spore	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1297424&lvl=3&lin=f&keep=1&srchmode=1&unlock	An obligately anaerobic bacterial strain designated T-1-35(T) was isolated as a dominant cultivable cellulose-degrading bacterium from soil of a Japanese rice field as an anaerobic filter-paper degrader. Cells of strain T-1-35(T) stained Gram-positive and were non-spore-forming rods with rounded ends, 0.8-1.0×3.5-15.0 µm, and motile by means of two to four polar flagella.
Anaerobacillus	Spore	Spore	Uncultured	yes	ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=472963&lvl=3&lin=f&keep=1&srchmode=1&unlock	An anaerobic, spore-forming bacterium (strain Z-0521) was isolated from the iron-reducing microbial community enriched from sample of bottom sediments from low-mineralized soda lake Khadyn, Tuva upper Yenisey region (Russia). Cells of strain Z-0521 are motile straight Gram-positive rods, 0.7–1.1 (µm in diameter and 3.0–7.0 µm length. It is a mesophilic halotolerante obligate alkaliphilic bacterium with a pH range for growth 8.5–10.7

Ammoniphilus	Spore	0	Unclassified	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=66863&lvl=3&lin=f&keep=1&srchmode=1&unlock	Gram-variable, strictly aerobic, rod shaped, haloalkalitolerant spore-forming, obligately oxalotrophic and motile bacterial genus from the family of Paenibacillaceae with peritrichous flagella. In the cell wall of Ammoniphilus is meso-diaminopimelic acid
Allobacillus	Spore	Spore	Unclassified	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1955271&lvl=3&lin=f&keep=1&srchmode=1&unlock	A novel bacterial strain designated B3A(T), isolated from shrimp paste, was investigated by a polyphasic taxonomic approach. Cells stained Gram-positive and were aerobic, non-pigmented, sporulating and rod-shaped with a polar flagellum.
Alkaliphilus	Spore	Spore	Alkaliphilus metalliredigens QYMF Unclassified	yes	ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=185693&lvl=3&lin=f&keep=1&srchmode=1&unlock	isolated from methanogenic butyrate-oxidizing mixed cultures. The cells were straight to slightly curved, gram-positive rods that were motile by means of multiple flagella and formed endospores. Growth was observed in the temperature range 15-45 degrees C (optimum 37 degrees C) and pH range 5.5-9.0 (optimum pH 7.5).
Alkalicoccus	0	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1121087&lvl=3&lin=f&keep=1&srchmode=1&unlock	Gram-stain-positive, cocci-shaped, non-spore-forming and moderately halophilic bacterium, designed BZ-SZ-XJ29T, was isolated from a salt lake of China. On the basis of 16S rRNA gene sequence similarity, the closest phylogenetic relatives were Bacillus saliphilus 6AGT (97.3 % 16S rRNA gene sequence similarity) and five other species of the genus Bacillus(95.4–96.3 %). However, strain BZ-SZ-XJ29T shared only 89.5 % 16S rRNA gene sequence similarity with Bacillus subtilis subsp. subtilis DSM 10T, indicating that this isolate might not be a member of the genus Bacillus.
Alkalibacterium	Spore	Spore	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1130080&lvl=3&lin=f&keep=1&srchmode=1&unlock	Alkalibacterium thalassium sp. nov., Alkalibacterium pelagium sp. nov., Alkalibacterium putridalgicola sp. nov. and Alkalibacterium kapii sp. nov. The isolates are Gram-positive and non-sporulating. They have motility with peritrichous flagella depending on the strains. They lack catalase and quinones. Under anaerobic conditions, lactate yields were 64-93 % of the glucose consumed; residual products were formate, acetate and ethanol with a molar ratio of approximately 2 : 1 : 1. The pH of the fermentation medium markedly affected the product composition; at higher pH, the yield of lactate decreased (15-48 % at pH 9.0) and yields of other products increased, retaining the molar ratio.
Alkalibacte	Spore	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1120975&lvl=3&lin=f&keep=1&srchmode=1&unlock	A. saccharofermentansare Gram-positive and rod-shaped cells,0.5·1.5–2.5lm, with pointed ends; they form singly orin pairs, or rarely, form short chains. They are nonmo-tile cells divided by a septum. Spores are not observed.The cells are thermosensitive; there is no growth afterheating for 10 min at 80°C.
Agathobacter	0	Spore	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1712665&lvl=3&lin=f&keep=1&srchmode=1&unlock	Three strains of a butyrate-producing bacterium were isolated from the rumen contents of grazing sheep and cows. The strains were anaerobic, with Gram-positive cell walls, straight-to-slightly-curved, rod-shaped, non-spore-forming and single flagellate. C14 : 1, C14 : 0, C16 : 0 and C16 : 1 were the predominant fatty acids.
Acidaminobacter	Total	Total	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1120920&lvl=3&lin=f&keep=1&srchmode=1&unlock	Rod-shaped bacteria with pointed ends; Gram-stain-negative. Endospores not formed. Chemo-organotrophic fermentative metabolism. Strictly anaerobic. Amino acids are major energy sources. Acetate is the major fermentation product. Lactate, succinate, butyrate, and ethanol not formed. Growth on many substrates stimulated by or dependent on the presence of hydrogen-utilizing bacteria or archaea. Mesophilic.
Acetobacterium	0	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=52689&lvl=3&lin=f&keep=1&srchmode=1&unlock	Gram-positive, non-sporeforming rods. The isolate utilized H(2)/CO(2), CO, fructose, glucose, ethanol, ethylene glycol, glycerol, pyruvate, lactate, betaine and the methyl-groups of several methoxylated benzoic derivatives such as syringate, trimethoxybenzoate and vallinate. The optimum temperature for growth was 29 degrees C, whilst slow growth occurred at 2 degrees C.
Acetoanaerobium	0	Total	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1482736&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cells are nonsporeforming rods. Cells stain Gram-negative but have an atypical Gram-negative wall structure. Obligate anaerobe. Chemo-organotrophic. Ferment carbohydrates, producing acetate and sometimes other volatile acids. Ferment yeast extract, producing acetate and several volatile acids. At slower growth rates produce acetate from H2 and CO2. May require yeast extract for growth
Acetivibrio	Spore	Spore	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=290052&lvl=3&lin=f&keep=1&srchmode=1&unlock	A. ethanolgignens were obligately anaerobic, gram-negative, nonsporeforming, motile, slightly curved rods with lateral flagella that emanated from the concave sides of the cells and formed fascicles
Acetanaerobacterium	0	Spore	Unclassified	non		isolated from waste water sludge of the Xinanzhang paper mill, Beijing, China. The strains were Gram-positive, non-spore-forming and motile. Cells were thin rods (0.2-0.4x4.0-8.0 microm). Growth of the strains was observed at 20-42 degrees C and pH 5.0-7.5.
Tetrasphaera	0	Spore	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1193182&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Cells are cocci, occurring singly or in pairs but predomi-nantly as tetrads or clusters, or exhibit morphologicalchange from rod to coccus and V- and T-shaped formations.Aerobic, non-motile, non-endospore-forming and Gram-positive to Gram-variable. Catalase-positive but negative forindole production.
Sva0996 marine group	Spore	Total	uncultured	unknown		marine environments
Streptomyces	Spore	Spore	Unclassified	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1211820&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Found predominantly in soil and decaying vegetation, most streptomycetes produce spores, and are noted for their distinct "earthy" odor that results from production of a volatile metabolite, geosmin.
Serinicoccus	0	Spore	Unclassified	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=265976&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Gram-positive, strictly aerobic, moderately halophilicbacteria. Oxidase-negative, catalase-positive, not acid-fast.No formation of spores. Cells are non-motile cocci.

Rubrobacter	Total	Spore	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=909625&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Rubrobacter forms non-motile pleomorphic (rods and/or cocci) Gram-positive cells. Mesophilic, moderately thermophilic or thermophilic. Catalase and oxidase positive.Aerobic and organotrophic. Nitrate is reduced to nitrite. The peptidoglycan type is A3 with llysine as principal amino acid. Menaquinone 8 (MK-8) is the major respiratory lipoquinone.
Pseudonocardia	0	Total	Unclassified	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1123023&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	The vegetative mycelium varies in thickness(0±3±2±0lm) and in the degree of branching. Somestrains form swollen hyphal segments. Aerial myceliummay or may not be present. The mycelium may or maynot be fragmented ; if fragmented, both types ofmycelium exhibit cell division in different directions. Insome species, the mycelium is covered by an electron-dense outer layer. Spore sizes vary ; the spores arenormally smooth and form chains by acropetal bud-ding or septation from the substrate or aerial my-celium, or else are formed in longitudinal pairs onvegetative hyphae and in longitudinal pairs or singlyon aerial hyphae. Non-motile.
Pseudarthrobacter	0	Spore	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=410837&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	
Ornithinimicrobium	0	Spore	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=125288&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	A Gram-staining-positive, non-spore-forming actinobacterium, strain JC311T, isolated from marine green alga of the genus Ulva was studied to examine its taxonomic position.
Ornithinicoccus	0	Total	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1748220&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Two Gram-positive coccoid, non-motile bacteria with L-ornithine as diagnostic diamino acid of the peptidoglycan and an interpeptide bridge of L-Orn<--Gly(1,2)<--D-Glu were isolated from a sample of garden soil.
Nocardioides	Spore	Spore	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1517589&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	A short-rod-shaped, non-spore-forming endophytic actinobacterium, was isolated from a surface-sterilized leaf of Acrostichum aureum in Fangchenggang, Guangxi Zhuang Autonomous Region, China, designated strain CBS4Y-1T and examined by a polyphasic approach to determine its taxonomic position. This actinobacterium was Gram-staining-positive and aerobic.
Nitriliruptor	Spore	Spore	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1069448&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	A novel bacterial strain, designated ANL-iso2(T), was obtained from an enrichment culture inoculated with a mixture of soda lake sediments by using isobutyronitrile (iBN) as the carbon, energy and nitrogen source at pH 10. The enrichment resulted in a stable binary culture containing iBN-degrading Gram-positive rods and a satellite Gram-negative gammaproteobacterium Marinospirillum sp. strain (ANL-isoa) scavenging the products of nitrile hydrolysis. Cells of the iBN-degrading strain, ANL-iso2(T), were short, non-motile, non-spore-forming rods.
Nesterenkonia	0	Spore	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1463631&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	A Gram-staining-positive, aerobic, motile and non-spore-forming actinobacteria, designated strain F10(T), was isolated from a deep-sea sediment of the western Pacific Ocean. Phylogenetic and phenotypic properties of the organism supported that it belonged to the genus Nesterenkonia.
Mycobacterium	0	Spore	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1463631&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	A Gram-stain-positive, non-endospore-forming, haloalkaliphilic actinobacterium, strain CK5T, was isolated from a soil sample, collected at Cape King (Antarctica), and its taxonomic position was investigated by using a polyphasic approach. Cells were cocci with orange pigmentation, non-motile and grew optimally at 25 °C and pH 9.0-9.5 in the presence of 2 % (w/v) NaCl.
Mycetocola	0	Spore	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=76637&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	The taxonomic positions of 10 tolaasin-detoxifying bacteria, which were isolated from the cultivated mushroom Pleurotus ostreatus, were investigated. These strains are Gram-positive, obligately aerobic, non-sporulating and irregular rod-shaped bacteria.
Modestobacter	Spore	0	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=2213158&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Gram-positive, non-spore-forming. Short rods or cocci with a tendency to remain aggregated and form short, multiseptate filaments. Aerobic heterotrophs able to grow in oligotrophic medium.
Micromonospora	Spore	Spore	Unclassified	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=2203715&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Micromonospora species have long been recognized as important sources of antibiotics and also for their unusual spores. Micromonospora exhibits a complex life cycle, differentiating into both substrate mycelia and spores, but no aerial mycelia are produced (Vobis, 1992). The species often form indistinct colonies that can be confused with other actinomycetes.
Microbacterium	Spore	Spore	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=2615184&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	The genus Microbacterium belongs to the family Microbacteriaceae within the surborder Micrococcineae. Microbacterium are Gram-positive, nonspore-forming, rod-shaped bacteria. Members of the genus Microbacterium can be isolated from a wide range of environments including soil, insects, human clinical specimens, marine environments, plants, dairy products, etc.

Actinobacteria	Marmoricola	0	Spore	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=397278&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	yellow-coloured, marine actinobacterium, designated SST-45(T), was isolated from sandy sediment under the surface of a beach and taxonomically characterized by physiological, chemotaxonomic and phylogenetic methods. The cells of the isolate were Gram-positive, aerobic, non-sporulating, non-motile, spherical cells that occurred singly, in pairs, in clusters or as short chains.
	Marisediminicola	0	Spore	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=2711233&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Cells are Gram-positive, motile, short rods. Neither substratemycelium nor aerial mycelium is formed. Catalase isproduced but oxidase is not produced. The cell-wallpeptidoglycan belongs to the B2bgroup with ornithine asdiamino acid. MK-10 is the major menaquinone. The polarlipids are diphosphatidylglycerol, phosphatidylglycerol andsome glycolipids. The main cellular fatty acids are anteiso-C15: 0,iso-C16 : 0and anteiso-C17: 0.TheG+C content of theDNA is approximately 67 mol%. The type species is Marisediminicola antarctica
	Luedemannella	Total	Total	Unclassified	yes		Three actinomycete strains were isolated from soil samples collected in Bangladesh. The cul-tures formed spherical sporangia on short sporangiophores directly above the surface of thesubstrate mycelium. The sporangia developed singly or in clusters and each sporangium con-tained several nonmotile spherical to oval spores with a smooth surface.
	Kocuria	0	Spore	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1461025&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Members of Kocuriaare Gram-positive, aerobic, non-encapsulated, non-halophilic, non-endospore-forming cocci. Aquatique env. Some high salinity.
	IMCC26207	Total	spore	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=1641811	ulture reached the stationary phase after 2 weeks of incubation with a maximum cell density of approximately 8 × 105 cells/ml. Cells of strain IMCC26207 looked pure in epifluorescence microscopy and transmission electron microscopy, showing a coccus-shaped morphology with diameters of 0.4–0.5 µm, dividing by binary fission
	Ilumatobacter	Spore	Spore	Uncultured		https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1331058&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Cells are Gram-positive, aerobic, non-motile. Cells are rod-shape. The cell-wall peptidoglycan contains LL-DAP, glycine, alanine and hydroxyglutamate (molar ratio, ca. 1.4 : 2.4 : 1.1 : 1.0). Sediments
	Iamia	Spore	Spore	Uncultured	non		Cells are Gram-positive, non-endospore-forming rods thatare non-motile. Colonies grow aerobically, but notanaerobically. In media devoid of sodium chloride, growthis observed. Oxidase- and catalase-positive. Isolated from sea cucumber Holothuria edulis
	Georgenia	Total	Spore	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=154116&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cells exhibit a rod±coccus cycle. Rods and cocci occursingly or in small clusters. Cocci are 1lm in dia-meter, rods are 2lm in length and 1lm in width.Gram-positive, non-sporulating, non-motile. Growthoccurs under both aerobic and anaerobic conditions.Oxidase-and catalase-positive. Isolated from medieval wallpaintings, forest soil, activated sludge, salt lake, deep-sea sediment, desert or from enrichment culture
	Gaiella	Total	Spore	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1002870&lvl=3&lin=f&keep=1&srchmode=1&unlock	Gaiella occulta is a nonpigmented, Gram-negative staining, nonmotile, aerobic, and chemoorganotrophic, with an optimal growth tempera-ture of 35–37°C, optimum pH for growth between 6.5 and 7.5, and was isolated from a 150 meter deep water aquifer in Portugal (Albuquerqueet al., 2011; Foesel et al., 2016). The strain forms short rod-shaped cells 1.0–3.0 µm in length by 0.3–0.5 µm in wid
	Frankia	0	Spore	Unclassified	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1836972&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Frankiais one of the few bacterial genera to havedeveloped a symbiosis with plants that extends therange of soil conditions suitable for growth. The nitrogen fixing actinorhizal symbiosis allows 24 generaof plants belonging to eight dicotyledonous plantfamilies to colonize soils that are poor in nitrogen, e.g.sandy beaches, mine spoils, burned forests or glacialmoraines (Benson & Silvester, 1993). tive cells. Frankia spores exhibited low levels of endogenous respiration that were at least ten-fold lower than the endogenous respiration rate of
	Fodinicola	Spore	0	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=2681555&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Gram-positive, aerobic, non-motile, catalase-positive, oxi-dase-negative actinomycetes that form branched substratemycelium and sparse to abundant, white aerial mycelium.The aerial hyphae break up into irregular rod-likeelements.
	Euzebya	Total	Total	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1608957&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Gram-stain-positive, aerobic, chemo-organotrophic, rod-shaped, non-spore-forming strain, which produced convex, circular, pink-pigmented colonies, designated as DY32-46T, was isolated from seawater collected from the Pacific Ocean. Some strains are also isolated from surface seawater of the East China Sea
	Egicoccus	Spore	Spore	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1670830&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Cellare non-endospore-forming cocci witha diam-eter of 0.5–0.8mm. Colonies are dry, rough and circular whencultured on marine agar 2216E at 30uC for 14 days. Coloniesare light yellow. Growth occurs at 20–40uC(optimumat30uC), at pH 6.0–10.0 (optimum at pH 8.0–9.0) and with0–9 % (w/v) NaCl (optimum at 3–5 %) on marine 2216E. Isolated from saline-alkaline soil

	Dietzia	0	Spore	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=37914&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Dietziae are aerobic, Gram-positive, non-acid-alcohol fast, nonsporing, catalase-positive actinomycetes that form cocci that germinate into short rods or rod-shaped cells, which exhibit snapping division and produce V-shaped forms. Circular, raised or convex, glistening, orange to coral red colonies with entire edges are formed on agar media
	Demequina	Spore	Spore	Unclassified	non	https://www.ncbi.nlm.nih.gov/Tax	Gram-positive and are non-motile, non-spore-forming rods
	Cutibacterium	Spore	Spore	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1249618&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	skin bacteria
	Cryobacterium	0	Spore	Unclassified	non	ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=670052&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Pleomorphic nonmotile rod. Non endospore forming. Branch- ing occurs in the early growth phase. Rod forms occur in old culture. Occasionally gram variable in the old culture. Grows optimally at 9 to 12°C and not at 18°C. Aerobic.
	Corynebacterium	0	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1716&lvl=3&lin=f&keep=1&srchmode=1&unlock	Genus of bacteria that are Gram-positive and most are aerobic. They are bacilli (rod-shaped), and in some phases of life they are, more specifically, club-shaped, which inspired the genus name. Animal microbiota
	Conexibacter	Spore	Spore	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=912552&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cells are small rods (0·6–0·7×0·9–1·2 μm), occurring singly or in pairs. Gram-positive and non-sporulating. Motile by long, peritrichous flagella. Aerobic. Catalase- and oxidase-positive.
	Collinsella	0	Spore	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=102106&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cells occur in chains of coccid cells. Gram-positive and obligately anaerobic. Spores and flagella are absent. Fermentation products of glucose are H,, ethanol, formate and lactate.
	CL500–29 marine group	0	Spore	Uncultured	/		
	Cellulomonas	0	Spore	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=2071633&lvl=3&lin=f&keep=1&srchmode=1&unlock	In young cultures, slender, irregular rods ~~0.3–0.7 μm × ~1.0–4.0 μm or more which may be straight, angular, or slightly curved; some of the rods are arranged at an angle to each other giving V formations. Occasional cells may show primary branching. A mycelium is produced by one species. Cultures a week or more old usually contain mainly short rods, but a small proportion of the cells may be coccoid. Nonsporeforming.
	Blastococcus	Spore	Total	Uncultured	non	ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=38502&lvl=3&lin=f&keep=1&srchmode=1&unlock	Gram-positive. Coccoid units, often reproducing by bud-ding and multiple fission, giving rise to a variety of cellforms and aggregates. Single cells may be motile rods andvibrioid or non-motile cocci that tend to form aggregates.
	Bifidobacterium	Spore	Spore	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1678&lvl=3&lin=f&keep=1&srchmode=1&unlock	bifidobacteria are Gram-positive, non-motile, non-spore forming, anaerobic, saccharolytic microorganisms with a Y-shaped or 'bifid' morphology, and a high G + C DNA content [18].
	Arthrobacter	0	Spore	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1077972&lvl=3&lin=f&keep=1&srchmode=1&unlock	Arthrobacter is a genus of obligate aerobes bacteria characterized by a rod–coccus growth cycle. Arthrobacter spp. commonly are found in soils, aerial surface of plants, and wastewater sediments; they do not form endospores and are highly proteolytic.
	Aquipuribacter	0	Spore	Unclassified	non	/	Cells exhibit a rod–coccus cycle, are aerobic and non-endospore-
	Adlercreutzia	0	Spore	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1235794&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cells are Gram-positive, obligately anaerobic, non-spore-forming, non-motile coccobacilli, 0.6–0.761.5–2.7mm.Colonies on BL agar plates are 1–2 mm in diameter, greyto off-white–grey, circular, entire, slightly convex andsmooth. Asaccharolytic. No metabolic end product isdetected in peptone-yeast extract medium supplementedwith glucose.
	Actinotalea	Spore	Spore	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=458839&lvl=3&lin=f&keep=1&srchmode=1&unlock	Gram-positive, strictly aerobic and slightly halophilic.Oxidase-negative, catalase-positive and acid-fast-negative.Do not form spores. Cells are non-motile rods with roundends.
Tenericutes	Haloplasma	Spore	Spore	Inordinaticella fortuita	/		Haloplasma contractilewas isolated from Shaban Deep , oneof the most extreme environments on Earth . Tenericutes have been detected in the gut and gonad microbiomes of fish, sea star, oysters and mussel
	Acholeplasma	Spore	Total	Uncultured	/		wall-less bacteria in the Mollicutes class. They include saprotrophic or pathogenic species
Halanaerobiaeota	Orenia	/spore	0	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=926561&lvl=3&lin=f&keep=1&srchmode=1&unlock	Halophilic anaerobic bacterium from Lake Sivash lagoons
	Halanaerobium	/total	Total	Uncultured	non	ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=54121&lvl=3&lin=f&keep=1&srchmode=1&unlock	Fermented products
	Halanaerobacter	/spore	0	Uncultured	non	ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=706427&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cells are Gram-negative, colourless, non-sporulating rods, motile by peritrichous flagella ; the cells are long, flexible, 0-34.4 pm width and 5-8 pm length, in young cultures ; short degenerate cells and spheroplasts in old cultures.
	Unidentified	Spore	/	Uncultured	/	/	
Aegiribacteria	Waddlia	Spore	Total	Unclassified	similar	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=765953&lvl=3&lin=f&keep=1&srchmode=1&unlock	Chlamydiae are evolutionarily well-separated bacteria that live exclusively within eukaryotic host cells. They include important human pathogens such as Chlamydia trachomatis as well as symbionts of protozoa. As these bacteria are experimentally challenging and genetically intractable, our knowledge about them is still limited.
iae	Neochlamydia	0	Total	Unclassified	similar		
	Chlamydiales CRIB32	0	Total	Unclassified	similar		
	Unidentified	Spore	Spore	Unclassified	similar		

Chlamyd	Unidentified	Spore	/total	Unclassified	/		Members of the bacterial candidate phylum WPS-2 (or Eremiobacterota) are abundant in several dry, bare soil environments. In a bare soil deposited by an extinct iron-sulfur spring, we found that WPS-2 comprised up to 24% of the bacterial community and up to 108 cells per g of soil based on 16S rRNA gene sequencing and quantification.
Candidatus Eremiobacterot a	Unidentified	Spore	Spore	Unclassified	/		Unculture bacteria
Elusimicrobia	Crenothrix	/	Spore	Uncultured	/	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=360316&lvl=3&lin=f&keep=1&srchmode=1&unlock	The phylum Elusimicrobia, previously known as "Termite Group 1", has been shown to be widespread in different ecosystems like marine environment, sewage sludge, contaminated sites and soils, and toxic wastes. The high abundance of 'Elusimicrobia' representatives is only evidenced for the lineage of symbionts found in termites and ants. Crenothrix polyspora is a genus of filamentous bacteria which utilize iron in their metabolism, and cause staining, plugging and taste and odor problems in water systems.
	Xuhuaishuia	0	Total	Unclassified	non	/	Gram-negative, non-spore-forming bacteria. Dividing bybinary fission. Type strain Brevirhabdus pacifica, isolatedfrom deep-sea sediment in a hydrothermal ventfield. Does not require naturalseawater or artificial sea-salts for growth. The pH and tem-perature ranges for growth are pH 6.5–8.5 and 10–40uC(optimum pH 7.5 and 30–35uC).
	Woeseia	Total	0	Uncultured	/	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1738655&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Cells are Gram-stain-negative, rods or bent rods, non-motileand facultatively anaerobic. Catalase is weakly positivebut oxidase is negative. Does not grow without salt. Type strain, Woeseia oceani. Isolated from coastal sediment from Xiaoshilsland, Weihai, China. Optimal growth occurred at 28–358C (range 8–428C) and pH 7.0–8.0(range pH 6.0–9.0) with 1–3 % (w/v) NaCl (range 0.5–8 %).
	Wenzhouxiangella	Spore	Spore	Uncultured Unclassified	non	/	Cells are Gram-stain negative, non-spore-forming,non-motile, non-phototrophic, non-alkaliphilic, mod-erate halophilic, obligately aerobic and chemo-heterotrophic rods. Type strain, Wenzhouxiangella marina isolated from the culturebroth of a marine microalga,Picochlorumasp. 122
	Variovorax	0	Spore	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=34072&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Cells are straight to slightly curved rods that are 0.5 to 0.6 by 1.2 to 3.0 pm and occur singly or in pairs. Motile by means of degenerate peritrichous flagella. Variovorax ginsengisoli is a Gram-negative, non-spore-forming, rod-shaped, motile bacterium from the genus Variovorax, which was isolated from soil from a ginseng field in Pocheon in South Korea.
	Undibacterium	Spore	Spore	Uncultured	non	/	Cells are non-motile, non-spore-forming rods (approx.2mm in length). Gram-negative and oxidase-positive,showing an oxidative metabolism. Type strain, Undibacterium pigrum isolated from drinking water,
	Uliginosibacterium	0	Total	Uncultured Unclassified	non	/	Type strain, Uliginosibacterium gangwonense. yellow-pigmented, Gram-negative, aerobic, isolated from a wetland, Yongneup, of the Inje region, Korea. Temperature range for growth is 4–35uC(optimum, 25–30uC) and pH range for growth is 4.0–8.0(optimum, pH 6.0–7.0).
	UKL13-1	0	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1690484&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Only sequences
	Tropicimonas	Spore	Total	Unclassified Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=441112&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cells are Gram-negative, motile by means of peritrichousflagella and rod-shaped (0.5–1.0mm long and 0.3–0.5mmwide). Obligatory halophiles. Type strain Tropicimonas isoalkanivoransgen isolated from Semarang Port in Indonesia
	Thlothrix	0	Total	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1030&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Cells are rods, 0.7-2.6 pm in diameter and 0-7-5-0 pm in length, that exist in multicellular filaments. Found in sulfide-containing water and in wastewater- treatment systems. Type strain Thiothrix nivea
	Thiorhodovibrio	0	Total	Thiorhodovibrio winogradskyi	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=77007&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Cells vibrioid- to spirilloid-shaped, 1.7 5.0 gm long, 0.7-2.1 gm wide. Anaerobically grown cultures are pink to light pinkish-purple. pH Range: 7.0 7.4. Growth temperature: 14-37 °C, optimum temperature 33 °C. Type species Thiorhodovibrio winogradskyi isolated from saline littoral sediments
	Thiorhodospira	0	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=765914&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Cells are vibrioid- or spiral-shaped, multiply by binary fission and are motile by means of a monopolar flagellar tuft. They are Gram-negative and belong to the y-Proteobacteria.
	Thiomicrospira	Spore	0	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=933&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Cells vary from straight rods with sharp edges tospirilla, 0±4±0±50±8±1±5lm, motile by means of 1±3polar Øagella at one end. Gram-negative. Cell wall isundulating and unstable in low osmotic environmen. The type species is Thioalkalimicrobium aerophilum. obligately alkaliphilic and obligatelychemolithoautotrophic sulfur-oxidizingbacteria from soda lakes
	Thiomicrorhabdus	Spore	Total	Unclassified Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=2039723&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Gram-stain-negative. Cells when grown in liquid media areрod-shaped. Typical cell lengths are 0.8–2.7 µm and diametersare 0.3–0.6 µm, wider thanThiomicrospiraspecies. Do notform endospores or exospores. Type species:Thiomicrorhabdus frisia. Coastal region of north-western Germanyand north-eastern Netherlands, from where the organismwas obtained

Thiohalocapsa	0	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=69359&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Cells nearly spherical to oval, 2 x 2-3 pm in size, may occur in regular platelets of 4-1 6 cells, multiplication by binary fission, non-motile, Gram-negative. Type species is Thiohalocapsa halophila isolated from a red layer in a laminated mat occurring underneath a gypsum crust in the mediterranean salterns of Salin-de-Giraud (Camargue, France)
Thiogranum	Total	0	Uncultured	non	/	Obligately aerobic and chemolithoautotrophic. Gram-stain-negative cells. Grow by the oxidation of reduced sulfurcompounds and the fixation of carbon dioxide. Non-phototrophic. Thetype species isThiogranum longum grows at 19–35uC; optimalgrowth at 32uC. The initial pH for growth is pH 6.0–7.5,with an optimum at pH 6.5. The NaCl concentration forgrowth ranges from 1 to 5 % (w/v), with an optimum at3 %
Thioflexothrix	0	Total	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1570016&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	T. psekupsiiD3 produces immenseamounts of exopolysaccharides (EPSs), mostly consisting of galactose polymers, duringgrowth.
Thiocystis	0	Total	Unclassified Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=61597&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Cells are rod-shaped or ovoid to spherical, before cell division often diplococcus-shaped. Cells occur singly or in pairs, may grow in irregular aggregates sur- rounded by slime. Multiply by binary fission, motile by means of flagella. Gram-negative. Type species is Thiocystis violacea
Thiocapsa	Total	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1058&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Cells are spherical, 1-0-3.0 pm in diameter, diplococci before multiplication by binary fission and are non- motile. Tetrads may be formed after consecutive division in two perpendicular planes. Type species is Thiocapsa roseopersicina. purple sulfur bacteria from the family of Chromatiaceae. It is gram negative and non-motile. The bacterium is either rose colored or milky white based on the growth conditions.
Thiobacillus	Total	Total	Unclassified Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1123393&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Contains autotrophic sulfur-oxidizing bacteria obtainingtheir energy from the oxidation of reduced sulfur oxyanions,namely thiosulfate and tetrathionate plus other sulfur spe-cies such as polythionates, elementary sulfur and/or methyl-ated sulfur compounds. Type species Thiobacillus thioparus isolated from a fild in USA
Thiobaca	0	Total	Thiobaca trueperi Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=127458&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Cells are rod-shaped, occurring as single cells or inpairs. Motility is consistent with the presence of flagella. Cells stain Gram-negative. Cells are rod-shaped, 1±5lm in diameter, 3±4±5lmlong and motile. Gas vesicles are not formed. Cellsuspension is brown in colour. Photosynthetic mem-branes are of the vesicular type. The optimum temperature forgrowth is between 25 and 30∞C, the maximum being36∞C. The G≠C content of the DNA is 63 mol %
Thioalkalivibrio	Total	Total	Unclasified Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=106633&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Thialkalivibrio(Sorokin et al. 2001) includesobligately and facultatively alkaliphilic strains. Most ofthe strains grow optimally in soda-rich media. Somestrains have a chloride-dependent growth and can growup to saturating concentrations of NaCl. Gram-negative, non-spore-forming,aerobic, obligately chemolithoautotrophic, halophilic,and facultatively alkaliphilic bacterium. Cells are 0.3–0.4·1–2lm and made motile by a single polar flagellum.
Thioalkalispira	Total	0	Uncultured	non	/	Obligately chemolithoautotrophic. Cells have aSpirillum-like morphology, with dimensions 0±3±0±45˙1±4lm (occasionally up to 15lm in length).Motile by means of a single polar Øagellum. Gram-negative cell wall. typespecies of the genus. Thioalkalispira microaerophila. Alkaliphilic, with the pH rangefor growth between 8 and 10±4 (optimum aroundpH 10). Moderately halophilic, with total Na+contentsuitable for growth between 0±2 and 1±4 M (optimum0±5 M).
Thioalkalimicrobium /Thiomicrospira	Spore	0	Unclasified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=933&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Cells are pleomorphic when grown in liquid media, rangingfrom very thin, curved rods. Growth occurs from pH 5.9–8.0 to pH 8.4–10.0 but rangevaries with species–pH optima are pH 7.0–10.0, varying byspecies. Grow from 3.5 to 42C with optima of 25–40C,varying by species. NaCl is required for growth, with min-ima of 40–250 mM, maxima of 1200–3000 mM and optimaof around 430–600 mM.
Thioalkalicoccus	0	Total	Unclasified	non	/	Cells are spherical or oval in shape, multiply by binaryÆssion and are Gram-negative. Cells are cocci of1±3±1±8lm diameter, usually non-motile and sur-rounded by a thin capsule. Type species isThioalkalicoccuslimnaeus. Mesophilic, obligate alkaliphilicbacterium with optimum growth at pH 8±8±9±5 (rangepH 8±10) and 20±25∞C.
Thermomonas	0	Total	Unclasified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=141949&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Gram-negative, non-spore-forming rods, usually oc-curring in Ælaments. The temperature range for growthis 18±50∞C, optimal growth occurring between 37 and50∞C. The type species of the genus isThermomonas haemolytica. The cells are motile by meansof polar monotricheous Øagellation or are non-motile.Non-spore-forming. Colonies on complex standardmedia at 50∞C are whitish}translucent (at 37∞C theyare transparent), circular, smooth and convex with anentire edge.
Tabrizicola	Spore	Total	Unclasified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=909926&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Cells are Gram-negative and rod-shapedAlphaprote-obacteria. They are catalase and oxidase positive andfacultatively anaerobic. pecies of this genus are charac-terized by chemoheterotrophic growth with a limitedvariety of organic substratesas carbon and energy sources.The species of this genus are unable to grow phototro-phically in the light, photosynthetic pigments are notproduced
Syntrophus	Total	Total	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=56780&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Syntrophus aciditrophicus is a gram-negative and rod-shaped bacterium. It is non-motile, non-spore-forming and grows under strictly anaerobic conditions, thus an obligate anaerobe.

Syntrophorhabdus	Spore	Total	Uncultured Unclasified	non	ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=909663&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Obligately anaerobic bacterium. Nonmotile, rod-shapedcells. Sulfate, sulfite, thiosulfate, nitrate, nitrite, elemental sul-fur, or ferric iron cannot serve as an electron acceptor, whileanthraquinone-2,6-disulfonate can. The type species is Sy-trophorhabdus aromaticivorans. Gram negative,nonmotile. Spore formation was never observed. The temper-ature range for growth is 25 to 37°C (optimum, 35 to 37°C).The pH range is 6.6 to 7.4 (optimum, 7.0). Growth occurs inthe presence of 0 to 1.25% NaCl but does not occur in thepresence of more than 1.5% NaCl.
SWB02	0	Total	Uncultured			
Sva0081 sediment group	Total	Total	Uncultured Unclasified			
Stenotrophomonas	Spore	Spore	Unclasified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=40323&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	The type species isStenotrophomonas maltophilia. Cell size: 0.5–0.81.5–3 µm. Colonies are yellow and circularon trypticase soy agar. On nutrient agar, colonies are orange–yellow or yellow.
Spiribacter	Spore	0	Unclasified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1335757&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Spiribacter aquaticus. Cells are Gram-stain-negative, non-motile, aerobic, curvedrods-nearly closed rings and short spiral shapes, 0.41.5–1.8 µm. Colonies are circular, entire, yellow-brown and 0.5–1.0 mm in diameter on SMM medium after 5 days of incu-bation at 37C. Moderately halophilic, able to grow inmedia with 7.5–25 % (w/v) NaCl, with optimal growth at12.5 % (w/v) NaCl. No growth occurs in the absence ofNaCl. Able to grow in the pH range 7–9 and able to growfrom 20 to 40C, with optimal growth at pH 8 and at 37C.
Sphingorhabdus	0	Total	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1343899&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	The type species of the genus is Sphingorhabdus planktonica. The general characteristics are the same as those given in thedescription of the genus. Colonies are yellowish orange andtransparent. Cells are 1.17±0.20mm long and 0.47±0.07mmwide. Cells absorb at 427 nm, 453 nm and 481 nm, indicatingthe presence of carotenoids. Grows at pH 6.5–9 (optimumpH 7–8).
Sphingopyxis	0	Total	Unclasified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=165697&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Cells are Gram-negative, non-sporulating rods,measuring 0±3±0±71±0±3±5lm. Motile or non-motile.Colonies are yellow or whitish-brown. Strictly aerobicand chemo-organotrophic. Catalase-positive. The type species isSphingobium yanoikuyae
Sphingomonas	Spore	Spore	Uncultured Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=13687&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Cells are 0.3–0.8x10–2.7mm. Colonies are off-white-,yellow- or orange-pigmented. Sphingomonas aerolata. Cells are small rods,. Growth isobserved on Czapek–Dox, R2A, CasMM, PYES and TSA,but not on MacConkey agar. Cells occur singly or sometimesin short chains. Cells grow at 4–28uC, but not at 37uC.Gram-negative as determined by Gram staining, KOH andaminopeptidase tests. Motile. Endospores not observed.Colonies are circular, slightly convex, opaque and orange-pigmented. Aerobic.
Sphingobium	Spore	Spore	Unclasified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=165695&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Colonies (1.5–3.0 mm in diameter) are pale yellow,smooth, circular and slightly elevated on R2A plates. Aerobic,non-motile, catalase-positive but oxidase-negative. Tem-perature range for growth is 4–30uC (optimum, 28uC).Growth occurs with NaCl concentrations in the range 0–2 %(optimum, 0.5 %) and at pH 5–9 (optimum, pH 7.0). T
Sorangium	/	Spore	Unclassified	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=448385&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Sorangium cellulosum is a soil-dwelling Gram-negative bacterium of the group myxobacteria.[1] It is motile and shows gliding motility. Under stressful conditions this motility, as in other myxobacteria, the cells congregate to form fruiting bodies and differentiate into myxospores.
Smithella	Total	Total	Unclassified Uncultured uncultured bacterium zdt-14e13	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=92485&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Weakly motile Gram-negative rods. Strictly anaerobic. Type species : Smithella propionica. Gram-negative rods, 0-5 pm in diameter, with most cells 3-5 pm long, but some cells as long as 10 pm. Contain granules of poly-P-hydroxybutyrate. Weakly motile. Strictly anaerobic, Mesophilic.
Silanimonas	/	Total	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1123253&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Cells are strictly aerobic, Gram-negative, non-spore-form-ing rods. Oxidase- and catalase-positive. Nitrate is notreduced. The type species isSilanimonas lenta. Cells are 0?3–0?5mm wide and 0?8–1?8mm long.Some, but not all, cells have a polar flagellum. The pHrange for growth is 6?0–10?0, with an optimum at pH 9?0.The temperature range for growth is 25–53uC, with anoptimum at 47uC.
Shewanella	/	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1028752&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Gram negative, motile rods with positive oxidase and catalase reactions. Shewanella spp. are ubiquitous in natural environments, occurring mainly in marine environments, iced fish, proteinaceous foods, and occasionally clinical samples
SEEP–SRB1	Total	Total				The SEEPSRB1 group is a subgroup of the broad DSS clade.
Saccharospirillum	Total	/	Unclasified	non		Saccharospirillum aestuarii(aes.tu.a9ri.i. L. gen. n.aestuariiof a tidal flat).Cells are Gram-reaction-negative, chemoheterotrophic,oxidase- and catalase-positive, non-motile, non-flagellated,facultatively anaerobic, non-pigmented, curved rods (0.5–1.061.4–2.6mm). Saccharospirillum aestuarii(aes.tu.a9ri.i. L. gen. n.aestuariiof a tidal flat).Cells are Gram-reaction-negative, chemoheterotrophic,oxidase- and catalase-positive, non-motile, non-flagellated,facultatively anaerobic, non-pigmented, curved rods (0.5–1.061.4–2.6mm).
Sandarakinorhabdus	/	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1123240&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Cells are non-spore-forming, non-motile rods that do notform a capsule and are Gram-negative. The type species isSandarakinorhabdus limnophila. Facultatively oligo-trophic, grows as single cells and forms orange–red coloniesof 1–3 mm with mucilaginous texture on agar plates.
			Uncultured		https://www.ncbi.nlm.nih.gov/Tax	Vegetative cells are rod-shaped with blunt rounded ends(Sorangium-

Sandaracinus	/	Total	Unclassified	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=927083&lvl=3&lin=f&keep=1&srchmode=1&unlock	type). Cultures in nutrient-rich media arecoloured yellowish-orange. Produce shallow and wavy agardepressions on P-agar. Scattered and sessile fruiting-body-like aggregates on agar bear refractive myxospore-like cells,which are shorter than vegetative cells. The type species is
Sandaracinobacte	/	Total	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1715348&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cells are thin, long rods, forming chains. Motile by means of subpolar flagella, gram negative. Divide by binary division. Cultures are intensely yellow-orange because of carotenoid pigments. The type species is Sandaracinobacter sibiricus. Gram-negative, yellow-orange-pigmented, thin, long rods, 0.3 to 0.5 by 1.5 to 2.5 pm or more. Motile by means of subpolar flagella (up to three).
Salinarimonas	/	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1123229&lvl=3&lin=f&keep=1&srchmode=1&unlock	Type strain Salinarimonas rosea. In addition to the characteristics reported for the genus,cells are halotolerant, motile by means of a single polarflagellum and approximately 0.4–1.0mm wide by 1.20–1.45mm long. Growth occurs at 15–37uC (optimum, 28–30uC), at pH 6.0–9.0 (optimum, pH 7.0–8.0) and in NaClconcentrations of 0–5 % (w/v) (optimum, 3 %).
Ruegeria	/	Total	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=97050&lvl=3&lin=f&keep=1&srchmode=1&unlock	Most species are strict aerobes, but some spe-cies can grow facultative anaerobically via nitrate reduction.Accumulation of polyhydroxybutyrate is variable. The type species for the genus isRuegeria atlantica. Cells are Gram-negative, aerobic, non-motilerods that are 1.5–2.0mm long and 0.6–0.8mm wide. Growthis detected at 5–30uC with an optimum at 25uC. pH rangefor growth is 6–11 with an optimum at pH 7.
Rubrimonas	/	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=89524&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cells are aerobic, Gram-negative, short rods that are motile by means of polar flagella. Catalase and oxidase are produced. The type species is Rubrimonas cliftonensis. Colonies are circular, smooth, slightly convex, entire, glistening, opaque and pink. Cells are 1-0-1-5 x 1.2-2-0 ym and divide by binary fission. Optimum growth occurs at pH 7-5-8-0 and at 27-30 "C. Growth occurs in the presence of 0-5-7-5 YO (w/v) NaC1. No growth occurs in the absence of NaCl. Nitrate reductase, phosphatase and urease are pro- duced.
Rubribacterium	/	Total	Unclassified	non	/	Rubribacterium polymorphum gen. nov., sp. nov.po.ly.mor'phus—Gr. adj. poly many; Gr. n. morphusshape, body; M.L. adj. polymorphum many shapes.Young cells are oval, later polymorphic. When intra-cellular storage compounds are accumulated, the cellsbecome spindle-shaped with an irregularly swollencentral part. Cell size is 0.7 × 1.0–1.2 µm.
Rubellimicrobium	/	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=687525&lvl=3&lin=f&keep=1&srchmode=1&unlock	Rubellimicrobium roseum(ro'se.um. L. neut. adj.roseum rose-coloured, the colour of colony of thetype strain).Aerobic, non-motile, non-spore-forming, irregularrods (0.8–191.8–2.2lm), Gram-negative bacte-rium contains poly-b-hydroxybutyrate. Colonies arepink-coloured, circular and clearly protuberant aftergrowth on ISP 2 agar for 6 days at 28°C. Growthtemperature occurs in very narrow range (25–30,optimally around 28°C) and pH range is 6.5–9(optimum 7).
Roseovarius	/	Total	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=74031&lvl=3&lin=f&keep=1&srchmode=1&unlock	Gram-negative rods with one or both cell poles pointed, multiplying by monopolar growth, i.e. by a budding process. The type species for the genus is Roseovarius tolerans. Optimal growth occurs at 8-33.5 "C with salt concentrations of 1.0-8.0 % NaCl or 10-130%, ASW. The optimum pH is 5.9->9.0.
Roseomonas	Spore	Total	alpha proteobacterium BAC153 Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1449094&lvl=3&lin=f&keep=1&srchmode=1&unlock	Is a genus of Gram negative bacteria. The cells are coccoid rods when viewed microscopically. Certain species are known to be opportunistic infections for humans. The type species is Roseomonas gilardii.
Roseococcus	Total	Total	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=455361&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cells are gram negative, coccoidal, pink, and motile by means of polarly inserted flagella. The type species is Roseococcus thiosulfatophilus. The type species is Roseococcus thiosulfatophilus Habitat: freshwater cyanobacterial mat of a thermal alkaline sulfide spring (54°C; pH 9.3; 7.4 mg of sulfide per liter; 1.6 mg of oxygen per liter)
Roseibaca	Total	Total	Unclasified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1666912&lvl=3&lin=f&keep=1&srchmode=1&unlock	Gram-negative non-motile rods. One or both cell poles arenarrower. Cell growth is monopolar indicating a buddingprocess. Stem-like structures are often produced. he type species is Roseibaca ekhonensis. Temperaturerange for growth is 10–30uC. Optimum growth occurs at16uC and pH 7.0–9.5. NaCl tolerance ranges from 0–1.0 to3.5–4.0 %, with an optimum of 2.5 %. Positive forperoxidase and cytochrome oxidase, and weakly-positivefor catalase activity.
Rhodovibrio	Spore	Total	Unclassified	non	ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1089552&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cells are vibrioid- to spiral-shaped, 06-0.9 pm wide, motile by means of polar flagella, multiply by binary fission and are Gram-negative. The type species is Rhodovibrio salinarum.
Rhodovarius	/	Total	Uncultured	/	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1979269&lvl=3&lin=f&keep=1&srchmode=1&unlock	
Rhodomicrobium	/	Spore	Uncultured		https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1068&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cells are ovoid, motile by peritrichous flagella, multiply by budding, and have intracytoplasmic membranes parallel to the cytoplasmic membrane. Rhodomicrobium lacus (la'cus. L. gen. masc. n. lacus of a lake).Colour of phototrophically grown culture is reddish brown and the culture is non- flocculent. Cells are Gram- stain-negative, swollen- rod shaped, motile by peritrichous flagella and multiply by short tube- like budding.
Rhodoferax	/	Total	Uncultured Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=2576305&lvl=3&lin=f&keep=1&srchmode=1&unlock	Gram negative, vibroid or slightly curved rods, 0.2–1.061.0–5.0mm; polar flagellaoccur. May grow brown pigmented or translucent aerobic,facultative anaerobic or with low oxygen concentrations at4–30uC and at pH 5.0–9.0.
Rhodocista sp. SCSIO 13435	/	Total				

Rhodocista	/	Spore	Unclassified	non		Cells are vibrioid or spiral, 0.6–0.8mm wide and0.8–1.5mm long. Motile by means of a single polarflagellum. Gram-negativea-Proteobacterium.
Rhodobaculum	/	Total	Unclassified	non		Rhodovulum steppense(step.pen9se, N.L. n.steppumsteppe;L. neut. suff.-ensesuffix used with the sense of pertainingto; N.L. neut. adj.steppensepertaining to the steppe,widespread in steppe soda lakes).Cells are ovoid to rod-shaped, 0.3–0.8mm wide and 1.0–2.5mm long, motile by means of polar flagella, multiply bybinary fission, and contain vesicular internal photosyn-thetic membranes.
Rhodobacter	/	Total	Uncultured Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1415162&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Type strain Rhodobacter capsulatus. Cells are ovoid to rod shaped and motile or nonmotile, divide by binary fission, and have vesicular photosynthetic mem- branes. All species require thiamine, and most of them require biotin and a different third vitamin as growth factors.
Rhizobacter	/	Total	Uncultured Unclassified	non		Straight to slightly curved, non-spore-forming, uncapsu-lated, Gram-negative rods. The type species is Rhizobacter dauci. Good growth is obtained at 28–30°C; no growth at 35°C. Growth occurs at pH between 5.0 and 9.0. Utilizes d-glucose as a sole source of carbon and energy.
Rheinheimera	/	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1123053&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Cells are Gram-negative, Øagellated, rod-shaped tococcoid, oxidase- and catalase-positive. Growth isaerobic, chemoheterotrophic and occurs at temper-atures from 4 to 30∞C, with optimum growth at20±25∞C. The type species is Rheinheimera baltica. Strains are not halotolerant (no growthat"6 % NaCl) ; NaCl stimulates growth, but is notrequired for growth. Optimal growth was observed at1 % NaCl. Growth temperature ranges from 4 to30∞C, with an optimum at 20±25∞C.
Reyranella	Spore	Spore	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1205680&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Gram-stain-negative, microaerophilic rods that are pos-itive for oxidase activity and negative for catalase activity. The type species is Reyranella massiliensis. Reyranella soli(so9li. L. neut. gen. n.soliof soil, the sourceof the type strain).Cells are Gram-stain-negative, aerobic, non-motile rods(0.8–1.0 in width and 1.3–2.0mm in length). Colonies areconvex, grey–white and opaque. Grows within thetemperature range of 20–37uC (optimum, 30uC), atpH 4–9 (optimum, pH 6–7) and with 0–1 % NaCl(optimum, 0 %).
Ralstonia	Spore	Spore	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=48736&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	The genus Ralstonia comprises plant pathogens capable of growth and polyhydroxyalkanoate production on plant oils and fatty acids. They belong to the class of the Betaproteobacteria, family of the Burkholderiaceae.
Psychrobacter	Total	/	Unclasified		https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=261164&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Psychrobacter is a genus of mostly psychrotrophic cocco-bacilli, Gram-negative, catalase, and oxidase-positive species. A common species found in milk, Psychrobacter immobilis, are aerobic, but some strains can also be anaerobic. Psychrobacter alimentarius(a.li.men.ta9ri.us. L. adj.alimen-tariusrelating to food).Cells are cocci, 1.4–2.0mm in diameter. Colonies on MAare smooth, low convex, circular to slightly irregular andcream in colour. Aerobic. Growth occurs at 4 and 35uC,but not above 36uC. Optimal pH for growth is 6.5–7.5;growth occurs at pH 5.0, but not at pH 4.5. Growth occursin the presence of 0–12 % (w/v) NaCl, with optimumgrowth at 2–3 %.
Psychroglaciecola	/	Total	Unclassified	non	/	Cells are rod-shaped, non-spore-forming, Gram-stain-negative, strictly aerobic, and are motile by a single polarflagellum.The type species is Psychroglaciecola arctica. Colonies are pink, smooth, circular and convex with entiremargins at 18uC on R2A agar (Difco). Growth occurs at 4–28uC (optimum 18uC) and at pH 5.0–8.0 (optimumpH 7).
Pseudoxanthomonas	/	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=83618&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Members of the genus are non-spore-forming rods, usually0?4–0?860?9–1?5mm, which stain Gram-negative. Most speciesare mesophilic (optimum temperature 30–37uC), slightlyalkalophilic (optimum pH over 7 and preferentially around8) and motile by means of a single polar flagellum; how-ever, one species is thermophilic (optimum temperature50uC) and non-motile.
Pseudorhodoplanes	/	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1235591&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Cells are Gram-stain-negative, facultatively anaerobic,non-motile and rod-shaped. Reproduction of cells is bybudding. Growth occurs after incubation on R2A agar for72 h at 30uC. Able to grow at pH 5.5–8, at 15–35uC andin the presence of 0–3.5 % (w/v) NaCl. The type species isPseudorhodoplanes sinuspersici.
Pseudorhodobacter	/	Total	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=238783&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	The cells are gram-negative rods, 0.6 to 1.6mmwide and 1.0 to 4.0mm long, and do not form spores.Non-motile. Aerobic chemoorganotrophic bacteriumwith strictly respiratory type of metabolism with oxygenas terminal electron acceptor. No photosyntheticgrowth occurs under both oxic and anoxic conditions. The type species is Pseudorhodobacter ferrugineus. Growth occurs in the pres-ence of NaCl concentrations ranging from 0 to 3% (op-timum NaCl concentration, 1%).
Pseudomonas	Total	Spore	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=286&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Pseudomonas species are Gram-negative, aerobic bacilli measuring 0.5 to 0.8, µm by 1.5 to 3.0 µm. Motility is by a single polar flagellum.
Pseudolabrys	/	Spore	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=331696&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Cells are Gram-negative, aerobic, non-motile and short rod-shaped. he type species is Pseudolabrys taiwanensis.
			Uncultured		https://www.ncbi.nlm.nih.gov/Tax	Cells are Gram-negative,aerobic, non-spore-forming,catalase-positive,

Pseudohongiella	/	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1249552&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Cells are Gram-negative,aerobic, non-spore-forming,catalase-positive, oxidase-negative, and motile rods withsinglepolarflagellum. hetype species is P. spirulinensis Cells are usually0.73–0.8lm in diameter and 1.24–4.26lm long.Colonies on MA are pale-yellow, non-translucent, andcircular with regular edges. Growth occurs at pH6.0–10.0 and 10–43°C
Pseudoalteromonas	Total	/	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=53246&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Pseudoalteromonas species are gram-negative, non-spore- forming, straight or curved rods that are 0.2 to 1.5 by 1.8 to 3 Fm. The cells of most species are motile by means of single unsheathed polar flagella. The type species is Pseudoalteromonas haloplanktis
Pseudofulvimona	/	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=634155&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cells are non-motile, non-spore-forming rods (approx.2mm in length). Gram-stain-negative, oxidase-positive,showing an oxidative metabolism. The type species is Pseudofulvimonas gallinarii.
Propionivibrio	/	Total	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1123006&lvl=3&lin=f&keep=1&srchmode=1&unlock	Gram-negative rod-shaped cells that may be motile by one polar flagellum. Type species Propionibacter pelophilus.Growth on glucose and aspartate possible at initial pH of 6-5-85, the optimum being around 7.5-8.0. Final pH of glucose-fermenting cultures is 5.5. Temperature op- timum 27-30 "C; no growth at 35 "C.
Proteobacteria bacterium B210	Spore	/	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi	Cells are Gram-stain-negative, rod-shaped, gliding motileand facultatively anaerobic. Positive for catalase but negativefor oxidase.
Porphyrobacter	Total	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1111&lvl=3&lin=f&keep=1&srchmode=1&unlock	Gram-negative pleomorphic motile rods or cocci, reproduc- ing by polar growth or budding. The type species is P. neustonensis
Polynucleobacter	/	Total	Unclassified		https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=44013&lvl=3&lin=f&keep=1&srchmode=1&unlock	The type species isPolynucleobacter necessarius. Contains obligate endosymbionts living in ciliates of thespecies
Polymorphobacter	Total	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1070431&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cells are Gram-stain-negative, motile, non-sporulating and polymorphic. The type species is Polymorphobacter multimanifer. Growthoccurs between24 and 30uC (optimum, 25uC), betweenpH 6.0 and 9.0 (optimum, approximately pH 7.0), andwith between 0.0 and 1.0 % NaCl (optimum, 0.5 %).
Polaromonas	/	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=52972&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cigar-shaped, gram negative rods that are 0.8 by 2.0 to 3.0 pm, Encapsulated. Aerobic. Chemoorganotro- phic and catalase and oxidase positive. Requires amino acids, but not vitamins, for growth. Motile by means of a polar fla- gellum. Cells may contain gas vesicles. Psychrophilic. The max- imum growth temperature of known strains is 15°C.
Phreatobacter	/	Spore	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1122261&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cells are Gram-negative, strictly aerobic, motile rods.Oxidase-positive and weakly catalase-positive. Thetypespecies isPhreatobacter oligotrophus. Growth occursat 20–45uC (optimum at 25–37uC) and at pH 5.5–9.5(optimum pH 6–8); no growth occurs in the presence ofNaCl.
Phenylobacterium	/	Total	Unclasified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=31967&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cells stain Gram-negative, are non-spore-forming straightto slightly curved rods, coccobacilli or cocci measuring 0.7–1.061.0–2.0mm and occur singly, in pairs or short chains. Cells of some species have prosthecae. he type species isPhenylobacterium immobile. Growth isobserved between 10 and 40uC, with optimal growtharound 37uC.
Phaselicystis	Spore	Total	Uncultured	yes		Vegetative cells are long and cylindrical rods with bluntends typical of thePolyangiaceae; movement by gliding onthe agar surface in long and fine radial veins. The swarm istough, film-like and slimy, characteristic of theCystobacteraceae. Colonies are Congo-red-negative, withedges with sophisticated networking of migrating cells onlean media; the agar is slightly etched and corroded.Myxospores are refractive, slender rods, shorter thanvegetative cells, enclosed in a sporangial wall. Fruitingbodies are ovoid to sausage- or bean-shaped, glisteningsporangioles that are clustered in chains or a flat mat, orsolitary. he type species isPhaselicystis flava.
Peredibacter	Spore	Total	Bacteriovorax sp. EPC3 Unclassified Uncultured	non		Consists of predatory, Gram-negative, bacteriovorousspecies that require a Gram-negative host as prey tocomplete a biphasic life cycle. Members of the genus aresoil-dwellers. Type strain Peredibacter starrii
Pelomonas	Spore	Spore	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=431059&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cells are Gram-negative rods, motile with one polarflagellum. Colonies are grey, round and opaque; oldcolonies (cultured for more than 2 weeks) are brown innitrogen-free medium. The type species is Pelomonas saccharophila. Cells are3?0–4?0mm long and 0?5mm in diameter. Growth occursat 4–40uC with an optimum at 25–32uC; unable to growat 41uC.
Pelobacter	/	Total	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1122946&lvl=3&lin=f&keep=1&srchmode=1&unlock	taxonomic entity consisting of strictly anaerobic, Gram-negative, nonsporeforming, rod-shaped bacteria that use only a very limited number of substrates.
Pedomicrobium	/	Spore	Uncultured	non		Pedomicrobium is a ubiquitous bacterium dominant in biofilms of man-made aquatic environments such as water distribution systems and bioreactors.
Paucibacter	/	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=270368&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cells are rod-shaped and motile by means of a single polarflagellum. Gram-negative, oxidase-, alkaline phosphatase-,chymotrypsin- and leucine arylamidase-positive. Weakcatalase-positive reaction. Grow heterotrophically underaerobic conditions. The type species is Paucibacter toxinivorans. Cell size is0?5–0?761?3–5?0mm. Fails to grow on rich media, suchas TSA. Grows well at 20–30uC but does not grow at37uC.

Neisseria	/	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1470200&lvl=3&lin=f&keep=1&srchmode=1&unlock	Neisseria spp. are a Gram-negative non-spore-forming diplococcus that has a flattened shape; its size ranges between 0.6–0.8 µm. They are oxidase-positive, non-acid-fast cocci or plump rods. They can reach a diameter of around 0.6–0.8 µm and sometimes up to 1.0 × 2.0–3.0 µm.
Nannocystis	/	Spore	Unclassified	yes	/	Nannocystis konarekensis. The vegetative cells are Gram-negative, rod-shaped, shortwith blunt, rounded ends (Nannocystistype) and non-motile, 1.1–1.5 µm in diameter and 2.0–3.25 µm long. Cellscan move by gliding on solid surfaces. Myxospores are 0.8–0.9 µm wide and 1.7–1.8 µm long. The colour of colonies isbright orange on P and CY agar and in liquid medium. OnVY/2 agar, transparent, distinctive swarming activity. OnCY and water agar, agar corrosion is extensive but thestrain cannot liquefy agar. Orange-coloured scatteredsporangioles can be observed on water agar withE. colibait.Growth temperature is between 25–37C (optimum 37C);no growth at 44C. Growth occurs at pH between 6.5–10.0(optimum 8.0–10.0).
Methyloversatilis	/	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=999628&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cells are Gram-negative, non-motile rods that multiply bybinary fission. Thetype and only species is Methyloversatilis universalis. Cells are 1?2±0?460?4±0?1mm in size and occur singly orin pairs. Growth occurs at pH 6–9, with optimal growth atpH 7?5–8. Temperature optimum is 30–37uC.
Methylophaga	Total	Total	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=182774&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cells are non-sporulating, Gram-negative rods, motile bymeans of a single polar flagellum. Thick periplasmic spaceof 20–30 nm. Strictly aerobic moderate halophile. Somespecies are alkaliphilic. The type species is Methylophaga marina. obligately methylotrophic, Gram-negative, strictly aerobic, motile, rod-shaped bacteria
Methylomicrobium	/	Total	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=39774&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cells are short rods, 0.5–1.561.5–3.0mm. Motile by asingle polar flagellum or three peritrichous flagella.Reproduce by binary fission and unable to form cysts orspores. he type species isMethylomicrobium agile.
Methyloceanibacter	Spore	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1384459&lvl=3&lin=f&keep=1&srchmode=1&unlock	Gram-negative, non-motile rods. Aerobic and reproduceby binary fission. Facultative methylotrophs. The type species is Methyloceanibacter caenitepidi. ells are 0.7–1.162.8–10.4mminsize. Colonies on agar medium with methanol are 0.5–0.8 mm in diameter, white, round and convex with entireedges. Does not reduce nitrate to nitrite. Able to grow at19–43uC and with 0–9 % NaCl. Optimal conditions forgrowth are 35uC, pH 6–8 and 1.0 % NaCl.
Methylobacterium	Spore	Spore	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=407&lvl=3&lin=f&keep=1&srchmode=1&unlock	aerobic, oxidase-positive, gram-negative bacilli that produce characteristic pink or coral pigmented colonies on a variety of media
Mesorhizobium	/	Total	Unclassified	unknown	ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=68287&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cells are gram-negative, aer- obic, non-spore-forming rods, motile, usually with one polar or subpolar flagellum. The type species is Mesorhizobium loti
Parasutterella	/	Spore	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=762966&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cells are Gram-negative, non-motile, strictly anaerobic,non-spore-forming cocci to coccobacilli. Oxidase-, urease-and catalase-negative. The typespecies isParasutterella excrementihominis, ells are approximately 0.4–1.160.9–2.6mm in size. After 3 days anaerobic incubation onanaerobe basal agar (pH 6.0) colonies are translucent,entire, circular, convex and pinpoint
Paracoccus	/	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=265&lvl=3&lin=f&keep=1&srchmode=1&unlock	Spherical cells (diameter, 0.5 to 1.3 km) or short rods (length, 0.9 to 1.3 pm) occur singly, in pairs, in chains, or in clusters. Intracellular granules of poly-p-hydroxybu- tyrate are present. No resting stages are known. Gram negative. Nonmotile.
Pajaroellobacter	Spore	Total	Uncultured	non		The causative agent of epizootic bovine abortion proved elusive for over fifty years until a novel Deltaproteobacteria was identified as the etiologic agent in 2005. Prior to that time, numerous attempts to visualize the bacteria using classic staining techniques, including Gram stain, were unsuccessful (Kimsey et al., 1983, Stott et al., 2002). The current inability to cultivate P. abortibovis using in vitro techniques, and its resistance to purification from necropsy tissues, has interfered with traditional morphological and biochemical-based methods of bacterial characterization.
OM27 clade	Spore	Total	Uncultured	/	/	/
Oligoflexus	/	Spore	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=708132&lvl=3&lin=f&keep=1&srchmode=1&unlock	Gram-negative. Cells are non-motile and non-spore-forming. Aerobic. Cell shape is pleiomorphic, such as afilamentous, flexible, long fusiform shape or a helical shapesimilar to spirilla. Cells grow under low nutrient condi-tions. The type species isOligoflexus tunisiensis. Growth occurs at 20–37uC, withan optimum temperature of 25–30uC, and at pH 7.0–9.5,with an optimum at pH 7.0–8.0. Sulfate reduction is notobserved
Oceanicaulis	/	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=153232&lvl=3&lin=f&keep=1&srchmode=1&unlock	Gram-negative, non-spore-forming. Cells are rod-shapedor vibrioid. Cultivated cells are 1mmby3mm. Cells possessone prostheca, which extends centrally along the long cellaxis from one pole. The type species isOceanicaulis alexandrii.
Novosphingobium	Spore	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=165696&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cells are Gram-negative, non-sporulating rodsmeasuring 0±3±0±5 1±0±3±0lm. Motile or non-motile.Colonies are yellow or whitish-brown. Strictly aerobicand chemo-organotrophic. Catalase-positive. he type species isNovosphingobiumcapsulatum
Nitrincola	Total	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=267850&lvl=3&lin=f&keep=1&srchmode=1&unlock	Alkaliphilic, halotolerant and heterotrophic. Cells arenon-pigmented, asporogenous, motile, Gram-negative rods.NO{2and O2can be used as electron acceptors. Nitrincola lacisaponensis.
Nevskia	Spore	Total	Nevskia terrae Unclasified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1122603&lvl=3&lin=f&keep=1&srchmode=1&unlock	Nevskia terrae(ter9rae. L. gen. n.terraeof the soil).Cells are strictly aerobic, Gram-negative rods (0.4–0.6mmin width and 1.0–2.5mm in length). Motile with 1–3subpolar flagella. Colonies are ivory, circular and convex with clearmargins after 8 days. Grows at 15–35uC (optimum 28–30uC) and at pH 5–7 (optimum pH 6–7).

	Marinobacter	Total	Total	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=2742&lvl=3&lin=f&keep=1&srchmode=1&unlock	The cells of the type strain of Marinobacter hydrocarbonoclasticw. are rod shaped (2 to 3 pm long and 0.3 to 0.6 pm wide in logarithmic growth phase) and harbor numerous surface blebs when grown on eicosane in mineral medium. Cells are gram negative, nonsporeforming, and motile by means of a single unsheathed polar flagellum in media containing 0.2 to 1 M NaC1.
	Magnetospirillum	/	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=13134&lvl=3&lin=f&keep=1&srchmode=1&unlock	Magnetospirillum (formerly Aquaspirillum) magnetotacticum is a Gram-negative, helical (clockwise), magnetotactic, microaerophilic spirillum. The typical habitat of Magnetospirillum species consists of shallow fresh water and sediments, characterized by low concentrations of oxygen for growth
	Lysobacter	Spore	Total	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1444315&lvl=3&lin=f&keep=1&srchmode=1&unlock	Lysobacter capsici(cap9si.ci. N.L. gen. n.capsiciofCapsicum, referring to the isolation of the type strain fromthe rhizosphere ofCapsicum annuumL.).Cells are Gram-negative, facultatively anaerobic, rod-shaped, non-spore-forming and non-motile but havinggliding activity, 0.3–0.5mm wide by 2.0–20mm long. Cellsoccur singly and in pairs and can form irregular filament-like structures. Colonies grown on 2 % TSA at 30uC for3 days are 2.0–4.0 mm in diameter, creamy white toyellow, smooth and circular. The temperature range forgrowth is 15–37uC (very weak growth at 4uC); no growthoccurs at 45uC.
	Luteimonas	/	Total	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1122183&lvl=3&lin=f&keep=1&srchmode=1&unlock	type species is Luteimonas mephitis. aerobic, Gram-stain-negative, non-motile, rod-shaped bacterium isolated from a marine sediment sample collected at Kending, Taiwan.
	Loktanella	Total	Total	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=195913&lvl=3&lin=f&keep=1&srchmode=1&unlock	Gram-negative, rod-shaped cells that are strictly aerobic,moderately halotolerant and chemoheterotrophic. They donot form spores and the optimal growth temperature is25uC. Motility is not observed. The type species isLoktanella salsilacus. Cells are Gram-negative, short rods (<1mm by 3–4mm),often forming pairs or short chains. Strains grow at 5–30uC;weak growth is observed at 37uC and no growth occurs at45uC.
	Leptothrix	/	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=395495&lvl=3&lin=f&keep=1&srchmode=1&unlock	Leptothrix is a genus of Gram-negative bacteria in the class Betaproteobacteria.The name is from the Greek leptos thrix. They occur in standing or slow-flowing, ferruginous, neutral to slightly acidic fresh waters with only low concentrations of organic matter.
	Legionella	Spore	Total	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=445&lvl=3&lin=f&keep=1&srchmode=1&unlock	Legionnaires' disease (LD) is a type of pneumonia caused by the Legionella spp. Legionella pneumophila is the most common cause of the disease and, along with Legionella longbeachae, causes disease in both immunocompetent and immunosuppressed people. The >50 other named Legionella spp. rarely cause LD and do so exclusively in immunocompromised patients. The Legionella spp. are aerobic Gram-negative bacilli that are found mainly in aqueous environments.
Proteobacteria	Lautropia	/	Total	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=887898&lvl=3&lin=f&keep=1&srchmode=1&unlock	Gram-negative cocci occurring in at least three forms: (1) encapsulated cocci, 1-2 pm in diameter, often occurring in clusters of lo-> 100 cells; (2) unencapsulated cocci, 1-2 pm in diameter, motile by the action of a tuft of three to nine flagella; and (3) large, > 5 pm in diameter, sphaeroblast-like cells. Facultative, but grows best under aerobic conditions with no requirement for CO,. Meso- philic, growing at temperatures between 30 OC and 44 "C.
	Lamprocystis	/	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=765909&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cells spherical to ovoid, 1.9–3.8 µm in diameter, diplococcusshaped before cell division and may occur in irregular aggregates, multiply by binary fission, nonmotile or motile by means of a single flagellum, form gas vesicles in central part. Gram negative and belong to the Gammaproteobacteria. At high sulfide concentration (4–6 mM) and light intensity (1000–2000 lux), cells may grow as long, branching cell aggregates embedded in slime. Type species: Lamprocystis roseopersicina
	Klebsiella	/	Spore	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=570&lvl=3&lin=f&keep=1&srchmode=1&unlock	Straight rods, 0.3–1.0 × 0.6–6.0 µm, arranged singly, in pairs or short chains; often surrounded by a capsule. Conform to the general definition of the family Enterobacteriaceae. Gram negative. Nonmotile (except K. mobilis). Facultatively anaerobic, having both a respiratory and a fermentative type of metabolism. Klebsiella pneumoniae
	JTB255 marine benthic group	Total	Total	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1738654&lvl=3&lin=f&keep=1&srchmode=1&unlock	JTB255 are ubiquitous and consistently rank among the most abundant 16S rRNA gene sequences in diverse marine sediments. They account for up to 22% of bacterial amplicons and 6% of total cell counts in European and Australian coastal sediments.
	Jannaschia	Total	/	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=188906&lvl=3&lin=f&keep=1&srchmode=1&unlock	Gram-negative, irregular rods with a tendency to formchains during growth. On LBSS agar, strains develop small,whitish, shiny colonies within 3–5 days. They do not formspores. Growth is poor at 15uC and optimal at 30uC. ThepH tolerance for growth is 7?0–8?0. In media devoid of saltsor containing only sodium chloride, no growth is observed.Growth is observed with 1–7 % (w/v) sea salts.The type species is Jannaschia helgolandensis.
	Jahnella	/	Total	Polyangium thaxteri	/		
	Hyphomicrobium	Spore	Total	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=2665159&lvl=3&lin=f&keep=1&srchmode=1&unlock	Hyphomicrobium album (al'bum. L. neut. adj. album, white, referring to the color of the colonies).Gram stain negative and facultative anaerobic. Cells are rod shaped with bud forming at the tip of a prosthe-cate.
	Hyphomonas	/	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1280949&lvl=3&lin=f&keep=1&srchmode=1&unlock	Rod-shaped to oval cells measure 0.5–1.0 µm × 1.0–3.0 µm. Species have a biphasic life cycle and generate a single polar prosthecum. Cell division occurs by budding. Buds are produced at tips of polar prostheca, which are 0.2–0.3 µm in diameter and one to five times the length of the cell body. Young daughter cells are oval to pear shaped, lack prostheca, and are smaller than the mother cell. Motile by means of a single polar to lateral flagellum. Pleomorphic, Gram-negative, not acid-fast, aerobic, non-spore-forming, and chemoorganotrophic.

Hydrogenophaga	Total	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1349752&lvl=3&lin=f&keep=1&srchmode=1&unlock	Straight to slightly curved rods, 0.3–0.6 × 0.6–5.5 µm, occurring singly or in pairs. Gram negative. Motile by means of one or, rarely, two polar to subpolar flagella. Colonies are yellow due to the presence of carotenoid pigments. Aerobic. Oxidase positive and catalase positive, except for H. pseudoflava, which is catalase variable. Type species: Hydrogenophaga flava
Herbaspirillum	Spore	/	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=757424&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cells are generally vibrioid, sometimes spirilloid, approximately 0.6–0.7 µm in diameter. The cell length varies from 1.5–5.0 µm depending on the culture medium. Gram negative. Motile, having 1–3 flagella at one or both poles. The organisms have a strictly respiratory type of metabolism, and sugars may be oxidized but not fermented. The three named species presently included in the genus have the ability to fix atmospheric N2 under microaerobic conditions and grow well with N2 as the sole nitrogen source, even in the presence of 10% sucrose. Type species: Herbaspirillum seropedicae
Hoeflea	/	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1940281&lvl=3&lin=f&keep=1&srchmode=1&unlock	Hoeflea oleisp. nov.Hoeflea olei (o9le.i. L. gen. n. olei of oil)Colonies are circular with entire margin, and texture ismoist with flat to raised elevation. On nutrient agar, colo-nies are reddish-brown and measure 1–3 mm in diameter.Cells are straight rods (1–2mm)2–5mm), Gram-stain-negative, motile and non-endospore-forming. Aerobic,microaerobic and phototrophic anaerobic growth is poss-ible.
Hirschia	/	Total	Unclassified	non	ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=582402&lvl=3&lin=f&keep=1&srchmode=1&unlock	Rod-shaped or oval cells, 0.5–1.0 × 0.5–6.0 µm with one or two polar hyphae (prosthecae) with a diameter of 0.2 µm. Buds are formed at the tips of the hyphae. Daughter cells are motile by a single polar flagellum. Gram negative. Aerobic and chemoorganotrophic. PHB is not stored. Utilize various sugars and organic acids. Type species: Hirschia baltica
Halothiobacillus	Total	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=555778&lvl=3&lin=f&keep=1&srchmode=1&unlock	Obligately chemolithoautotrophic Gram-negative rods, 0.3–0.6 × 1.0–2.5 µm, occurring singly or in pairs. Energy for growth is obtained from the oxidation of reduced inorganic sulfur compounds (thiosulfate, tetrathionate, trithionate, sulfur, and sulfide, but not thiocyanate); unable to oxidize ferrous iron. Tolerant of high concentrations of solutes (e.g., 4 M NaCl; 0.25 M sodium thiosulfate), with some strains showing moderate halophily with a requirement for NaCl (optima 0.4–1.0 M NaCl). Optimum temperature for growth 30–42°C and optimum pH 6.5–8.0. Type species: Halothiobacillus neapolitanus
Halomonas	Total	Spore	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=768066&lvl=3&lin=f&keep=1&srchmode=1&unlock	The cells are Gram-stain-negative and non-endospore-forming rods. Most strains are motile. Colonies are cream, cream-yellow, yellow, white, brown, or orange pigmented. Chemoorganotrophic. Strictly aerobic or facultatively anaerobic. Catalase-positive and oxidase-variable. Halophilic or halotolerant. Some species are haloalkaliphilic or psychrotolerant. Optimal growth at 0–15% (w/v) NaCl, pH 6.0–10.0, and 20–40°C. Type species: Halomonas elongata
Halofilum	Spore	/	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1611323&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cells of Halofilum ochraceum are rod shaped, long, Gram-stain-negative, facultatively anaerobic, chemoheterotrophic, catalase-positive, and oxidase-negative. Optimal growth is observed at 33–37°C, pH 7.5–8.0, and 8–10% (w/v) NaCl. No growth occurs in the absence of NaCl. Nitrate is reduced, but nitrite is not. Type species: Halofilum ochraceum
Halochromatium	/	Total	Uncultured	non	/	Cells straight to slightly curved rods, single or in pairs, multiply by binary fission. Motile by polar flagella, Gram negative, belong to the Gammaproteobacteria, and contain internal photosynthetic membranes of vesicular type with bacteriochlorophyll a and carotenoids as photosynthetic pigments. Type species: Halochromatium salexigens
Haliangium	Spore	Total	Uncultured	yes	ser/wwwtax.cgi?mode=Info&id=502	Vegetative cells are Gram-negative rods with bluntends, 0.5–0.6 by 3.0–8.0mm. Motile by gliding on solidsurfaces, such as agar. Yellow colonies spread andare usually slightly sunk into the agar surface. Fruitingbodies are yellow- to brown-colored, and consist ofone or more of sessile sporangioles (15–150mm) in dense packs. Obligate aerobic and moderatelyhalophilic, requiring 1–3% NaCl for optimal growth. The typespecies is Haliangium ochraceum. Cellsare gliding rods, 0.5–0.6 by 4.0–4.5mm. No growth at45°C. Fruiting bodies contain one or more middle-sized sporangioles (50–150mm).
Haemophilus	Spore	/	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=888728&lvl=3&lin=f&keep=1&srchmode=1&unlock	Haemophilus is a genus of Gram-negative, pleomorphic, coccobacilli bacteria belonging to the family Pasteurellaceae. While Haemophilus bacteria are typically small coccobacilli, they are categorized as pleomorphic bacteria because of the wide range of shapes they occasionally assume. These organisms inhabit the mucous membranes of the upper respiratory tract, mouth, vagina, and intestinal tract. The genus includes commensal organisms along with some significant pathogenic species
Geoalkalibacter	Spore	/	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=483547&lvl=3&lin=f&keep=1&srchmode=1&unlock	Type species Geoalkalibacter subterraneus(sub.ter.ra9ne.us. L. adj.sub-terraneusunderground, subterranean).Cells are non-spore-forming, strictly anaerobic and slightlyrod-shaped. Cells are Gram-negative and 0.5–0.661–5mm. Motile by polar flagella.
Geobacter	/	Total	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=28231&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cells are rod-shaped with rounded ends that grow as single cells, in pairs, or in chains. Cells are 1–4 µm × 0.6 µm, nonmotile, with no spore formation. They are Gram-negative strict anaerobes and belong to the Deltaproteobacteria. Type species: Geobacter metallireducens
FukuN57	/	Total	Uncultured	/	/	/
Formivibrio	/	Total	Unclasified	/	/	This anaerobic species has been described for strains that ferment certain organic acids, such as citrate, mesaconate and pyruvate to acetate and formate, while S-citramalate is fermented to acetate, formate and hydrogen; the G + C content of DNA is 6 1 mol YO (Tanaka et al., 1991).

Filomicrobium	/	Total	Uncultured	non	ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1608628&lvl=3&lin=f&keep=1&srchmode=1&unlock	Fusiform cells, 0.5–0.7 × 1.5–4.0 μm, with two or three polar prosthecae, which are about 0.2 μm in diameter and have a length of up to 40 μm or more. Buds are formed at the tips of the prosthecae (Fig. BXII.α.208). Nonmotile. Gram negative. Aerobic and chemoorganotrophic. Type species: Filomicrobium fusiforme
Escherichia–Shigella	Total	Spore	Uncultured	non	/	Gram-negative, facultative anaerobic, non-spore-forming, nonmotile, rod-shaped and genetically closely related to E. coli. Straight rods, 1–3 × 0.7–1.0 μm, that conform to the general definition of the family Enterobacteriaceae and contain the enterobacterial common antigen. Gram negative. Nonmotile. Non-pigmented. Facultatively anaerobic, having both a respiratory and a fermentative type of metabolism.
Enhygromyxa	Spore	/	Unclassified	yes	ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=215803&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cells are straight rods with blunt, rounded ends (So-rangium type). Gram-negative. Spherical myxosporeswith diameters 0.5–0.7 μm are formed. Vegetative cellsmove by gliding, tend to concentrate at the swarm pe-riphery and the agar gel within the swarms is sometimescleaved, but agar gel is not liquefied. Fruiting bodies areorange to brownish-orange. They are solitary aggregates,with thin slimy wall. Strictly aerobic chemoorganotrophs.Mesophilic, neutrophilic and slightly halophilic. The type species is Enhygromyxa salina.
Erythrobacter	/	Total	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1044&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cells are ovoid to rod-shaped, 0.2–0.4 × 1.0–5.0 μm, multiply by binary fission, and are motile by means of polar or subpolar flagella. Gram negative and belong to the Alphaproteobacteria. Cell suspensions and colonies are orange or pink. Type species: Erythrobacter longus
Enhydrobacter	Spore	Spore	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=553217&lvl=3&lin=f&keep=1&srchmode=1&unlock	Coccobacillary to rod-shaped cells, 0.5–0.7 × 1.0–5.0 μm. Unicellular; pairs and short chains occur. No resting stages known. Gram negative. Facultative aerobic. Chemoheterotrophic. Oligotrophic. Grow very slowly. Sugars are fermented anaerobically, and organic acids are used aerobically. Type species: Enhydrobacter aerosaccus
Ellin6067	/	Spore	Uncultured	/	/	/
Ectothiorhodospira	/	Spore	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=195064&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cells rod-shaped or vibrioid, also appearing as true spirals, 0.7–1.5 μm in diameter, motile by a polar tuft of flagella, multiply by binary fission, and may contain gas vesicles. Gram-negative and belong to the Gammaproteobacteria. Type species: Ectothiorhodospira mobilis
DSSD61	/	Total	Unclassified	/	/	/
Dokdonella	/	Spore	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1300342&lvl=3&lin=f&keep=1&srchmode=1&unlock	Dokdonella kunshanensis(kun.shan.en9sis. N.L. fem. adj.kunshanensisof pertaining to Kunshan city, Jiangsuprovince, China, from where the type strain was isolated).Cells are Gram-negative, aerobic, non-spore-forming, non-motile rods. Colonies after 3 days on R2A agar are smooth,circular, convex and yellowish. Grows with 0–1.0 % (w/v)NaCl (optimum 0.5 %), at 15–37uC (optimum 30uC) andat pH 6.0–9.5 (optimum pH 7.0)

Diaphorobacter	/	Spore	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1236961&lvl=3&lin=f&keep=1&srchmode=1&unlock	Rods, non-spore-forming, Gram-stain-negative. Motile with a single polar flagellum or nonmotile. Aerobic or facultative aerobic. Mesophilic with optimum growth conditions at a temperature around 28°C and pH 7. No special growth requirements. Catalase-positive.Type species: Diaphorobacter nitroreducens
Devosia	/	Total	Unclassified	non	/	Rods, 0.4–0.8 × 2.0–8.0 μm. Motile by means of several polar flagella. Endospores are not formed. Gram negative. Aerobic. Oxidase and catalase positive. Type species: Devosia riboflavina
Desulfuromusa	Total	Total	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=37625&lvl=3&lin=f&keep=1&srchmode=1&unlock	Slightly curved or rod-shaped cells 0.4–0.8 × 1–6 μm. Motile by means of a single, subpolarly inserted flagellum. Gram negative. Spore formation not observed. Strictly anaerobic, having mainly a respiratory type of metabolism; however, fermentative growth may occur with fumarate or malate as the sole substrate. Type species: Desulfuromusa kysingii
Desulfovirga	/	Total	Uncultured	non	/	Rod-shaped cells, 0.8–2 × 2.2–4 μm. Occur singly or in pairs. Spore formation is not observed. Stains Gram-negative. Motile by a single polar flagellum. Strictly anaerobic, having a respiratory type of metabolism. Type species: Desulfovirga adipica
Desulfurivibrio	Total	Total	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=589865&lvl=3&lin=f&keep=1&srchmode=1&unlock	The genus Desulfurivibrio was originally classified as a member of the Desulfobulbaceae family in the Deltaproteobacteria. Its closest relatives are members of the genus Desulfobulbus, which include mostly propionate-oxidizing sulfate-reducing bacteria. In contrast, Desulfirivibrio is incapable of sulfate or sulfite reduction, and its main metabolic trait is sulfur disproportionation. It is a moderately salt-tolerant and obligately alkaliphilic sulfidogenic anaerobe isolated from soda lake sediments. The genus includes a single species Desulfurivibrio alkaliphilus, which is the type species of the genus. Type species: Desulfurivibrio alkaliphilus
Desulfovermiculus	Total	/	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1121433&lvl=3&lin=f&keep=1&srchmode=1&unlock	Rod- or oval-shaped curved cells, 0.5–1.5 × 1.0–3.0 μm. Occur singly or in pairs. At stationary growth phase, cells elongate to 15–20 μm. Spore formation is not observed. Gram-stain-negative. Motility might occur depending on the species. Strict anaerobe with a respiratory type of metabolism. Mesophilic. The optimal temperature for growth is 37°C. Type species: Desulfovermiculus halophilus
Desulfotignum	Total	/	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1304886&lvl=3&lin=f&keep=1&srchmode=1&unlock	Rod-shaped, sometimes slightly curved, cells, 0.5–1.0 μm × 1.4–4 μm. Occur singly or in pairs. Spore formation is not observed. Gram-stain-negative. Motility might occur. Strictly anaerobic, having both a respiratory and a fermentative type of metabolism. Chemoorganoheterotroph or chemolithoautotroph. Type species: Desulfotignum balticum
Desulfosarcina	Total	Total	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=859321&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cells are oval-to-rod shaped. Occur singly or in pairs. Spore formation is not observed. Stain Gram-negative. Nonmotile or motile by means of a single polar flagellum. Strictly anaerobic, having both a respiratory and a fermentative type of metabolism. Mesophilic, with optimal growth temperature of 28–34°C. Type species: Desulfosarcina variabilis

Desulfofustis	Total	Total	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1121409&lvl=3&lin=f&keep=1&srchmode=1&unlock	Straight or slightly curved rods. Motile. Spore formation not observed. Gram-stain-negative. Strictly anaerobic, having a respiratory type of metabolism. Mesophilic. Optimal growth temperature, 28°C. Type species: Desulfofustis glycolicus
Desulfoconvexum	Total	/	Unclassified	non	/	Cells are curved rods or vibrioids (1.5 µm × 2.0–3.5 µm). Occur singly or in pairs. Spore formation is not observed. Gram-stain-negative. Motile. Strictly anaerobic, having a respiratory and fermentative type of metabolism. Type species: Desulfoconvexum algidum
Desulfococcus	Total	Total	Desulfococcus biacutus Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1121405&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cells are spherical or lemon-shaped, 1.4–2.3 µm in diameter. Occur singly or in pairs. Spore formation is not observed. Cells stain Gram-negative and often contain granules of poly-β-hydroxybutyrate. The cells are nonmotile. Mesophilic. The optimal temperature range for growth is 28–35°C. The optimal pH range for growth is 6.7–7.6. Type species: Desulfococcus multivorans
Desulfocapsa	Total	Total	Uncultured	non	/	Cells are round-ended or elongated rods with pointed ends, 0.8–0.9 µm × 2.0–3.5 µm. Cells are motile by means of single subpolarly inserted flagella. Gram-stain-negative. No spores are formed. Metabolism is strictly anaerobic chemoorganoheterotrophic and chemolithoautotrophic. Type species: Desulfocapsa thiozymogenes
Desulfobulbus	Total	Total	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=577650&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cells are ovoid-to-rod shaped, or lemon shaped with pointed ends, 0.6–1.5 µm × 1.5–2.5 µm. Occur singly, in pairs, or in chains. Spore formation is not observed. Stain Gram-negative. Cells are motile by a single polar flagellum or nonmotile. Strictly anaerobic, having both a respiratory and a fermentative type of metabolism. Type species: Desulfobulbus propionicus
Desulfobacterium catecholicum group	Total	Total	bacterium ROME95Asa Uncultured	/	/	/
Desulfobacterium	Total	Total	Uncultured	non	/	Cells are oval-to-rod shaped, measuring 0.7–1.5 × 2.0–2.5 µm. Occur singly or in pairs. Spore formation is not observed. Stain Gram-negative. Motile. Strictly anaerobic, having a respiratory type of metabolism. Type species: Desulfobacterium indolicum
Desulfobacca	Total	Spore	Uncultured	non	ser/wwwtax.cgi?mode=Info&id=880	Cells are oval-to-rod shaped, which occur singly or in pairs. Gram-stain-negative. Endospores are not observed. Nonmotile. Strict anaerobe with a respiratory type of metabolism. Type species: Desulfobacca acetoxidans
Desulfatitalea	Total	/	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1185843&lvl=3&lin=f&keep=1&srchmode=1&unlock	Rod-shaped cells, 0.5–0.6 µm × 1.7–3.8 µm. Occur singly or in pairs. Spore formation is not observed. Gram-stain-negative. Motile. Strictly anaerobic, having a respiratory type of metabolism.Mesophilic. Optimal growth temperature, 34–42°C. Type species: Desulfatitalea tepidiphila
Desulfatirhabdium	/	Total	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1121394&lvl=3&lin=f&keep=1&srchmode=1&unlock	Oval- to rod-shaped rods, 1–1.3 µm × 2.6–3.5 µm. Occur singly or in pairs. Spore formation is not observed. Gram-stain-negative. Strictly anaerobic, having a respiratory and fermentative type of metabolism. Type species: Desulfatirhabdium butyrativorans.

Desulfosalsimonas	Total	/	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=332175&lvl=3&lin=f&keep=1&srchmode=1&unlock	Rod-shaped cells, 0.8–1.0 µm × 2.0–4.0 µm. Occur singly or in pairs, sometimes forming filaments. Spore formation is not observed. Gram-stain-negative. Motility might occur. Strictly anaerobic, having a respiratory type of metabolism. Chemoorganoheterotroph, using short-chain fatty acids, lactate, pyruvate, fumarate, and alcohols as carbon sources and electron donors. Type species: Desulfosalsimonas propionicica
Desulforhopalus	Total	Total	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=2569543&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cells are oval-to-rod shaped with rounded ends, 1.5–1.8 µm × 3–5 µm. Longer cells occur in the stationary phase. Cells may contain gas vesicles. Occur singly, in pairs, or short chains. Spore formation is not observed. Stain Gram-negative and nonmotile. Strictly anaerobic, having both a respiratory and a fermentative type of metabolism. Type species: Desulforhopalus vacuolatus
Desulfopila	Total	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1121416&lvl=3&lin=f&keep=1&srchmode=1&unlock	Rod-shaped cells, 0.3–1.2 µm × 1.0–3.8 µm. Occur singly or in pairs. Spore formation is not observed. Stain Gram-negative. Motility might occur depending on the species. Strictly anaerobic, having a respiratory and a fermentative metabolism. Type species: Desulfopila aestuarii
Desulfonatronum	/	Total	Desulfonatronum thiosulfatophilum Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=935942&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cells are vibrios with sizes 0.4–0.9 µm × 1.0–4.0 µm, cell arrangement is single, in pairs, or in short or filamentous long chains of not separated cells, reminiscent of spirilla with a snake-like motion. Stain Gram-negative. Motile due to single polar flagellum. Strict anaerobe
Desulfonatronovibrio	Total	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1121413&lvl=3&lin=f&keep=1&srchmode=1&unlock	Gram-stain-negative, vibrio-shaped cells, occur singly or S-shaped in pairs. Motile by means of a single polar flagellum. Spore formation is not observed. Multiplication by binary fission. Strict anaerobes. Possess a respiratory, and some species also fermentative, type of metabolism. Sulfate and other partially oxidized sulfur compounds serve as terminal electron acceptors that are reduced to sulfide. Desulfonatronovibrio hydrogenovorans
Desulfonatronobacter	Total	Total	Desulfonatronobacter acidivorans bacterium YC-ZSS-LKJ160 Unclassified	non	/	Rod-shaped cells, 0.5–1.0 µm × 0.8–5.0 µm. Occur singly or in pairs. Spore formation is not observed. Stain Gram-negative. Motility might occur depending on the species. Strictly anaerobic, having a respiratory type of metabolism. Type species: Desulfonatronobacter acidivorans
Desulfomonile	/	Spore	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=706587&lvl=3&lin=f&keep=1&srchmode=1&unlock	Single rod-shaped cells with rounded ends, 0.5–0.7 × 3–10 µm, each containing an invagination of the cell wall that resembles a collar. Cells may be slightly curved or bent at the collar region. Endospores not formed. Gram-stain-negative. Nonmotile. Strict anaerobe. A medium containing a reducing agent is required for growth. Type species: Desulfomonile tiedjei
Desulfomicrobium	Total	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=525897&lvl=3&lin=f&keep=1&srchmode=1&unlock	Rod-shaped cells, 0.5–1.0 × 0.9–5.0 µm. Occur singly or in pairs. No spore formation. Gram-stain-negative. Nearly all species are motile. The genus currently accommodates six species. All Desulfomicrobium spp. are strict anaerobes with respiratory and fermentative types of metabolism. Type species: Desulfomicrobium baculatum

Desulfatiglans	Total	Spore	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1121399&lvl=3&lin=f&keep=1&srchmode=1&unlock	Rod-shaped cells that occur singly or in pairs. Spore formation is not observed. Gram-stain-negative. All species are nonmotile. Strict anaerobe with respiratory type of metabolism. Type species: Desulfatiglans anilini
Delftia	Spore	Total	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=883100&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cells are straight to slightly curved rods, 0.4–0.8 × 2.5–4.1 µm (occasionally up to 7 µm), occurring singly or in pairs. Motile by means of polar or bipolar tufts of one to five flagella. Do not produce endospores. Gram negative. Oxidase and catalase positive.Type species: Delftia acidovorans
Deltaproteobacteria bacterium RIFOXYB12_FULL_58_9	Total	/	Unclassified	/	/	/
Defluviicoccus	Spore	Total	Uncultured	non	/	roduces mucoid beige colonies<5 mm in diameter after3–4 weeks incubation at 25uC on GS medium. Gram-negative, chemoheterotrophic, non-spore-forming, non-motile and aerobic cocci/coccobacilli (mean cell diameter1?5–4?0mm), which are usually arranged in clusters ortetrads. Cells stain very faintly and appear empty afterstaining. Oxidase-negative and catalase-positive. The typespecies isDefluvicoccus vanus.
Deefgea	/	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1121375&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cells are Gram-negative, rod-shaped (0.7–0.863–4mm)and facultatively anaerobic. They occur mostly singly(Fig. 1a) and are motile by means of a single flagellum(Fig. 1b) or rarely two polar flagella. The type species isDeefgea rivuli.
Curvibacter	Spore	Spore	Unclasified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1336244&lvl=3&lin=f&keep=1&srchmode=1&unlock	Gram-negative, vibrioid or slightly curved, rod-shaped cells,0?3–0?961?1–1?8mm, with an anticlockwise helix; may ormay not have flagella. Catalase-, oxidase- and phosphatase-positive. The temperature range necessary for growth is9–40uC and the pH range for growth is 5?5–8?5. No growthoccurs in the presence of 3 % NaCl. The G+C content ofthe DNA is 62?2–66?0 mol% and the quinone type is Q-8.The major cellular fatty acids are 16 : 0, 16 : 1 and 18 : 1 andthe major 3-hydroxy fatty acid is 3-OH 8 : 0. The typespecies isCurvibacter gracilis.
Cupriavidus	Spore	Spore	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1304882&lvl=3&lin=f&keep=1&srchmode=1&unlock	Coccoid rods, 0.7–0.9 × 0.9–1.3 µm. Gram negative. Motile by two to ten peritrichous flagella. Chemoheterotrophic. An organic nitrogen source is not required. Glucose not utilized. Strictly respiratory metabolism with oxygen as the terminal electron acceptor. Oxidase positive. Type species: Cupriavidus necator
Crenothrix	/	Spore	Uncultured	/	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=360316&lvl=3&lin=f&keep=1&srchmode=1&unlock	Two stratified freshwater lakes a substantial part of upward-diffusing methane was oxidized by filamentous gamma-proteobacteria related to Crenothrix polyspora. These filamentous bacteria have been known as contaminants of drinking water supplies since 1870, but their role in the environmental methane removal has remained unclear.

Coxiella	/	Total	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=360116&lvl=3&lin=f&keep=1&srchmode=1&unlock	Strictly intracellular bacteria, usually 0.2–0.4 µm × 0.4–1.0 µm. Best stained by Gimenez staining. They have no flagella or capsule. They live in close natural association with arthropod and vertebrate hosts. Type species: Coxiella burnetii
Craurococcus	Spore	/	Uncultured	non	/	Cells are coccoid, 0.8–2.0 µm in diameter, nonmotile, Gram negative, and divide by binary fission. Form pink irregular colonies when grown on agar media. Contain bacteriochlorophyll (BchI) a , spirilloxanthin and carotenoic acids. No growth occurs anaerobically in the light. Type species: Craurococcus roseus
Corallococcus	/	Total	Unclassified	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1144275&lvl=3&lin=f&keep=1&srchmode=1&unlock	Vegetative cells are slender rods with tapering ends, cigar-shaped, 0.6–0.8 × 3–8 µm. Myxospores spherical or ellipsoidal, often somewhat irregular, 1.3–2.4 µm in diameter. Fruiting bodies very variable in shape and size even within one culture: pustules, ridges, slabs, and irregular disks, often with finger-like projections, or raised and bizarre coralloid, of cartilaginous consistency. Type species: Corallococcus coralloides
Constrictibacter	Total	/	Unclasified	non	/	A member of the classAlphaproteobacteria, orderRhodospirillales, familyRhodospirillaceae. Cells are ovoidto rod-shaped and often occur in pairs or chains. Cells aremotile and do not form spores. Grows aerobically ormicro-aerobically. The type species isConstrictibacter antarcticus.
Comamonas	Spore	Spore	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1219032&lvl=3&lin=f&keep=1&srchmode=1&unlock	Straight or slightly curved rods or spirilla, 0.3–0.8 × 1.1–4.4 µm; occasionally longer (5–7 µm), irregularly curved cells or spirilla may occur. Cells occur separately or in pairs and are motile by means of polar or bipolar tufts of 1–5 flagella except for C. koreensis, which is nonmotile. Gram negative. Type species: Comamonas terrigena
Chthonobacter	/	Total	Unclassified	non	/	Cells are Gram-stain-negative, non-motile, non-gliding, aer-obic, oligotrophic and rod-shaped. Catalase and oxidase arepositive. Type strain Chthonobacter albigriseus Colonies are greyish-white after culti-vation on R2A, TSA or NA at 30C for 3 days. Growthoccurs at 20–42C
Chitinilyticum	/	Total	Chitinilyticum aquatile	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1121275&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cells are Gram-negative rods, motile by means of singlepolar flagella. PHB granules are stored as reserve material.Endospores are not formed. The type species isChitinilyticum aquatile. The type species isChitinilyticum aquatile
Chitinibacter	/	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1121274&lvl=3&lin=f&keep=1&srchmode=1&unlock	Strictly aerobic, Gram-negative rods that are straight toslightly curved (Fig. 2a) with round ends, 1?3–2?6mminlength and 0?5–0?9mm in width. Highly motile by meansof one polar flagellum or two polar flagella (Fig. 2b, c).Oxidase- and catalase-positive. The type species isChitinibacter tainanensis.
Caulobacter	/	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=190650&lvl=3&lin=f&keep=1&srchmode=1&unlock	Cells single, unbranched, with tapered poles; long axis typically distinctly curved, conferring a vibrioid shape, but may be straight in some isolates and the cell shape fusiform; 0.4–0.6 × 1–2 µm during growth in most media. Type species: Caulobacter vibrioides
Candidatus Paracaedibacter	/	Spore	Unclassified	/	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=91604&lvl=3&lin=f&keep=1&srchmode=1&unlock	/

Candidatus Symbiobacter	Total	/	Unclassified	/	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1436290&lvl=3&lin=f&keep=1&srchmode=1&unlock	/
Candidatus Nucleicultrix	Spore	Spore	Unclassified	/	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1414854&lvl=3&lin=f&keep=1&srchmode=1&unlock	Here, we report on the discovery of a bacterial symbiontthat is located inside the nucleus of itsHartmannellasp. host. This symbiont, tentatively named'CandidatusNucleicultrix amoebiphila', is only moderately related to known bacteria (B90% 16S and23S rRNA sequence similarity) and member of a novel clade of protist symbionts affiliated with theRickettsialesandRhodospirillales.
Candidatus Contendobacter	/	Total	Uncultured	/	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1400861&lvl=3&lin=f&keep=1&srchmode=1&unlock	The glycogen-accumulating organism (GAO) 'CandidatusCompetibacter' (Competibacter) usesaerobically stored glycogen to enable anaerobic carbon uptake, which is subsequently stored aspolyhydroxyalkanoates (PHAs).
Candidatus Competibacter	Total	Total	Uncultured	/	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1400863&lvl=3&lin=f&keep=1&srchmode=1&unlock	The glycogen-accumulating organism (GAO) 'Candidatus Competibacter' (Competibacter) uses aerobically stored glycogen to enable anaerobic carbon uptake, which is subsequently stored as polyhydroxyalkanoates (PHAs). This biphasic metabolism is key for the Competibacter to survive under the cyclic anaerobic-'feast': aerobic-'famine' regime of enhanced biological phosphorus removal (EBPR) wastewater treatment systems. As they do not contribute to phosphorus (P) removal, but compete for resources with the polyphosphate-accumulating organisms (PAO), thought responsible for P removal, their proliferation theoretically reduces the EBPR capacity.
Candidatus Alysiosphaera	Total	Total	Unclassified	/	/	Thiothrix, Meganema, Ca. Alysiosphaera and others, are primarily known to be problematic in WWTPs treating industrial wastewater as well as in plants without nutrient removal (Van Der Waarde et al., 2002).
Candidatus Accumulibacter	/	Total	Uncultured	/	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=327159&lvl=3&lin=f&keep=1&srchmode=1&unlock	is a common bacterial community member of wastewater treatment plants performing enhanced biological phosphorus removal[1] and is a polyphosphate-accumulating organism.
C1–B045	/	Total	Uncultured	/	/	C1-B045 clade that were first detected in deep-sea hydrothermal sediments (Teske et al., 2002).
Burkholderia–Caballeronia–Paraburkholderia	Spore	Spore	Uncultured	/	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?name=Paraburkholderia+caballeronis	The division of this group into Burkholderia, Caballeronia, Paraburkholderia, and Robbsia is strongly supported by genome analysis. These new genera broadly correspond to the various habitats/lifestyles of Burkholderia s.l., e.g., all the plant beneficial and environmental (PBE) strains are included in Paraburkholderia (which also includes all the N2-fixing legume symbionts) and Caballeronia, while most of the human and animal pathogens are retained in Burkholderia sensu stricto.
BRH–c57	/	Spore	Uncultured	/	/	/
Brevundimonas	Spore	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1255603&lvl=3&lin=f&keep=1&srchmode=1&unlock	Gram-negative, rod-shaped, subvibrioid or vibrioid cells, 0.4–0.5 × 1–2 µm. Cells of some species can form prosthecae (stalks). These species are characterized by an asymmetric cell division whereby fission results in a prosthecate (nonmotile) and a flagellated (motile) cell. Motility by means of single polar flagella. Nonsporeforming. A yellow or orange carotenoid pigment may be formed. Type species: Brevundimonas diminuta
Bradyrhizobium	Spore	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1304877&lvl=3&lin=f&keep=1&srchmode=1&unlock	Rods 0.5–0.9 × 1.2–3.0 µm. Commonly pleomorphic under adverse growth conditions. Usually contain granules of poly-β-hydroxybutyrate that are refractile by phase-contrast microscopy. Nonsporeforming. Gram negative. Motile by one polar or subpolar flagellum. Fimbriae have not been described. Aerobic, possessing a respiratory type of metabolism with oxygen as the terminal electron acceptor. Optimal temperature 25–30°C. Optimal pH, 6–7, although lower optima may be exhibited by strains from acid soils. Type species: Bradyrhizobium japonicum
Bosea	/	Spore	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=53254&lvl=3&lin=f&keep=1&srchmode=1&unlock	Rod shaped cells, 0.85 × 1.4–1.6 µm. Gram negative. Aerobic. Occurring singly. Motile by a single polar flagellum. Optimal temperature for growth: 30–32°C, range; 20–37°C. Optimal pH: 7.5–8.0; range, 6.0–9.0. Chemolithoheterotrophic, able to obtain energy from the oxidation of reduced sulfur compounds in the presence of organic carbon. No autotrophic growth occurs. Catalase- and oxidase-positive. Found in cultivated soil. Type species: Bosea thiooxidans
Blastomonas	/	Total	Unclassified	non	ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=34015&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Cells are ovoid or rod-shaped and reproduce by budding or asymmetric cell division. They occur singly or in pairs and may form rosette-like aggregates. No stalks and prosthecae are found. Gram negative. Nonsporeforming. Motile by means of polar flagella. Strictly aerobic. Chemoorganotroph and facultative photoorganoheterotroph. No growth occurs under anaerobic conditions in the light. Habitat is fresh water.Type species: Blastomonas natatoria
Blyi10	/	Total	Uncultured	/	/	/
Bdellovibrio	/	Total	Uncultured	/	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=264462&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Vibrios, 0.20–0.4 × 0.5–1.4 µm. Motile by a single, polar, sheathed flagellum. Gram negative. Prey upon Gram-negative bacteria. Have a predatory lifestyle characterized by two distinct phases, a free-living attack phase and an intraperiplasmic growth phase. Aerobic, having a strictly respiratory type of metabolism with oxygen as the terminal electron acceptor. Contain cytochromes a, c, and b. Mesophilic. Oxidase positive. Catalase variable. Occur in fresh and salt water, soil, and sewage. Type species: Bdellovibrio bacteriovorus
Bauldia	Total	Total	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=665467&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Gram-negative, aerobic heterotrophic budding bacteria with cellular appendages (prosthecae) less than 0.65 µm in length. Found in brackish and marine aquatic habitats as well as soils. Aerobic. Nonmotile. Type species: Bauldia litoralis

Bacteriovorax	/	Total	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=960&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Members of the genus Bacteriovorax exhibit the same general morphological and life cycle features as described for the genus Bdellovibrio. However, genetically the two genera vary considerably, with Bacteriovorax sp. having a lower mol% G + C content, little to no DNA–DNA hybridization (<4%), and <82% rDNA similarity with Bdellovibrio . Type species: Bacteriovorax stolpii
Azoarcus	/	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=29545&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Straight to slightly curved rods, 0.4–1.5 × 1.1–4.0 µm. Cell pairs often appear slightly S-shaped. In most species, some elongated cells (8–12 µm) occur in late-log or stationary-phase cultures on medium containing malic acid and N2 or ammonium as nitrogen source. Cells are highly motile by means of one polar flagellum. Accumulate poly-β-hydroxybutyrate granules. Gram negative. Some species are nitrogen fixers; these require microaerobic conditions for growth on N2. On semisolid nitrogen-free media, microaerophilic growth can be observed as veil-like pellicles developing several mm below the surface and moving to the medium surface during growth. In most species, colonies on VM agar1 supplemented with ethanol develop a nondiffusible yellowish pigment. Optimal temperature for growth 30–40°C; no growth occurs at 45°C. Type species: Azoarcus indigens
Aurantimonas	/	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=287752&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Aurantimonas manganoxydans. Cells are Gram negative, motile short rods that occur singly or in groups often arranged in stacks. The cell dimensions are 0.9 – 1.2 µm long and 0.5 – 0.8 µm wide. The cells form circular, opaque, convex colonies with smooth edges and develop a central brown colour in the presence of manganese. Manganese oxidation occurs after 192 to 240 hours and the organism will only oxidize manganese when the incubation temperature is below 30 °C. The organism grows optimally at 30 °C in a marine media with NaCl concentrations of 2.5 % (w/v).
Ascidiaceihabitans	/	Total	Unclassified	non	ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1510460&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Cells are Gram-stain-negative, aerobic, non-flagellated andcoccid, ovoid or rod-shaped. Catalase- and oxidase-positive.Nitrate is not reduced to nitrite. The type species is Ascidiaceihabitans donghaensis. collected from the East Sea at Yangyang, South Korea
Arenimonas	/	Total	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1121013&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Cells are Gram-negative, non-spore-forming, motile rodswith single polar flagellum. Cells are mesophilic andneutrophilic. Cells are oxidase-positive but catalase-negative. type species isAspromonas composti.
Aquimonas	/	Total	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1121008&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Gram-negative, strictly aerobic, mesophilic, motile rods,occurring singly or in pairs. Oxidase- and catalase-positive,but negative for H2S production, acid production fromD-cellobiose and growth on maltose. The type species isAquimonas voraii.
Aquicella	Spore	Total	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=254246&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Rod-shaped cells, 0.4–0.8 µm in width, and 1.8–2.3 µm in length; filaments are also formed. Endospores are not observed. Stain Gram-negative. Motility and flagella are not observed. Type species: Aquicella lusitana
Aquamicrobium	/	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1460425&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	nonmotile. Respiratory metabolism using either O2 or nitrate under anoxic conditions. Nitrate reduced to nitrite or N2 . Mesophilic. Optimal pH 6–9. Salt tolerant, up to 3% NaCl. Utilize sugars, fatty acids, and heterocyclic aromatic compounds as carbon sources. Utilize nitrate or O2 as electron acceptors. Type species: Aquamicrobium defluvii
Aquabacterium	Spore	/	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=70586&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	All members of the genus are Gram-negative, rod-shaped bacteria and are motile by means of monotrichous flagella. They contain polyalkanoate and polyphosphate inclusion bodies and produce fibrillar matrix material. Some are not motile. The type species is Aquabacteriuni commune
Anaeromyxobacter	/	Total	Uncultured	yes	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=455488&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	This genus consists of myxobacterial species that are capable offacultative anaerobic growth using terminal electron acceptorssuch as nitrate, fumarate, and chlorophenolic compounds. Sul-fur compounds are not reduced. Type species Anaeromyxobacter dehalogenans
Amphiplicatus	/	Total	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1519374&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Cells are Gram-strain-negative, thermophilic and motilewith a single flagellum. Strictly aerobic. No spores areobserved. Catalase- and oxidase-positive. Nitrate is reducedto nitrite. The type species isAmphiplicatus metriothermophilus.
Aminobacter	/	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=83263&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Rods, 0.5–0.9 × 1.0–3.0 µm, with rounded ends. Occur singly (rarely in pairs). Motile by means of subpolar flagella. Nonsporeforming. Gram negative. Reproduction occurs by budding. Type species: Aminobacter aminovorans
Amaricoccus	Spore	Total	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=57001&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Cocci, 1.3–1.8 µm in diameter, usually arranged in tetrads. Gram negative. Nonmotile. Do not form spores or store polyphosphate granules. Oxidase positive. Aerobic, having a strictly respiratory type of metabolism with oxygen as the terminal electron acceptor. Growth occurs at 20–37°C and at pH 5.5–9.0. Chemoheterotrophic. A variety of organic compounds can be used as carbon sources, including D-fructose, glucose, mannitol, L-glutamic acid, D-mannose, D-sorbitol, sucrose, L-rhamnose, L-arabinose, L-leucine, and L-histidine. Isolated from activated sludge and sewage. Type species: Amaricoccus kaplicensis

Aeromonas	/	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=642&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Aeromonas are flagellated, facultative, anaerobic, non–spore-forming, catalase- and oxidase-positive, gram-negative rods in the family Aeromonadaceae. Gastroenteritis is the major clinical illness associated with Aeromonas species, although causation and estimates of disease burden are controversial.
Actinobacillus	/	Spore	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1120931&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Actinobacillus are Gram-negative small rods, occurring singly, in pairs or rarely in chains. In Gram-stained smear, the bacteria show both bacillus and coccus form together, known as 'morse code' appearances (dots and dashes appearance). The bacteria are nonmotile (both at room temperature and 37°C), nonsporing and nonacid fast.
Acinetobacter	Spore	Total	Unclassified	non	ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=469&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Acinetobacter spp. are ubiquitous Gram-negative coccobacilli and nonmotile (displaying a 'twitching motility' presumably due to polar fimbriae). Acinetobacter spp. are widely distributed in nature, in soil and water, as free-living saprophytes. They are found in virtually 100% of soil and fresh-water samples (less than 1–7.9×104 cfu 100 ml−1). They utilize a wide variety of substrates as sole carbon source in nature, plants, food, and foodstuffs (source for human carriage).
Acidovorax	/	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=2743470&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Cells are straight to slightly curved rods, 0.2 to 0.7 by 1.0 to 5.0 pm. They occur singly or in short chains and are motile by means of a single polar flagellum. Gram negative. Oxidase positive. Urease activity varies among strains. Some strains grow on Christensen urea agar, but lack urease according to API 20NE tests. No pigment is produced on nutrient agar. Aerobic. The type species is Acidovorax facilis
Acidiphilium	/	Total	Uncultured	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=349163&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Cells are straight rods, 0.3–1.2 × 4.2 µm, multiply by binary fission and exhibit a pleomorphic tendency at varied pH values and in the presence of different carbon sources. Motile by means of polar, subpolar, and lateral flagella. Do not form spores or capsules. Gram negative. 16S rRNA structures and signatures conform to the Alphaproteobacteria . Cell suspensions and colonies are white to cream, yellow, pink, red, or brown. Strictly aerobic, chemoorganotrophic and chemolithotrophic bacteria containing photosynthetic pigments, categorized into the aerobic bacteriochlorophyll-containing bacteria. Type species: Acidiphilium cryptum
Acidibacter	/	Total	Uncultured	non	/	Cells occur as single and paired non-motile rods,1.5–2.5lm in length, and stain Gram-negative. Does notform endospores. Acidophilic, mesophilic and obligatelyheterotrophic. Capable of reducing ferric iron. The type species isAcidibacterferrireducens
Achromobacter	Spore	Spore	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=762376&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Straight rods, 0.8–1.2 × 2.5–3.0 µm with rounded ends. Gram negative. Nonsporeforming. Motile with 1–20 sheathed flagella arranged peritrichously. Obligately aerobic and nonfermentative. Some strains are capable of anaerobic respiration with nitrate as the electron acceptor. Type species: Achromobacter xylosoxidans
Altererythrobacter	/	Total	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=361183&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Cells are Gram-negative and non-motile and the colour ofcell suspensions and colonies is yellow. Produce oxidaseand catalase. In addition to the description of the genus, the followingproperties are displayed. The typespecies isAltererythrobacter epoxidivorans. Cells are pleomorphic and 0.42–0.5860.58–0.63 (or 0.67–1.25)mm in size. Methanol-soluble pigment is characterized by absorption maxima at310, 447 and 473 nm. Growth occurs between 20 and 40uC(optimum 35uC), at pH 6–8.5 (optimum pH 6.5) andin the presence of 0.5–9 % NaCl (optimum 2 %) witheither 0.18 % (w/v) CaCl2or 0.59 % (w/v) MgCl2
alphal cluster	/	Total	Unclassified	/	/	/
Allorhizobium–Neorhizobium–Pararhizobium–Rhizobium	/	Total	Unclasified	/	/	TheresultsshowedthatR.galegae,R.vignae,R.huautlense,andR.alkalisoliformedaseparatecladethatclearlyrepresentedanewgenus,forwhichthenameNeorhizobiumisproposed.Agrobacteriumwasshowntorepresentaseparateclusterofmainlypathogenictaxaofthefamily Rhizobiaceae.A.vitisgroupedwithAllorhizobium,distinctfromAgrobacterium,andshouldbereclassifiedasAllorhizobiumvitis,whereasRhizobiumrhizogeneswasconsideredtobethepropernameforformerAgrobacteriumrhizogenes.Thisphylogeneticstudyfurtherindicatedthatthetaxonomicstatusofseveraltaxacouldberesolvedbythecreationofmorenovelgenera. http://dx.doi.org/10.1016/j.syapm.2013.12.007
Allochromatium	/	Total	Unclassified	non	ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=572477&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Cells straight to slightly curved rods, single or in pairs, multiply by binary fission, motile by polar flagella, Gram negative, belong to the Gammaproteobacteria, and contain internal photosynthetic membranes of vesicular type in which the photosynthetic pigments bacteriochlorophyll a and carotenoids are located. Type species: Allochromatium vinosum
Aliiroseovarius	Spore	Spore	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1173584&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Cells are Gram-stain negative, aerobic, ovoid- to rod-shaped and non-motile or motile by flagellum. Colonieson MA are smooth, convex and cream, pink, pinkish-beige, greenish-yellow or greyish-yellow. Bchlacan beproduced. Growth occurs in a wide range of NaClconcentrations (0.5–7.0 %). The type species isAliiroseovarius pelagivivens.
Aetherobacter	/	Total	Unclassified	yes	/	Aerobic and mesophilic members of theMyxococcales.Vegetative cells are phase-dark, long and slender rods(1.0–1.3 |2.9–6.0mm). Movement occurs by gliding onsurfaces. Swarm forms ring- or halo-like colonies in yeastagar. Colony edges are characterized by coherent migratingcells that penetrate deep into the medium. Myxospores are slightly refractive rods with blunted ends, shorter than thevegetative cells and enclosed in a sporangial wall. Fruitingbodies contain tiny ovoid sporangioles, usually compactor clustered. The type speciesis Aetherobacter fasciculatus.

	Xanthomonadaceae bacterium	/	Spore	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1288954&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Members of this genus are short Gram-negative rods of linear shape, which are generally 0.4–0.7 µm wide and 0.8–2 µm long. The bacteria exist singularly or in pairs, and they are motile due to a single flagellum (~1.7–3.0 µm long). These bacteria have a GC content of 63.3–69.7 mol.%. They are catalase positive, urease, oxidase negative (or weakly positive), nondenitrifying or nitrate reducers, and they can produce H2S but not indole or acetoin. The type species of the genus viz. S. maltophilia was first isolated from human pleural fluid and named as Bacteriaum bookeri by J. L. Edwards.
	Bacteriovorax sp.	/	Spore	Unclassified	non	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=960&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock	Members of this genus exhibit a biphasic life cycle,with the potential of displaying an actively predaciousform as well as a PI, saprophytic form capable ofgrowing on nutrient medium. Prey-dependent (wild-type) strains are comma-shaped rods, 0±5±1±4lminlength which demonstrate a predatory lifestyle in thepresence of susceptible prey bacteria. Type species Bacteriovorax stolpii

Chapter 3 — Assessment of Fungal spores and spore-like diversity in Environmental Samples by Targeted Lysis

Foreword:

This chapter will be submitted to the BMC Microbiology journal. Here, the lysis-resistant enrichment method was applied to lake sediments and microbial mats, to assess the environmental diversity of lysis-resistant fungal cells. My personal contribution as co-first author was sampling, sample processing, data analysis of the environmental fungal community and writing of the manuscript.

Assessment of Fungal spores and spore-like diversity in Environmental Samples by Targeted Lysis

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Abstract

Spores are a vital part of the life cycle of fungi with essential roles in ecology and reproduction. Spores help fungi not only to disperse to other environments but also to survive long periods of time awaiting favorable growth conditions. Fungal spores are part of the microbial seed banks, which have been shown to affect the microbial community composition, and contribute to the maintenance of diversity. However, until now methods to study the diversity of fungal spores target those dispersing in the air and are time consuming culture-based approaches. In this study, we assessed and compared the total and fungal seed bank diversity in the polyextreme environment of the Salar de Huasco, a high-altitude athalassohaline wetland in the Chilean Altiplano. To define the fungal seed bank, we applied a separation method based on lysis-resistance to enrich fungal spores and spore-like cells to obtain a proxy of the composition of the fungal seed bank. This approach was first evaluated in selected species, the results obtained showed that DNA from fungal spores and from yeast is only obtained after the application of the lysis-resistant enrichment method, while mycelium is always lysed. After the validation, environmental samples were collected from a salt flat and from microbial mats in small surrounding ponds. Both the lake sediments and microbial mats were dominated by Ascomycota and Basidiomycota, however, the diversity and composition of each environment significantly differed. Members of the phylum Chytridiomycota were enriched in the lysis-resistant fraction, while members of the phylum Rozellomycota were never detected in this fraction. Moreover, we show that the community composition of the lysis-resistant fraction

reflects the diversity of life cycles and survival strategies developed by fungi in the environment. To the best of our knowledge this is the first time that the fungal diversity is explored in the Salar de Huasco. In addition, the method presented here provides a simple and culture independent approach to assess the diversity of fungal lysis-resistant cells in the environment.

1. Introduction

The majority of fungal species form single or multicellular specialized structures to reproduce and as a strategy to survive unfavorable environmental conditions. These structures are a central structure in the life cycle of most fungal species. The most common strategy for survival and dispersal is the formation of spores. Fungal spores are specialized dormant cells that serve different purposes, as means of reproduction, dispersal, and survival (1–3). Accordingly, there is a large diversity of fungal spores that vary in shape, mode of formation, and state of dormancy, as well as in their level of stress resistance (4). Many fungal species form different types of spores within their life cycle, with sexual spores being used for resistance and asexual spores for clonal dispersion. However, both types are known to be resistant to abiotic and biotic stress factors, depending on the species (3). For example, the asexual spores (microconidia) of *Fusarium oxysporum* are as resistant to antifungal as vegetative cells (5), whereas the sexual spores of other species, like those of *Aspergillus spinosus*, *Paecilomyces variotii*, and *Talaromyces macrosporus,* show a heat-resistance similar to that of bacterial endospores (6). Along spores, fungi also exhibit a vegetative phase which may be unicellular, such as yeasts, or pluricellular, such as in the case of mycelial fungi. Some fungi can even transit from the yeast to the (pseudo)-hyphal stage, an ability termed dimorphism. This dimorphism is also part of their resistance to abiotic or biotic stresses. In this case, the yeast stage may be considered as an intermediate between spores and an actively metabolizing vegetative cell in regard to resistance. This is in particular the case of the so-called black fungi that are considered poly-extremophiles and among the most stress-resistant eukaryotes on Earth (7,8). Finally, in addition to spores, fungi also form resistance tissue called sclerotia that can remain latent in the environment for years. Some of those are minute structures that cannot be seen macroscopically (microsclerotia) (9).

Up to now, the majority of studies about the diversity of fungal spores in the environment have been focused on the fraction of fungal spores suspended in the air (10–14). While studies on the fungal diversity in soils, target the diversity of mycorrhiza or fungal pathogens or the overall diversity (15–20), making no distinction between mycelium and spores. On the other hand, studies that do target spore diversity (e.g. in the case of mycorrhizal fungi) apply methods such as spore washing (21,22), which are time consuming and susceptible to contamination during the multiple manipulation steps.

Fungal spores can survive long periods of time in the environment, becoming part of microbial seed banks, which have been shown to influence the microbial composition of an environment and to contribute to the maintenance of diversity (3,23). Thus, the development of more efficient methods that allow the assessment of the dormant fraction of fungi in non-aerial samples would enable the study of fungal population shifts and the diversity of the fungal seed banks in soil, water, microbial mats, and other types of environmental samples. In this study, we assessed whether a method previously validated for the physical separation of bacterial endospores by selective lysis (24), could also be used to investigate the diversity of fungal spores (and spore-like cells) in environmental samples, as a proxy to the fungal seedbank. This method uses physical and chemical treatments to lyse non-resistant cells and to enrich lysis-resistant structures. The procedure results in the enrichment of endospores from Firmicutes, but also lysis-resistant cell forms from other bacteria (25). Moreover, the use of the lysis-enrichment method has been recently extended to study lysis-resistant communities in archaea. While in bacteria, lysis-resistance was shown to be restricted to a few groups, in archaea, resistance to lysis was widely spread (26). This is likely due to the specific cellular adaptations (i.e., lipid composition, diversity of cell envelope composition) within this domain (27–30).

Taking into account the diversity in morphology, function, formation, and resistance of fungal specialized resistant structures (spores, sclerotia or zoospores), we hypothesized that lysis-resistance in fungi will be widespread, thus reflecting not only the presence of resistance structures, but also the multitude of life cycles in fungi. To test this hypothesis, we first validated the lysis-resistance enrichment method for its application in fungi using pure cultures of mycelium and resistant structures (sexual and asexual spores and yeast stage) from various clades: *Ulocladium alternata*, *Candida albicans*, *Aspergillus niger, Coprinopsis cinerea,* and *Mortierella antarctica.*

These fungi were selected to represent different life cycles and types of specialized resistant structures. After validation, we applied the method to environmental samples, with the aim of assessing the abundance and diversity of fungal resistant structures. The samples were collected in the polyextreme environment of the Salar de Huasco. The Salar de Huasco is a high-altitude athalassohaline wetland located in the Chilean Altiplano at an altitude of 3,800 m.a.s.l. In addition to the high altitude, daily temperature changes from - 10° to + 25°C, high solar radiation < 1100 W/m^2, and a negative water balance, are other extreme environmental conditions in this ecosystem (31,32). Furthermore, it has been shown that in this salt flat, there is a high biodiversity of endemic bacteria and archaea (33–35). Nevertheless, the fungal diversity has never been explored in these habitat, even though fungi are now recognized to be key microbial players in extreme environments (36).

2. Results

2.1. Effect of the lysis-resistant enrichment method on pure cultures

The resistance to lysis was evaluated based on the amount of DNA obtained after the lysis-resistant enrichment method (Additional Table 2) compared to the DNA extracted directly for each fungal species. Our results show that the amount of DNA extracted depended on the type of sample (mycelium, spores or yeast form), as well as on the type of extraction performed (direct DNA extraction or extraction after the lysis-resistant enrichment treatment). Here is important to remind the reader that due to the differences in growth of the mycelium and size of the spores, as well as differences in DNA concentration per cell, the DNA concentration obtained cannot be compared between fungal species. Both for *A*. *niger* and *U*. *alternata*, DNA was obtained from spores only after the lysis-resistant enrichment treatment (Fig. 1A and B), while no DNA was recovered from the mycelium samples with either extraction method. Similarly, DNA from the sexual and asexual spores of *C*. *cinerea*, was only recovered after the lysis-resistant enrichment treatment , and mycelial DNA was only recovered with the direct DNA extraction method (Fig. 1C). Concerning *M*. *antarctica*, no DNA was recovered from the spores, and DNA from the mycelium samples was only obtained by the direct DNA extraction method (Fig. 1D). On the other hand, for *C*. *albicans*, which was used only under its yeast stage, a substantial amount of DNA (3.56 ng/μl) was recovered using the direct DNA extraction method, while only a small

amount of DNA (0.172 ng/μl) was recovered using the lysis-resistance method (Fig. 1E). Microscopical images showed that spores can resist the lysis-resistance enrichment method (Fig. 1). In the case of *M. antarctica*, the quantity of spores was too small to allow for their detection through DNA extraction or microscopically from the filter.

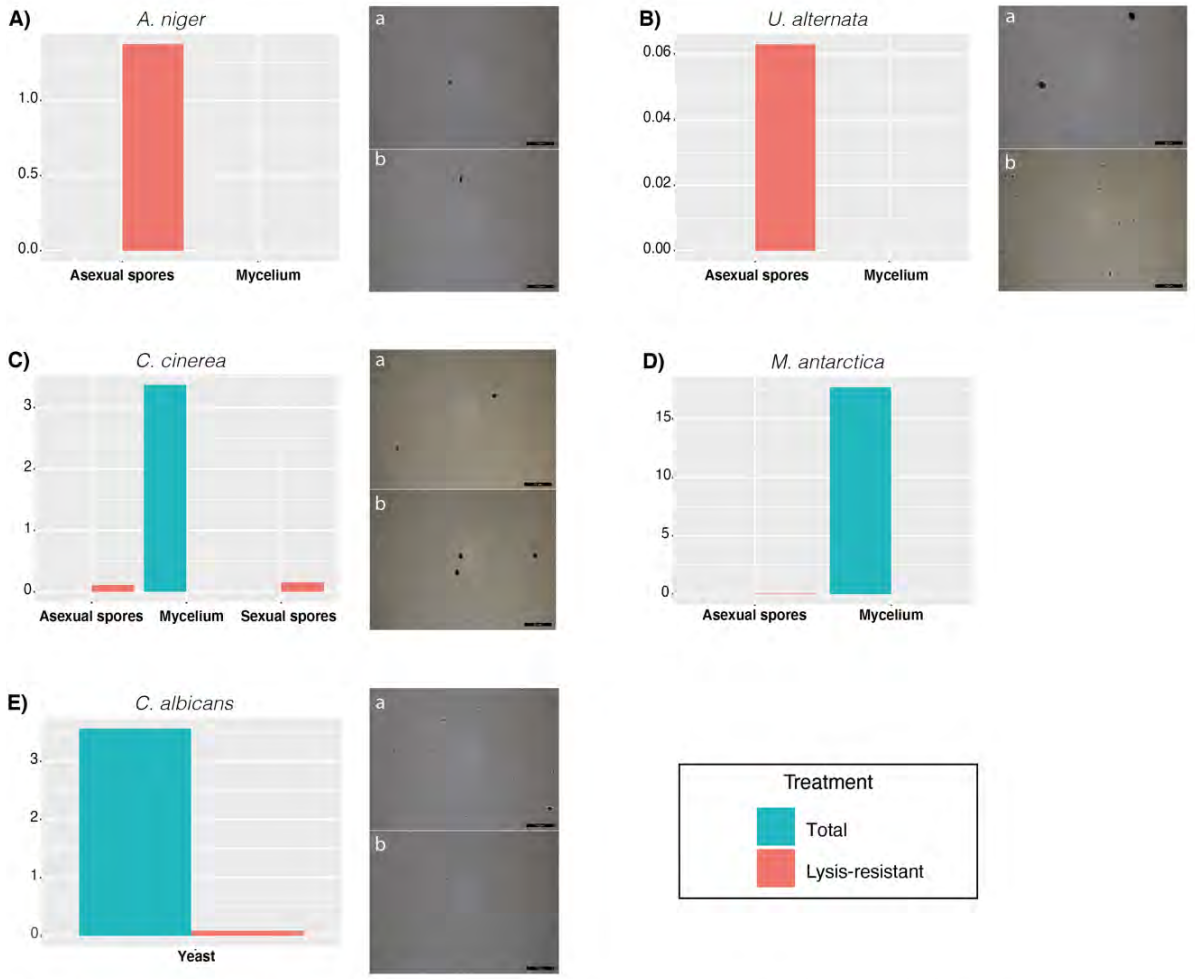

Figure 1. Validation of the lysis-resistant enrichment method. Barplot showing the DNA concentration in ng/ul from the direct DNA extraction (total in blue) and after the application of the lysis-resistant enrichment method (lysis-resistant in red) for mycelium, sexual spores, asexual spores and/or yeast of different fungal species. **A)** *Aspergillus niger*; **B)** *Ulocladium alternata*; **C)** *Coprinopsis cinerea*; **D)** *Mortierella antarctica*; **E)** *Candida albicans*. For A, B, C, and E: Microscopy pictures of the filters containing the spores used for direct extraction (a) or after the lysis-enrichment method (b) are shown. Due to the low spore concentration in Mortierella antarctica, which is also reflected in the low DNA obtained, no microscopic images could be obtained from this fungus.

2.2. Fungal diversity in the Salar de Huasco

At the Salar de Huasco, samples were collected from two different environments, lake sediments from the main saline lake and microbial mats from small ponds surrounding the main lake (Figure 2A). From the main saline lake, seven sediment core samples were collected along a salinity gradient ranging from 53 to 0.706 PSU. The pH in the

lake ranged between 8.22 and 8.43 (not following a specific gradient). From the small ponds surrounding the main saline lake, 20 microbial mat samples were collected from 18 ponds randomly selected. The samples varied in color, texture, and location. The pH in these ponds ranged between 7.67 and 9.74, and the salinity from 0.41 to 4.22 PSU. In both types of environments (lake sediments and microbial mats), the fungal diversity was assessed for the total fraction (direct DNA extraction) and for the lysis-resistant fraction (after the lysis-resistant enrichment method) (Fig. 2B). Representatives of the Ascomycota and Basidiomycota were the most abundant in the total community fraction in both environments. Furthermore, representatives of Chytridiomycota were present at a higher relative abundance in the microbial mats than in the lake sediments, while the contrary was observed for ASVs assigned to the Rozellomycota phylum. ASVs assigned to the phyla Mucoromycota, Monoblepharomycota, and Glomeromycota, were only present in the microbial mats, but their relative abundance was low (below 0.04; Fig. 2B). Moreover, although representatives of the Aphelidiomycota phylum were present in both environments, their relative abundance in the microbial mats was below 0.0014 and cannot be observed in figure 2B. Another difference between the fungal communities in lake sediments and microbial mats was the large fraction of unidentified ASVs present in the latter, as compared to the lake sediments, in which the unidentified fraction is almost negligible. When comparing the composition of the total and the lysis-resistant fraction of each environment only a few differences could be observed. The relative abundance of the Basidiomycota and Chytridiomycota was higher in the lysis-resistant fraction, while the phylum Rozellomycota was only present in the total community of both types of environments.

From this first assessment, we decided to evaluate each environment individually, to reduce the effect of the environment type and to better assess the effect of the enrichment method in the characterization of the lysis resistant community. This decision was supported by a PCoA analysis of the total community, which showed a clear separation of both environments (Fig. 3A), with the microbial mats samples presenting a broader dispersion than the lake sediment samples. Moreover, the beta diversity was statistically different (*p*-value 0.0202) between the two environments. However, a PCoA analysis of the lysis-resistant community showed an overlap of the two environments, with the microbial mat samples again showing a broader

distribution (Fig. 3B), and the beta diversity was not statistically different (*p*-value 0.327). This indicated that the lysis-resistant fraction is similar in both environments. Accordingly, the Venn Diagram in figure 3C shows that 21.84% of the ASVs were shared among the lysis-resistant fractions (green and blue ovals) of both environments and only 17.49% among the total community (red and orange ovals). However, considering the distinct biotic and abiotic environmental conditions of the lake sediments and microbial mats, the two environments were analyzed separately.

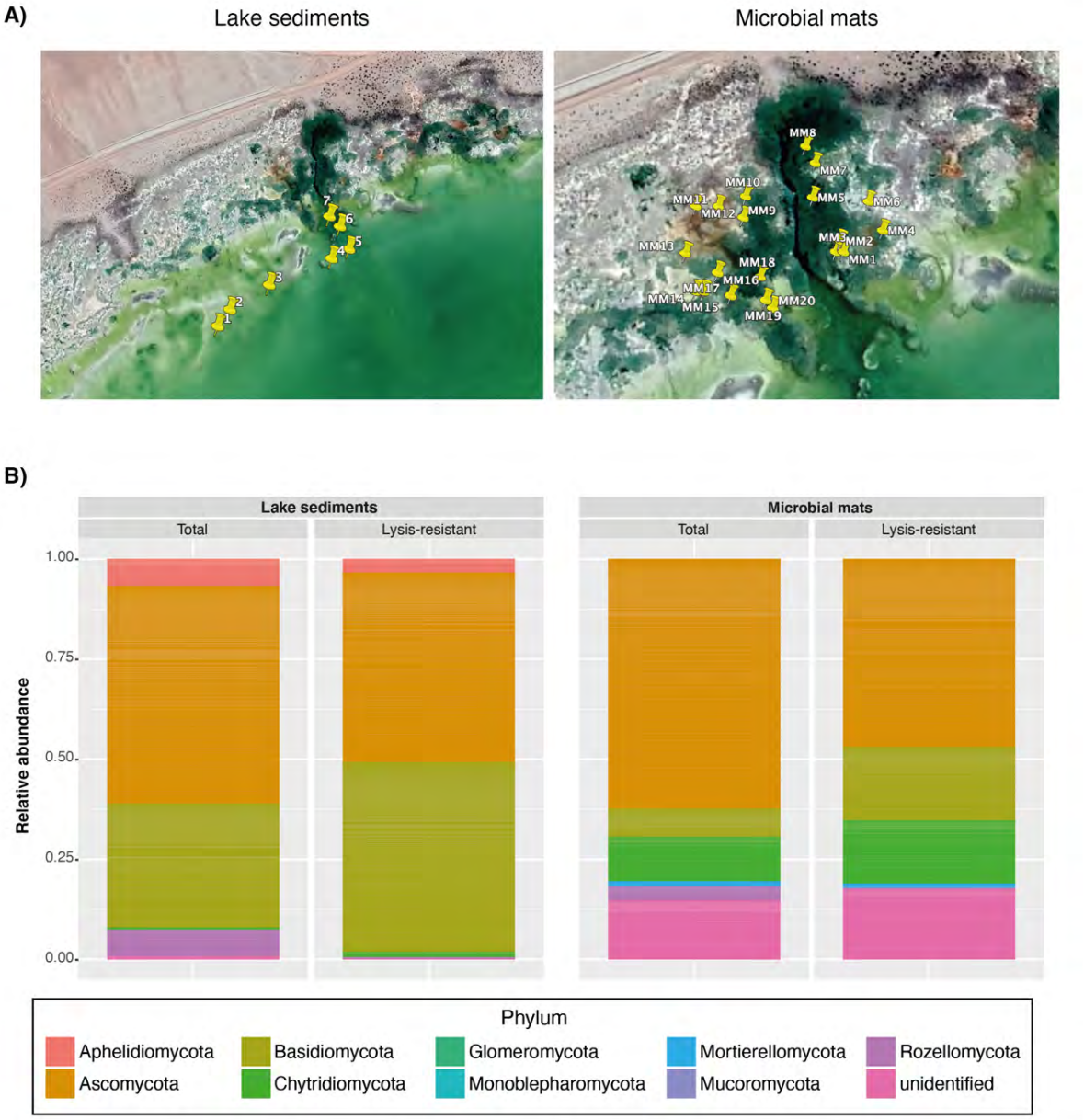

Figure 2. Sampling area at the Salar de Huasco and assessment of the fungal community composition per environment. **A)** Seven sediment cores collected from the main saline lake (right) and twenty microbial mat samples collected from the surrounding ponds (left). **B)** Bar plot showing the fungal community composition at the phylum level, in the total community (direct DNA extraction) and lysis-resistant fraction (after lysis-resistant enrichment treatment) in the lake sediments (right) and microbial mats (left).

2.3. Diversity of lysis-resistance in fungi

The community analysis was performed using all samples, but for the comparison of the total and the lysis-resistant fractions, only samples that could be paired were retained. For the lake sediments, sample 5 could not be analyzed as there was not enough sediment to perform the analysis. Furthermore, amplicons could not be generated from two samples of the lake sediments (total community 3.1.1 and 6.3.1) and of three microbial mat samples (lysis-resistant fraction 5 and 13, and total community 15). These samples were removed to only consider paired samples (lysis-resistant and total fraction) for the comparative analysis. Nonetheless, the community analysis was performed with all the samples.In figure 4A and B, a PCoA of the lake sediment and microbial mats, respectively, are presented. The total community and lysis-resistant fractions overlapped in both environments. Not only was there not a clear separation of the total community and the lysis-resistant fraction in the PCoA, but the Bray-Curtis beta diversity and the Shannon alpha diversity of the total community and the lysis-resistant fraction showed no significant statistical difference (beta diversity lake sediments *p*-value = 0.9309 and microbial mat *p*-value = 0.1369 and alpha diversity lake sediments *p*-value = 0.33 and microbial mat *p*-value = 0.087).

To better understand the effect of the lysis-resistant enrichment method on the community composition the relative enrichment of each phylum was plotted in figure 4C for the lake sediments and 4D for the microbial mats. This analysis shows whether the ASVs belonging to a given phylum were more abundant in the total community (positive values), higher in the lysis-resistant fraction (negative values) or equally abundant (zero). Only five phyla were present in the lake sediments, while nine phyla were present in the microbial mats. In the lake sediments, only Chytridiomycota was enriched in the lysis-resistant fraction (Fig. 4C). The Basidiomycota phylum was slightly enriched in the lysis-resistant fraction, while the Ascomycota, Aphelidiomycota, and Rozellomycota were enriched in the total community. The Mucoromycota, Mortierellomycota, Monoblepharomycota and Glomeromycota phyla were only present in the microbial mats (Fig. 4D). The phylum Chytridiomycota was slightly enriched in the lysis-resistant fraction in the microbial mats too. The Basidiomycota, Ascomycota, and Aphelidiomycota phyla show no enrichment in either fraction. These results reflect the differences observed in Figure 2B when comparing the community composition between the two fractions and environments. We then assessed the 50

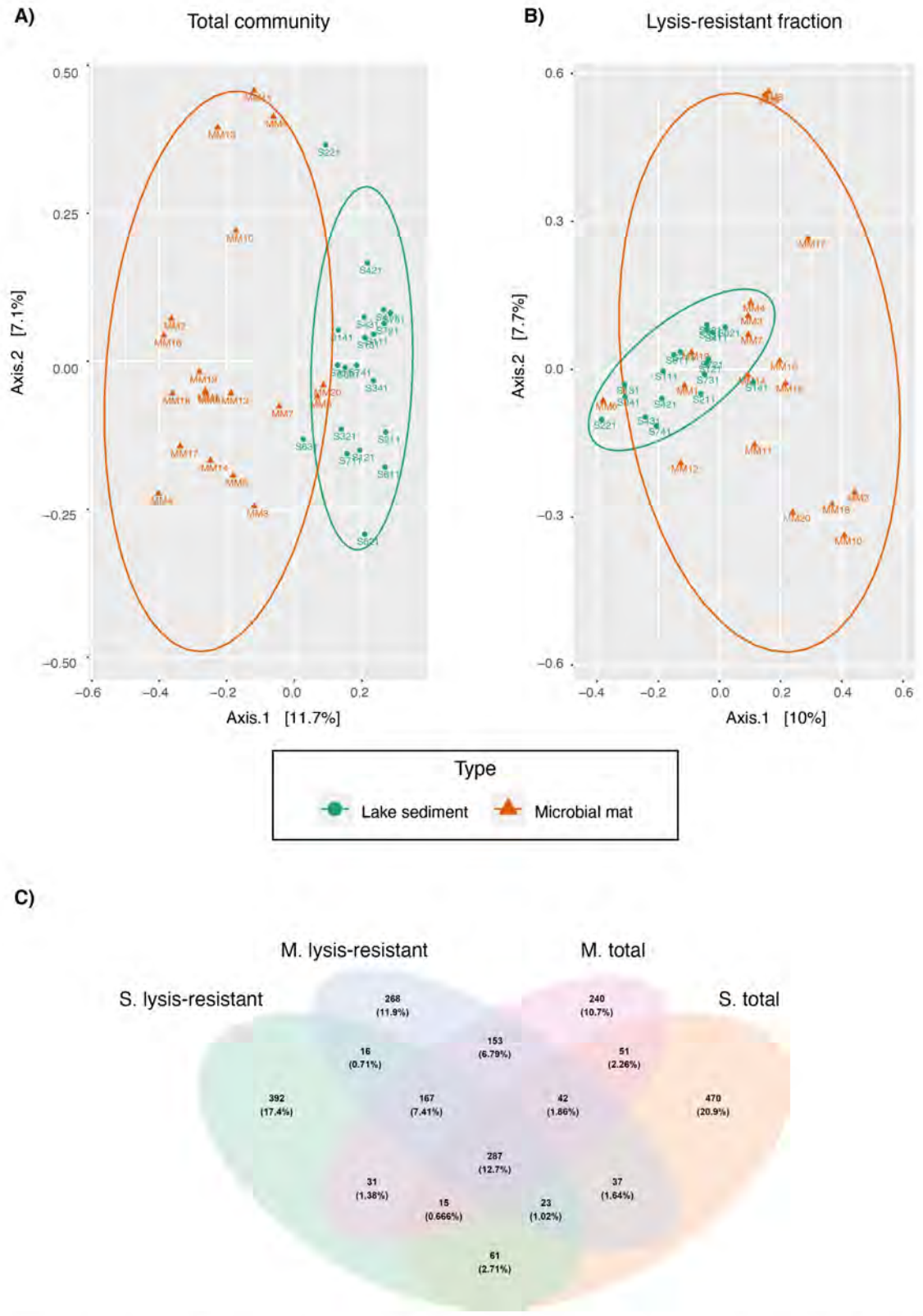

Figure 3. Principal Coordinates Analysis (PCoA) of the total community and the lysis-resistant fraction and Venn Diagram of the fungal community in the lake sediments and microbial mats. **A)** PCoA calculated based on the Bray-Cutis distances of the total community, grouped by environment, lake sediments (green) and microbial mats (brown). **B)** PCoA calculated based on the Bray-Cutis distances of the lysis-resistant fraction, grouped by environment, lake sediments (green) and microbial mats (brown). **C)** Venn diagram of the top 1000 ASVs for each fraction and environment, microbial mat (M. total and M. lysis-resistant) and lake sediments (S. total and S. lysis-resistant).

most abundant ASVs at the phylum level. In the lake sediments, only the Chytridiomycota phylum was not among the 50 most abundant phyla (Additional Figure 2). A higher diversity was observed in the microbial mats. The 50 most abundant ASVs in the microbial mats belonged to the Ascomycota, Basidiomycota, Chytridiomycota, Mortierellomycota, and Rozellomycota phyla additionally a large fraction belonged to unidentified ASVs (Additional Figure 3). In both environments the phylum Rozellomycota was only present in the total community, while all other phyla were present in both fractions. Thus, to better understand the differences in lysis-resistance, we assessed the same 50 most abundant ASVs at the class level in figure 5A (lake sediments) and 5B (microbial mats). These figures show that the differences in lysis-resistance are found at lower taxonomic ranks, in this case already at the class level. Additionally, these figures also show the broad fungal diversity present in the Salar de Huasco. In the lake sediments, most of the classes were present in both fractions, except for the class Sordariomycetes (Actinomycetes) and Exobasidiomycetes (Basidiomycota) that were only present in the lysis-resistant fraction, while the Eurotiomycetes (Ascomycota) and Leotiomycetes (Ascomycota) were only present in the total fraction (Fig. 5A). In the microbial mats, the classes Agaricomycetes (Basidiomycota), Cystobasidiomycetes (Basidiomycota), and Saccharomycetes (Ascomycota) were only found in the lysis-resistant fraction, while all other classes were present in both fractions (Fig. 5B). Furthermore, some differences were observed between the environments. For example, the classes Eurotiomycetes and Sordariomycetes that were only present in the lysis-resistant fraction in the lake sediments, were present in both fractions in the microbial mats. Contrary to this, the class Saccharomycetes was only present in the lysis-resistant fraction in the microbial mats but was present in both fractions in the lake sediments. These results show the differential effect of the lysis-resistant enrichment method in fungi.

Assessing the most abundant ASVs of a community leaves a large fraction of the diversity out of the analysis; thus the enrichment of all the different phyla at the class level was assessed separately. In the lake sediments, the only identified class within the Chytridiomycota phylum was Lobulomycetes. This class and unidentified sequences were enriched in the lysis-resistant fraction. Similarly, in the Rozellomycotina phylum, the only class identified was the Rozellomycotina

_cls_Incertae_sedis, which was enriched in the lysis-resistant fraction, while unidentified sequences were enriched in the total fraction. In the Aphelidiomycota phylum, only the Aphelidiomycetes class was identified, this class was enriched in the total fraction. Furthermore, in the Ascomycota phylum (Fig. 6A), only the Saccharomycetes and the Lecanoromycetes classes were enriched in the lysis resistant fraction. In the later, two genera were present, the *Caloplaca* and *Acarospora*. Both these genera are worldwide distributed and have been previously found in the Atacama Desert (37,38). Moreover, the Dothideomycetes class, that contains halophilic and halotolerant genera such as *Cladosporium* and *Aureobasidum*, was not enriched in either fraction (i.e., equal distribution in both fractions), however two known halotolerant and halophilic species, *Hortaea werneckii*, and *Aureobasidium pullulans* (39) were present in the lake sediments. In the Basidiomycota (Fig. 6B) phylum, five classes were enriched in the lysis-resistant fraction Pucciniomycetes, Microbotryomycetes, Malasseziomycetes, Exobasidiomycetes, and Cystobasidiomycetes, while the other four classes were enriched in the total fraction.

In the microbial mats, the Rozellomycota phylum was represented only by unidentified sequences enriched in the total fraction, similarly the Chytridiomycota phylum was represented only by unidentified sequences (enrichment in the lysis-resistant fraction). Furthermore, the classes Mortierellomycetes (Mortierellomycota phylum), Mucoromycetes (Mucoromycota phylum), Sanchytriomycetes (Monoblepharomycota phylum), and Glomeromycetes (Glomeromycota phylum) were the only identified classes in their respective phylum, and were all enriched in the total fraction. On the other hand, the Aphelidiomycetes class was the only identified in the Aphelidiomycota phylum and it was enriched in the lysis-resistant fraction. In the case of the Ascomycota phylum, only the classes Taphrinomycetes and Saccharomycetes were enriched in the lysis-resistant fraction (Fig. 6C). As in the lake sediments, the Dothideomycetes class was not enriched in either fraction but the known halotolerant species *Aureobasidium pullulans* was present. In the Basidiomycota phylum, six of eight classes were enriched in the lysis-resistant fraction. These were, Ustilaginomycetes, Tritirachiomycetes, Microbotryomycetes, Malasseziomycetes, and Cystobasidiomycetes (Fig. 6D). Some differences were observed when comparing the lake sediments and the microbial mats in figure 6 A-D. In the Ascomycota phylum, the Lecanoromycetes class was only present in the lake sediments, while the classes

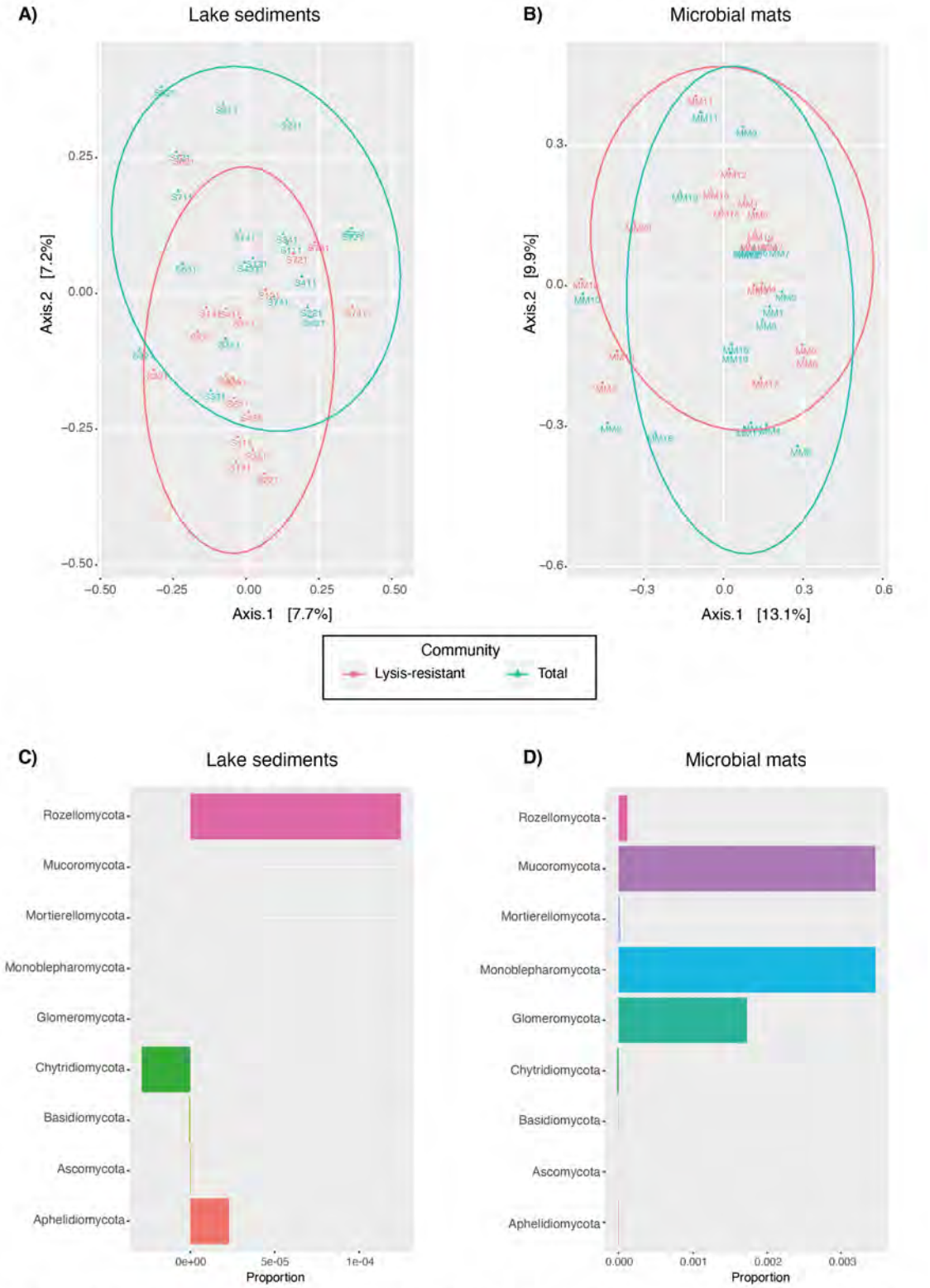

Figure 4. Principal Coordinates Analysis (PCoA) and bar plot of the enrichment in the different bacterial phyla per environment. **A)** PCoA calculated based on the Bray-Cutis distances of the lake sediments belonging to the lysis-resistant fraction (red) and the total community (blue). **B)** PCoA calculated based on the Bray-Cutis distances of the microbial mats belonging to the lysis-resistant fraction (red) and the total community (blue). **C)** Enrichment per phyla in the lake sediments, in the lysis-resistant or total fraction. Negative values show enrichment in the lysis-resistant fraction, positive values show enrichment in the total fraction, while 0 show equal abundance in both fractions. **D)** Enrichment per phyla in the microbial mats, in the lysis-resistant or total fraction. Negative values show enrichment in the lysis-resistant fraction, positive values show enrichment in the total fraction, while 0 show equal abundance in both fractions.

Taphrinomycetes and Pazizomycetes were only present in the microbial mats. Moreover, in the Basidiomycota phylum, the classes Puccinomycetes and Exobasidiomycetes were only present in the lake sediments, while the class Tritirachiomycetes was only present in the microbial mats. The predominance of unclassified sequences limits our ability to assess the effect of the lysis-resistant enrichment method on the identification of a seed bank for the fungal community. Thus, we calculated a lysis-resistant enrichment index, presented in figure 6E and F. By calculating the enrichment of each ASV, either in the total community (index value 0-0.2) or in the lysis-resistant fraction (index 0.8-1), we were able to evaluate the effect of the lysis-resistant enrichment method even in unclassified sequences. Figure 6E and F showed that most ASVs in both environments (lake sediments and microbial mats respectively) were either enriched in the total community or in the lysis-resistant fraction, with only a small proportion of them showing similar abundance in both fractions (index values 0.2-0.8). Therefore, although many of these ASVs could not be classified, we were able to evaluate their enrichment and show that most ASVs are either resistant to lysis or not.

3. Discussion

3.1. Use of the lysis-resistant separation method in Fungi

Our results show that, as expected, it is more difficult to extract DNA from spores. Indeed, DNA was extracted from spores of *U*. *alternata*, *C*. *cinerea* and A*. niger* only after the lysis-resistance enrichment method. This demonstrates the utility of the lysis-resistance enrichment method to detect Fungi, especially in extreme environments where the probability to find them under the form of spores is high. We further demonstrate that mycelium is not detected using the lysis-resistance enrichment method, but can only be detected using a direct DNA extraction method. In some cases, DNA could not be retrieved through direct DNA extraction. This shows that anything detected using the lysis-resistance enrichment method is more resistant than typical mycelium. Moreover, in the cases of *A*. *niger* and *U*. *alternata*, DNA from the mycelium was also not extracted using the direct DNA extraction method, indicating that the initial quantity of fungal material was probably too low. Moreover, with *C*. *albicans* (a non-melanized yeast), our results show that non-melanized yeasts will be better detected with a direct DNA extraction method, while only a small fraction of the

DNA of these yeast cells can be detected after the lysis-resistance treatment. Using different types of spores (asexual spores, sexual spores, melanized or not) alongside with yeast and different types of mycelium (melanized or not) allowed us to show the broad range of applicability of the lysis-resistant separation method for the fungal kingdom. Therefore, the combination of both types of methods (a direct DNA extraction and the lysis-resistant enrichment method before a DNA extraction) allows to improve the detection of fungi in different environments and broaden our understanding of the conditions they can tolerate under varied resistance forms.

3.2. Fungal diversity in the Salar de Huasco

To the best of our knowledge, this is the first time that the fungal community was assessed in the Salar de Huasco. In addition, we did not only assess the fungal diversity, but also the diversity of lysis-resistant fungal cells, as a proxy of specialized resistant cells such as spores, conidia, and zoospores. A large proportion of unidentified sequences was present in the data set, especially in the microbial mats. This is to be expected as fungal diversity has been less studied than bacterial diversity. Currently, 120,000 fungal species have been described but it is estimated that over 93% of the fungal diversity is still unknown, suggesting that the number of fungal species could be between 2.2 and 3.8 million (40). Additionally, aquatic fungal diversity has been less explored than fungal diversity in terrestrial environments, and studies in extreme environments such as the Salar de Huasco are rare (41). This highlights the importance of studies such as the one presented here to better understand the distribution and diversity of fungi in the biosphere. For instance, halotolerant and halophilic fungi such as *Hortaea werneckii*, and *Aureobasidium pullulans* (39) were found in the lake sediments, while the species *Aureobasidium pullulans* was also present in the microbial mats. Our results showed that the fungal community of both the lake sediments and the microbial mats samples were dominated by members of Ascomycota and Basidiomycota, while members of basal lineages had lower relative abundance. Similar results have been reported regarding the fungal diversity in the Atacama Desert (42). Furthermore, the results presented here are in agreement with the study made by (43) assessing the fungal diversity in sediments along an estuarine salinity gradient. They found in all salt concentrations a high abundance of Ascomycota followed in abundance by Basidiomycota. Moreover, they found that the

fresh water and medium salinity samples showed a higher abundance of basal lineages, which is also the case in our study when considering the microbial mats. The microbial mats, which in average presented a lower salinity concentration (1.67 PSU) than the lake sediments (36.96 PSU), showed higher abundance of Chrytidiomycota, Rozellomycota, and Mortierellomycota. The only exception is Aphelidiomycota (also basal fungi), which showed higher abundance in the lake sediments than in the microbial mats. Similarly, Rojas-Jimenez et al., 2019 assessed the fungal diversity in the Baltic Sea, sampling areas with different salinity concentrations. Ascomycota and Basidiomycota were dominant in the samples with a salinity of 19.2 to 33.5 PSU. These results are in agreement with the results found in the lake sediments.

3.3. Lysis-resistance in Fungi

The application of the lysis-resistant enrichment method to assess the diversity of fungal spores and spore-like cells, showed high similarity in the composition of the total and lysis-resistant fractions. This contrast with Bacteria, in which the production of a lysis-resistant cell appears to be restricted to some clades (45–49). However, the assessment of the fungal lysis-resistant diversity presents intrinsic challenges. For instance, all fungi produce at least one type of resistant structure and their production is not only limited to survival and dispersal, but also to reproduction. Thus, the lack of specific enrichment in the lysis-resistant fraction, relative to the total diversity, might reflect the presence of resistance structures associated with non-resistant structures (i.e., mycelium). Another challenge is that a high percentage of the fungal diversity is still unknown, and consequently, most of the diversity detected corresponded to unidentified sequences or sequences for which identification was only possible at the order or genus level. Nonetheless, by calculating a lysis-resistant enrichment index of each ASV (including those unclassified), the existence of a fungal seed bank distinct from the active (lysis prone fraction) community could be evaluated. The enrichment index showed that there is a bimodality in fungal lysis-resistance. This means that most ASVs were either enriched in the lysis-resistant fraction or in the total community (not being lysis-resistant), with only a small proportion of them showing equal abundance in either fraction.

Although the lake sediments and microbial mats differed in biotic and abiotic factors, two phyla showed similar enrichment patterns. The Rozellomycota was enriched in

the total community, while the Chytridiomycota was enriched in the lysis resistant fraction. The Rozellomycota phylum is constituted by mainly uncultured unicellular fungi, considered to be the most basal clade of Fungi (50,51). The enrichment of this phylum in the total fraction might be due to the absence of chitin in its parasitic stage and the absence of a cell wall in the wall-less zoospores (50,52). The absence of chitin and of the cell wall could make these cells highly susceptible to lysis. On the other hand, the Chytridiomycota phylum, enriched in the lysis-resistant fraction, is a basal phylum mainly composed of saprotrophs (degrading highly recalcitrant organic matter) and pathogens (infecting algae, plants, and amphibians) that inhabit freshwater, brackish, marine habitats and are also abundant in soils (53–55). In the microbial mats, the diversity of Chytridiomycota corresponded to unidentified sequences, while in the lake sediments, the Lobulomycetes class and unidentified sequences were enriched in the lysis-resistant fraction. Although little is known about the Lobulomycetes class, some members have been described as saprophytes or parasites isolated from soils (56), acidic fresh water (57), and from marine brown algae (58). The life cycle of these fungi might provide an insight in their resistance to the lysis treatment. For most Chytridiomycota, asexual reproduction occurs through the release of zoospores, which are motile asexual spores that use a flagellum for locomotion (53). These zoospores might be more resistant to lysis than the fungal rhizoids. Additionally, some members of the Chytridiomycota are parasites of algae and rotifers, and even vascular plants (59). In their parasitic state, they develop inside the host's cells, and as a result, the fungal cells might be protected from lysis. A similar enrichment of intracellular bacteria was found after the application of the lysis-resistant enrichment method. The bacterial phyla Tenericutes and Chlamydiae, which are composed of intracellular pathogens (60,61), were enriched in the lysis-resistant fraction (26).

As a whole, the most abundant phyla, Ascomycota and Basidiomycota were not enriched in either fraction in the lake sediments nor in the microbial mats. However, the specific enrichment of a few classes within both phyla are worth mentioning. In the Ascomycota phylum, the Saccharomycetes class was enriched in the lysis-resistant fraction of both the lake sediments and the microbial mats. This class is constituted of budding yeasts often considered to be "true yeasts", which can undergo meiosis and sporulation under nutrient-poor conditions (62,63). The higher resistance of yeast to lysis and the presence of spores might explain the enrichment of Saccharomycetes in both environments. Furthermore, due to the differences of the lake sediments and the

microbial mats, some of the Ascomycota classes enriched in the lysis-resistant fraction were only present in one environment. One of those is the Lecanoromycetes class, enriched in the lysis-resistant fraction of the lake sediments but absent in the microbial mats. This class is composed of lichenized fungi. The high enrichment of the Lecanoromycetes class might be due to the presence of fungal spores and or propagules (small aggregate of mycelium and phototrophic cells) used by the lichens to reproduce and disperse (64). The two genera identified (*Caloplaca* and *Acarospora*) do not inhabit aquatic environments, thus the presence of lichen propagules and or spores in the lake sediments could be due to the dispersal of spores from lichens present in the surroundings of the lake. The second Ascomycota class, which was enriched in the microbial mats but was not present in the lake sediments, was Taphrinomycetes. Members of the Taphrinomycetes class are dimorphic, parasitic or pathogenic fungi (65). The presence of fungal spores and the dimorphism of members in this class might explain their enrichment in the lysis-resistant fraction.

At the class level, more classes within Basidiomycota were enriched in the lysis-resistant fraction than for the Ascomycota phylum. The higher number of enriched classes in Basidiomycota compared to Ascomycota, might be due to the complex life cycles of the classes enriched in the Basidiomycota. Four of the six enriched classes in the Basidiomycota (Pucciniomycetes, Microbotryomycetes, Cystobasidiomycetes and Tritriachiomycetes) are members of the Pucciniomycotina subclass. This subclass accommodates a diverse array of fungi, ranging from plant pathogenic rust fungi, to insect associated fungi, mycoparasitic fungi and dimorphic yeast species (66,67). Additionally, some plant pathogens in the subclass display some of the more complex life cycles among fungi. For example, some of them are heteroecious and require two distantly related hosts to complete their life cycles, others are macrocyclic, possessing up to five distinct spore-producing states (68). Furthermore, in the lake sediments, the most enriched Basidiomycota class in the lysis-resistant fraction was the Pucciniomycetes. The Pucciniomycetes class is composed of mainly rust fungi, which are obligate plant pathogens (69). Its enrichment in the lysis-resistant fraction in the lake sediments might reflect their presence in spore form, as no living plant can be found in this hypersaline lake. Similarly, the classes Microbotryomycetes and Exobasidiomycetes, which were enriched in the lysis-resistant fraction, are also composed of plant pathogens. However, these classes might also present other ecological roles that are still unknown. In the microbial mats, the most enriched

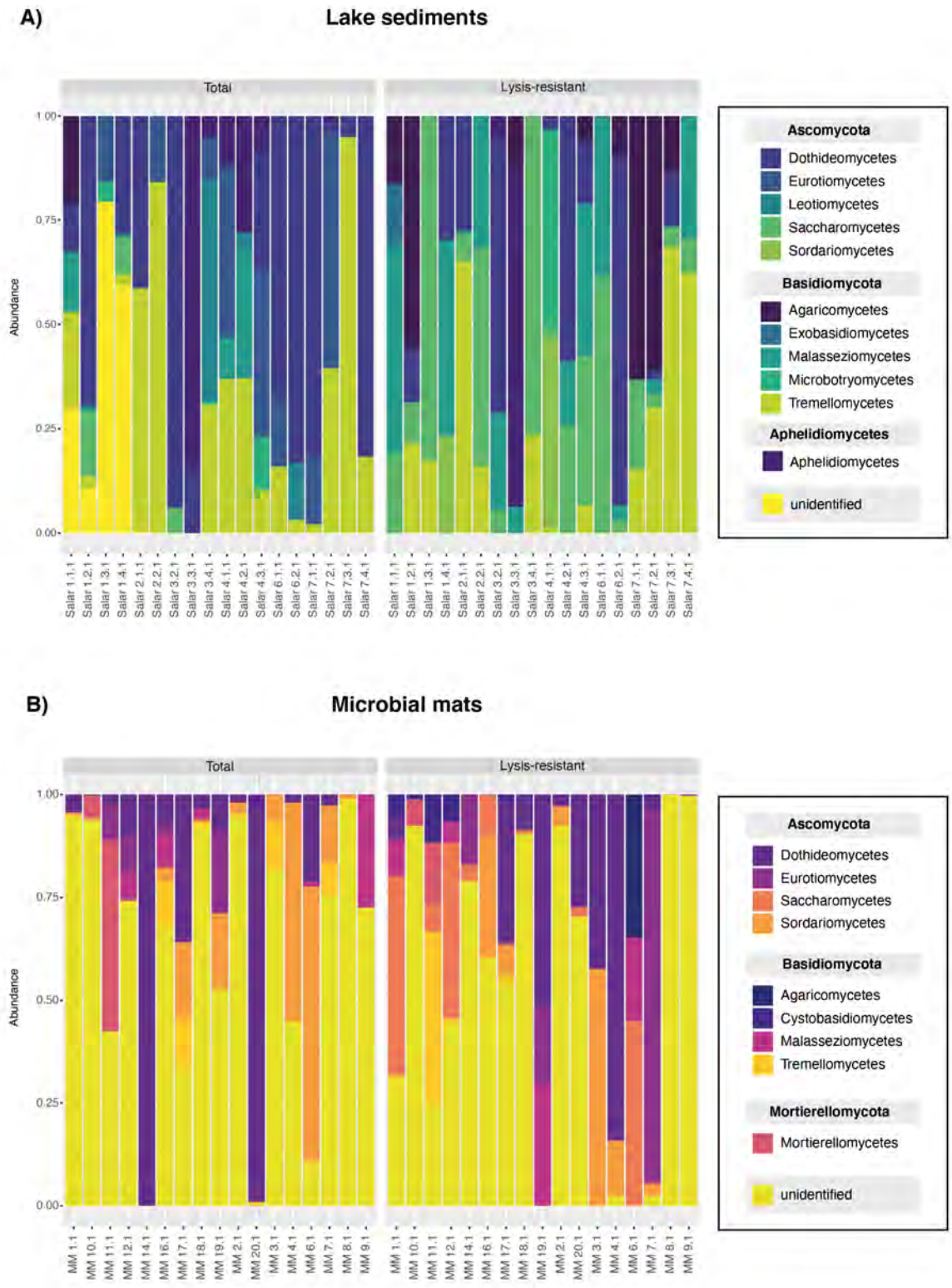

Figure 5. Relative abundance of the 50 most abundant ASVs per sampling point in the **A)** lake sediments and **B)** microbial mats. Left the total community and right the lysis-resistant fraction. Different colors represent different classes, in the legend on the right the classes are classified by phyla.

Basidiomycota class was the Tritirachiomycetes. In this class, the spores of *Paratritirachium curvibasidium* have been shown to be resistant to high temperatures (75°C) (70). The Ustilaginomycetes class was also enriched in the lysis-resistant fraction of the microbial mats. Members of this class are “smut fungi”, usually dimorphic, producing a saprobic haploid yeast phase and a parasitic dikaryotic hyphal phase. In their parasitic stage they occur mainly in angiosperms (71). The enrichment of the Ustilaginomycetes class in the microbial mats might be due to the increased resistance of the bi-layered yeast walls (72) or the high resistance of spores to lysis. Furthermore some classes of the Basidiomycetes phylum were enriched in the lysis-resistant fraction of both environments, such as the lichenized class, Cystobasidiomycetes (73), and the yeast class Malasseziomycetes. Although yeast showed less resistance than spores to the lysis-resistant enrichment method, the melanization of yeast in the Malasseziomycetes class (74) might increase their resistance to lysis of yeast. On the contrary, the dimorphic class Agaricostilbomycetes was enriched in the total fraction. Members of this class might have been present in this environment as mycelium and not yeast. These results illustrate the lysis-resistance diversity of fungi and that the enrichment in the total community or the lysis-resistant fraction, might reflect the fungal life cycle.

4. Conclusions

In conclusion, the results presented here corroborate our initial hypothesis. The widespread distribution of lysis-resistance in fungi reflects not only the presence of resistance structures, but also the multitude of life cycles in fungi. Furthermore, although lysis-resistance is broadly distributed in fungi, the application of lysis-resistant enrichment method showed that most of the ASVs are either enriched in the lysis-resistant fraction or in the total community. Thus, lysis-resistance is only characteristic for some taxa or for a particular stage in the life cycle. In order to better understand the composition and dynamics of the fungal seedbank, molecular studies still have to be coupled with culture-dependent methods and/or microscopy to provide additional information about the fungal lysis-resistant fraction in the environment. Regrettably, in this study it was not possible as the amount of environmental sample was limited, which is often the case when evaluating extreme environments. Nevertheless, to the best of our knowledge this is the first study evaluating the fungal

diversity in the extreme environment of the Salar de Huasco. Moreover, fungi are often overlooked in environmental studies, especially in extreme environments, in spite of being an integral component of every environment and representing an important resource to address major global challenges (Coleine et al., 2022; Drake & Ivarsson, 2018; Gadd, 2021; Grossart et al., 2019). Thus, this paper does not only contribute to the better understanding of the lysis-resistance of fungal cells, but also to the unveiling of their overall diversity in extreme environments.

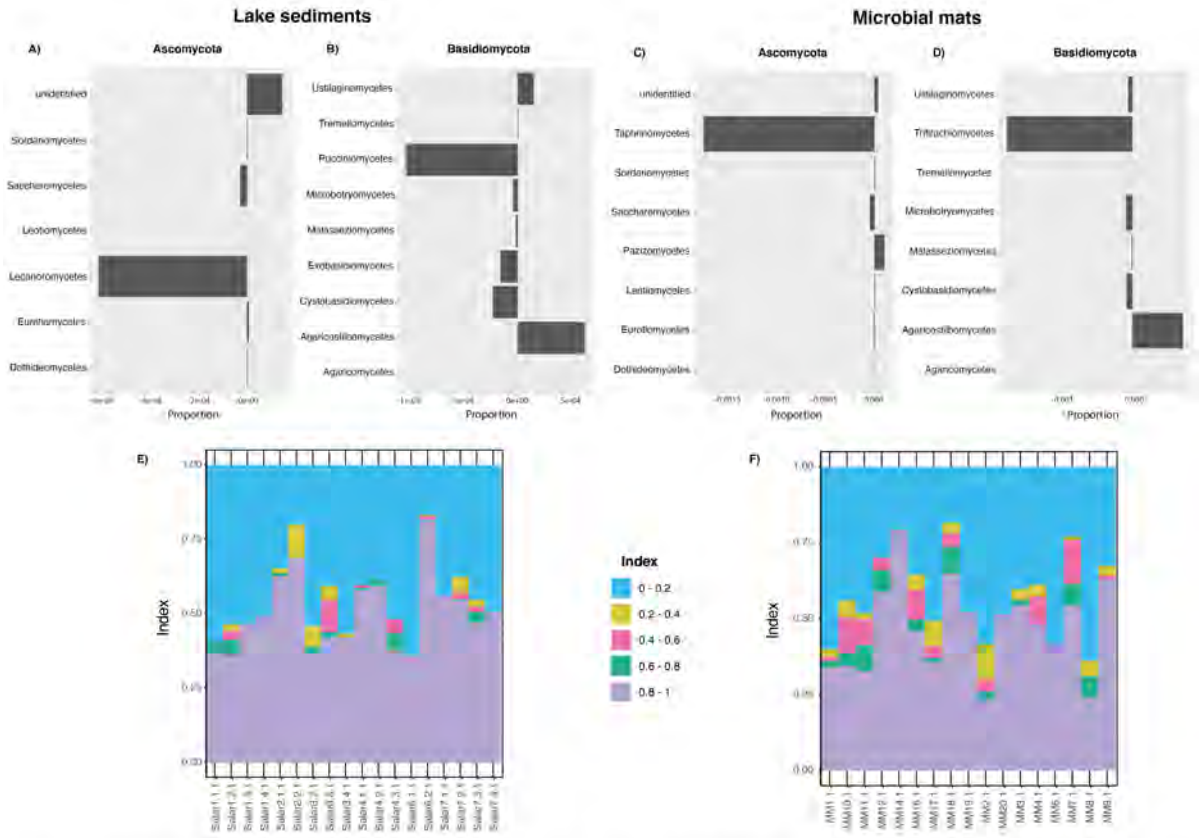

Figure 6. Enrichment of different fungal classes in the lysis-resistant or total fraction in the lake sediments and microbial mats (A-D). Evaluation of the composition of the lysis-resistant community calculated by a lysis-enrichment index (E and F). **A)** Enrichment of the classes in the Ascomycota phylum in the lake sediments. **B)** Enrichment of the classes in the Basidiomycota phylum in the lake sediments. **C)** Enrichment of the classes in the Ascomycota phylum in the microbial mats. **D)** Enrichment of the classes in the Basidiomycota phylum in the microbial mats. Negative values show enrichment in the lysis-resistant fraction, positive values show enrichment in the total fraction, while 0 show equal abundance in both fractions. **E)** Bar plots representing the relative fraction of the OTUs enriched in the lysis-resistant (enrichment index 0.8–1.0), the total community (enrichment index 0.0–0.2), or those shared between the two fractions in the lake sediments and **F)** microbial mats. The enrichment index was calculated by comparing the abundance (sequence counts) in the lysis-resistant fraction over the total abundance (sequence counts in lysis-resistant fraction + total community). In the distribution, ASVs enriched in the lysis-resistant fraction had an index value of 1, while those enriched in the total community fraction had an index value of 0.

5. Method

5.1. Valdation of the Lysis-resistant enrichment method for Fungi

5.1.1. Mycelium and spore collection

To produce different types of samples for each fungal strain, their respective growing medium and growing temperatures are summarized in Table 1. To collect the mycelium, all liquid cultures were performed in 15 ml of liquid medium and agitated at 100 rpm at the appropriate temperature. Liquid cultures were transferred into 50 ml Falcon tubes and centrifuged at 8,000 x g for 10 min. The supernatant was removed and the mycelial mass was weighted. Then the mycelial mass was resuspended in 5 ml physiological water (0.9% NaCl) and the sample was homogenized using an ULTRA-TURRAX® stem system (IKA, Germany) before being filtered through a 0.22 µm nitrocellulose membrane (Merck Millipore, Germany). To collect spores, fungi were grown on solid media and spores were collected using 5 ml of Tween 80 at 0.16% (Merck, Germany). To wash the samples, they were then centrifuged at 5,000 x g for 5 min. The supernatant was removed, 5 ml of physiological water was added and this procedure was repeated. Afterward, the number of spores collected for each sample was assessed using a Neubauer chamber (depth: 0,01mm, Carl Roth GmbH, Germany) prior to filtering the spores' solutions through a 0.22 µm nitrocellulose membrane (Merck Millipore, Germany). For *C. cinerea*, sexual spores were collected from the fruiting body. The fruiting body was immersed in 5 ml of physiological water and the sample was vortexed. The fruiting body was removed, leaving only the sexual spores in the physiological water, and the remaining solution was filtered through a 0.22 µm nitrocellulose membrane (Merck Millipore, Germany). For the yeast form of *C. albicans*, 2 colonies were put in 5 ml of physiological water before being filtered as for all other samples. All filters were kept at -20°C before further processing. For each fungal strain, each type of sample was analyzed in duplicates.

5.1.2. Lysis-resistant enrichment method and DNA extraction

To one filter containing the biomass, 900 µl of 1x TE (Tris-EDTA) buffer were added and the biomass was resuspended by vortexing. Then the sample was incubated at 65°C for 10 min. shaking at 80 rpm. Afterwards, 100 µl of 20 mg/ml lysozyme were added and the sample was incubated at 37°C for 60 min. shaking at 80 rpm. Later, 250 µl of 3 N sodium hydroxide (NaOH) and 250 µl of 6% sodium dodecyl sulfate

(SDS) solutions were added and the sample was incubated at room temperature for 60 min. shaking at 80 rpm. Next the sample was filtered in a 0.2 µm sterile nitrocellulose filter, which was then washed twice with 2 ml of sterile physiological water to remove any residue of the solutions used. Once the filter was dry, 450 µl of sterile water, 50 µl of 1x DNase reaction buffer and 0.5 µl DNase enzyme were added and incubated at room temperature for 15 min. directly on the filter. The addition of the DNase enzyme and DNase reaction buffer is a vital step to degrade the DNA released by the easy to lyse cells prior to the DNA extraction from the lysis-resistant fraction. After 15 min., the solution was filtered and once more the residue was washed with 1 ml of sterile physiological water that was filtered to wash away the degraded DNA as well as the DNAase solution. Finally, the filters (containing the lysis resistant fraction only) were stored at -20°C until the DNA extraction was performed (Wunderlin et al., 2016). The same DNA extraction method was used in both fractions (Additional Figure 1). The FastDNA®SPIN kit for soil (MP Biomedicals, USA) was used with a modified protocol that included three successive bead-beating rounds in the first step. The final DNA extracted from the successive bead-beating steps was pooled together (Wunderlin et al., 2013) and then precipitated with ethanol and resuspended in PCR-grade water. Finally the DNA was quantified using Qubit® dsDNA HS Assay Kit on a Qubit® 2.0 Fluorometer (Invitrogen, Carlsbad, CA, USA). Additionally, to assess the effect of the lysis-resistant enrichment method both in the mycelium and spores, microscopy images were taken from each sample (before the DNA extraction) the biomass in one half of a filter was resuspended in 1 ml of TE-Buffer. The liquid suspension was then observed under a Upright Leica DM4 B Microscope (Leica, Wetzlar, Germany) and the images were generated with a Leica Microscope Camera DFC7000T (Leica, Wetzlar, Germany).

5.2. Application of the lysis-resistant enrichment method in environmental samples

5.2.1. Sampling

The sampling at Salar de Huasco took place in September of 2019 and all samples were collected within 24 h. Seven sediment core samples were collected from the main saline lake, with a core of approx. 7 cm in diameter and approx. 4 cm in depth. The lake sediment samples were collected following a saline gradient, with salinity

ranging from 53 to 0.706 PSU (Figure 2A). After collection, the cores were cut on site to 1 cm subsamples and stored in sterile plastic Petri dishes. Alongside, 40 ml of microbial mats were collected in sterile 50 ml Falcon tubes from eighteen small ponds surrounding the main saline lake. At every sampling point, salinity, pH, conductivity, water temperature and dissolved oxygen (%OD) were measured using a Multiparameter Water Quality Meter-HI98194, Hanna Instruments (Rhode Island, USA) and the coordinates and altitude were recorded (Additional Table 1). The samples were transported back to the laboratory at room temperature (approx. 20-25°C), where they were stored at 5°C until being processed a week later.

Table 1: The fungal strains, the types of samples taken as well as the growing media and conditions are listed here. Malt-extract agar (MA, malt-extract 12 g/l (SIOS Homebrewing, Switzerland), agar 15 g/l (Biolife, Italy)), Malt-extract broth (MB, malt extract 12 g/l (SIOS Homebrewing, Switzerland)), YMG (yeast extract 4 g/l (Merck, Germany), malt extract 10 g/l (SIOS Homebrewing, Switzerland), glucose 4 g/l (Merck, Germany), agar 15 g/l (Biolife, Italy)), Potato dextrose agar (PDA, 39 g/l, Carl Roth GmbH, Germany), Potato dextrose broth (PDB, potato extract 4 g/l (Merck, Germany), glucose 20 g/l (Merck, Germany)), Yeast extract potato dextrose agar (YEPDA, yeast extract 10 g/l (Merck, Germany), peptone 20 g/l (Thermo Fisher Scientific, USA), dextrose 20 g/l (Carl Roth GmbH, Germany), agar 20 g/l (Biolife, Italy), pH adjusted to 5.6).

Strain	Type of sample	Growing medium	Growth temperature
Ulocladium alternata (M138)	Melanized mycelium	MB	room T°
	Asexual melanized spores	MA	room T°
Aspergillus niger (M8)	Mycelium	MB	30°C
	Asexual	MA	30°C
Coprinopsis cinerea (M141)	Mycelium	MB	room T°
	Sexual spores (fruiting body)	YMG	30°C
	Asexual spores	YMG	30°C, in the dark
Mortierella antarctica (M162)	Mycelium	PDB	room T°
	Asexual spores	PDA	room T°
Candidas albicans	Yeast form	YEPDA	37°C

5.2.2. Lysis-resistant enrichment method

Before applying the lysis-resistant enrichment method to environmental samples one additional step was added to the procedure explained in Section 2.1.2. To avoid bias in the separation of the lysis-resistant fraction due to the attachment of the cells to inorganic matter, an indirect DNA extraction method was used. First, 3 g of material from each sample were weighted in ULTRA-TURRAX® disperser tubes. Then, 15 ml of Na-Hexametaphosphate (1%) were added and the mixture was homogenized two times at 3,000 rpm for 1 min. with the ULTRA-TURRAX® tube disperser workstation system (IKA, Germany). The sample was left to precipitate for 10 min. and the supernatant was collected. This initial step was repeated twice. The two 15 ml

supernatants were pooled, centrifuged at 20 x g for 1 min. to precipitate large particles, and the remaining supernatant was filtered through a 0.2 µm sterile nitrocellulose filter. For each sample, the filter was cut in half. One half was used to assess the total fungal community by directly extracting DNA and the second half was subjected to the lysis-resistant enrichment method to assess the lysis-resistant fraction. This way, the same filter was used to assess both communities (Additional Figure 1). The lysis-resistant enrichment method and DNA extraction were performed as described in the validation of the method (Section 5.1.2). The only difference was that only half a filter containing the biomass was used for the lysis-resistant enrichment method, instead of a full filter.

5.3. Sequencing and statistical analysis

The purified DNA extracts were sent to Fasteris (Geneva, Switzerland) for the ITS region amplicon sequencing using an Illumina MiSeq platform (Illumina, San Diego, CA, USA), generating 300 bp paired-end reads. The ITS region was amplified using the universal primers ITS3 KYO2 (5'-GAT GAA GAA CGY AGY RAA-3') and ITS4 (5'-TCC TCCGCT TAT TGA TAT GC-3') (75). Demultiplexed and trimmed sequence reads provided by Fasteris were processed using QIIME2 (76) with dada2 (77) for the denoising step. Read lengths were truncated to optimized total nucleotide lengths (based on q-scores) at 478 bases total composite length for the un-joined sequences. These truncated sequences allowed the joining of denoised paired-end reads by at least 12 identical bases to obtain full denoised sequences length of 466 bases. Sequences were grouped on Amplicon Sequence Variants (ASVs). The ASVs obtained were then taxonomically classified using QIIME2's vSEARCH-based consensus taxonomy classifier (78) with the UNITE database version 8.2, release date February 4, 2020 (79).

All statistical analysis were performed with RStudio version 1.3.1093 (80), the community and multivariate analysis were performed with the phyloseq (81) and vegan (82) packages. The Venn Diagram was calculated with the package Venn.Diagram (83). The relative abundance was calculated using the Total-Sum Scaling (TSS) normalization. The principal coordinate analysis (PCoA) was calculated based on the Bray-Curtis distances. For the Venn Diagram, PCoA and the enrichment analysis, the relative abundance was used. For the statistical test of the alpha diversity and the analysis of variance (ANOVA) the raw abundance data was used. The

proportion used in the distribution plots was calculated by phylum, the relative abundance of each ASV was divided by the sum of all relative abundance to obtain a proportion, the log base 10 of the proportion was used for the graphical representation. The enrichment proportion was calculated by first adding the relative abundance per genus once in the total fraction and once in the lysis-resistant fraction. The proportion was then calculated by subtracting the lysis-resistant abundance from the total abundance and dividing this by the sum of the lysis-resistant fraction and the total fraction.

Additional analyses were performed to evaluate the contribution of unclassified ASVs to the composition of the lysis-resistant community. For this, an “enrichment index” was calculated for each individual ASV by comparing the relative contribution of the abundance in the lysis-resistant fraction (based on absolute total sequence counts) as compared to the total abundance of the ASV (lysis/(lysis+total fraction)). At the end, an index ranging from 0 (ASVs only detected in the total community fraction) to 1 (ASVs only detected in the lysis-resistant fraction) is obtained for each environmental sample. The results were represented as cumulative graphs, in which the proportion of ASVs displaying the index value were grouped together.

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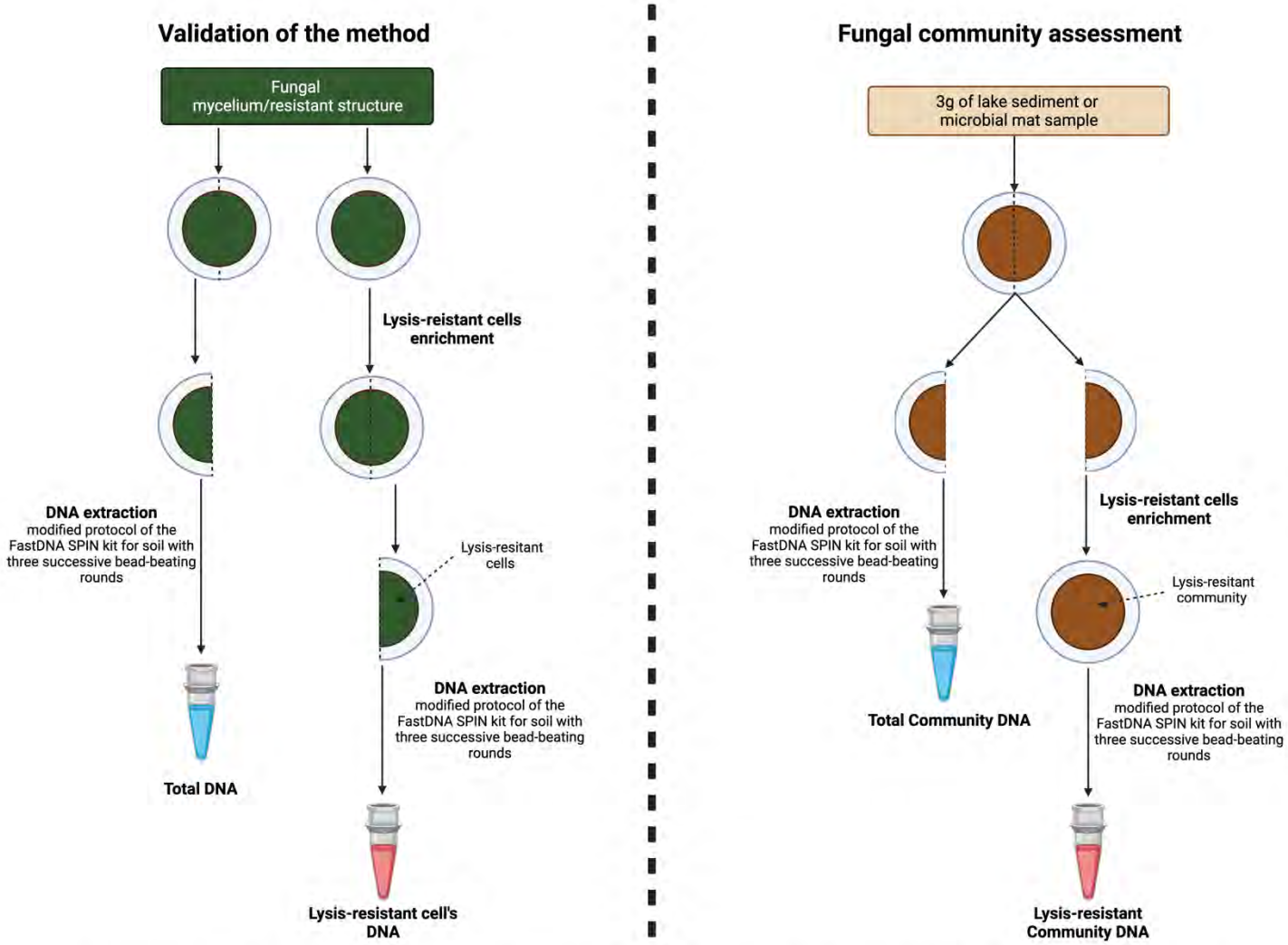
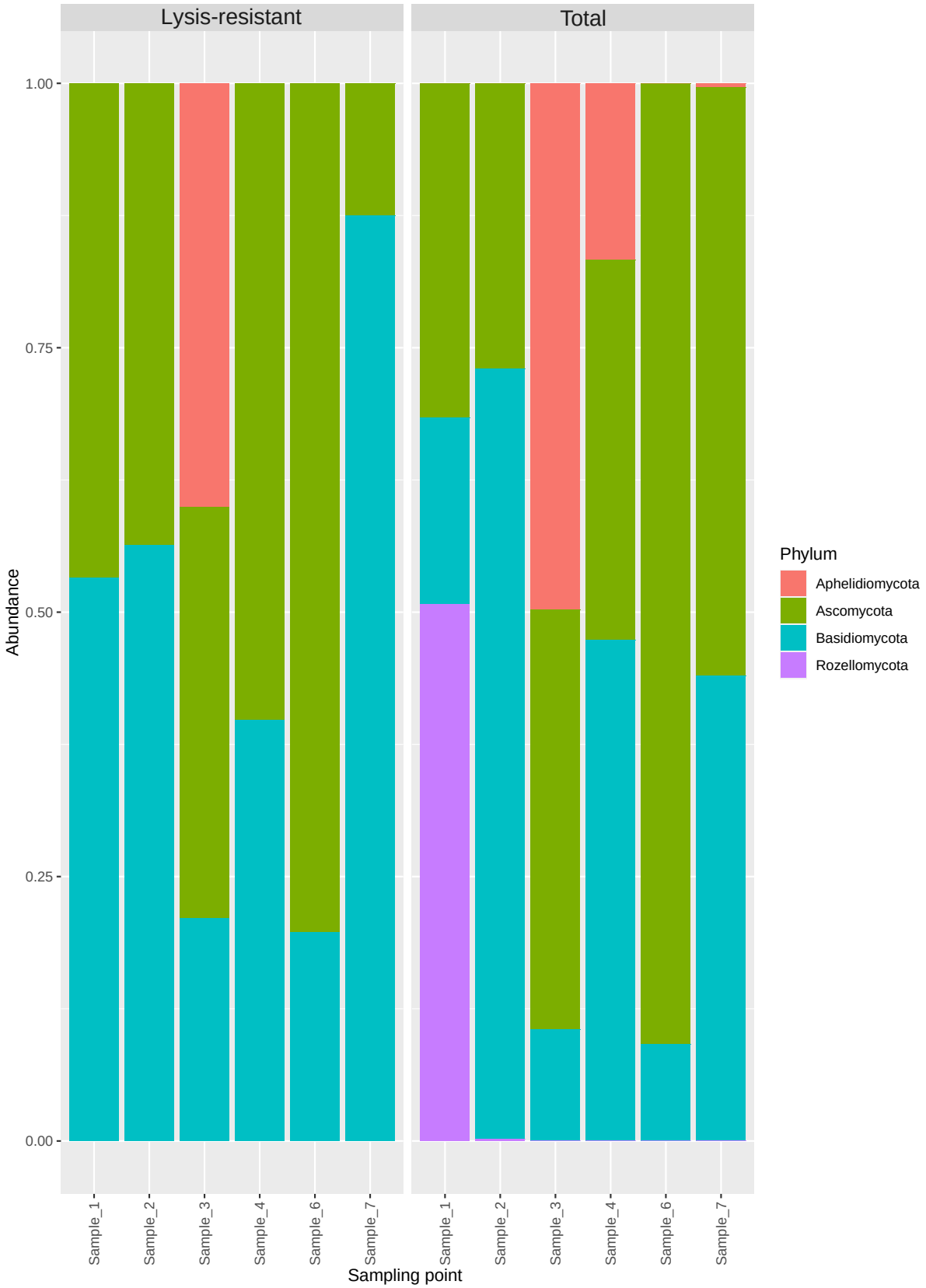
Additional Figures



Additional Figure 1. Graphical representation of the method used. Left, graphical description of the validation method and right graphical representation of the method used for the analysis of the fungal community



Additional Figure 2. Lake sediments 50 most abundant ASVs per sampling point at the phylum level. Left, lysis-resistant fraction and right total community, different colors represent different phyla.

Additional Figure 3. Microbial mats 50 most abundant ASVs per sampling point at the phylum level. Left, lysis-resistant fraction and right total community, different colors represent different phyla.

Supplementary table 1. Information on the abiotic parameters measured in situ for the different sampling locations.

Name	Pairs	Community	Type	Sampling_point	Description	Depth (cm from surface)	DNA sent to sequence (ng/ul)	Extracted DNA after precipitation (ng/ul)	Elevation (m.a.s.l)	Temperature (ºC)	Conductivity (us/cm)	Salinity (PSU)	OD%	pH	Coordinates
MM1-1	MM1	Total	Microbial mat	Pond_1	Submerged not structured	Surface	4	112	3797	16	1565	0.831	13.9	7.67	20.283139°S 68.888778°W
MM1-1S	MM1	Lysis-resistants	Microbial mat	Pond_1	Submerged not structured	Surface	0.374	0.374	3797	16	1565	0.831	13.9	7.67	20.283139°S 68.888778°W
MM10-1	MM10	Total	Microbial mat	Pond_9	White black mat. Almost fully dry	Surface	4	254	3791	16	3788	2.029	9.4	9.31	20.283306°S68.888778°W
MM10-1S	MM10	Lysis-resistants	Microbial mat	Pond_9	White black mat. Almost fully dry	Surface	2.3	2.3	3791	16	3788	2.029	9.4	9.31	20.283306°S68.888778°W
MM11-1	MM11	Total	Microbial mat	Pond_10	3rd region with vegetation only 3cm water	Surface	4	110	3791	16	3788	2.029	9.4	9.31	20.283444°S68.888778°W
MM11-1S-QM	MM11	Lysis-resistants	Microbial mat	Pond_10	4th region with vegetation only 3cm water	Surface	4	9.08	3791	16	3788	2.029	9.4	9.31	20.283444°S68.888778°W
MM12-1	MM12	Total	Microbial mat	Pond_11		Surface	4	158	3791	16	3788	2.029	9.4	9.31	20.283528°S68.888778°W
MM12-1S	MM12	Lysis-resistants	Microbial mat	Pond_11		Surface	0.47	0.47	3791	16	3788	2.029	9.4	9.31	20.283528°S68.888778°W
MM13-1	MM13	Total	Microbial mat	Pond_12		Surface	4	149	3792	16	3788	2.029	9.4	9.31	20.283722°S68.888778°W
MM13-1S	MM13	Lysis-resistants	Microbial mat	Pond_12		Surface	0.112	0.112	3792	16	3788	2.029	9.4	9.31	20.283722°S68.888778°W
MM14-1	MM14	Total	Microbial mat	Pond_13		Surface	4	35.4	3789	16	3788	2.029	9.4	9.31	20.283806°S68.888778°W
MM14-1S	MM14	Lysis-resistants	Microbial mat	Pond_13		Surface	4	4.9	3789	16	3788	2.029	9.4	9.31	20.283806°S68.888778°W
MM15-1	MM15	Total	Microbial mat	Pond_13		Surface	4	10.7	3791	16	3788	2.029	9.4	9.31	20.283778°S68.888778°W
MM15-1S	MM15	Lysis-resistants	Microbial mat	Pond_13		Surface	2.5	2.5	3791	16	3788	2.029	9.4	9.31	20.283778°S68.888778°W
MM16-1	MM16	Total	Microbial mat	Pond_14		Surface	4	140	3794	16	4395	2.365	21.5	9.51	20.283667°S68.888778°W
MM16-1S	MM16	Lysis-resistants	Microbial mat	Pond_14		Surface	4	13.8	3794	16	4395	2.365	21.5	9.51	20.283667°S68.888778°W
MM17-1	MM17	Total	Microbial mat	Pond_15		Surface	4	123		16.9	4395	2.365	21.5	9.51	20.283694°S68.888778°W
MM17-1S	MM17	Lysis-resistants	Microbial mat	Pond_15		Surface	4	6.54		16.9	4395	2.365	21.5	9.51	20.283694°S68.888778°W
MM18-1	MM18	Total	Microbial mat	Pond_16		Surface	4	308	3796	15.42	4395	2.365	21.5	9.51	20.283528°S68.888778°W
MM18-1S	MM18	Lysis-resistants	Microbial mat	Pond_16		Surface	4	4.34	3796	15.42	4395	2.365	21.5	9.51	20.283528°S68.888778°W
MM19-1	MM19	Total	Microbial mat	Pond_17		Surface	4	38.8	3794	19.4	4395	2.365	21.5	9.51	20.283583°S68.888778°W
MM19-1S	MM19	Lysis-resistants	Microbial mat	Pond_17		Surface	0.226	0.226	3794	19.4	4395	2.365	21.5	9.51	20.283583°S68.888778°W
MM2-1	MM2	Total	Microbial mat	Pond_2	Dry samples from the surface. Green mat	Surface	4	346	3791	16	1565	0.831	13.9	7.65	20.283111°S68.888778°W
MM2-1S	MM2	Lysis-resistants	Microbial mat	Pond_2	Dry samples from the surface. Green mat	Surface	4	19.3	3791	16	1565	0.831	13.9	7.65	20.283111°S68.888778°W
MM20-1	MM20	Total	Microbial mat	Pond_18		Surface	4	114	3788	16	1588	0.846	16.5	8.73	20.283583°S68.888778°W
MM20-1S	MM20	Lysis-resistants	Microbial mat	Pond_18		Surface	4	4.9	3788	16	1588	0.846	16.5	8.73	20.283583°S68.888778°W
MM3-1	MM3	Total	Microbial mat	Pond_3	Submerged. Black/green. High content of EPS	Surface	4	278		16	747	0.412	8.06	7.9	20.283167°S68.888778°W
MM3-1S	MM3	Lysis-resistants	Microbial mat	Pond_3	Submerged. Black/green. High content of EPS	Surface	0.616	0.616		16	747	0.412	8.06	7.9	20.283167°S68.888778°W
MM4-1	MM4	Total	Microbial mat	Pond_4	Highly structured. Submerged. Pink area	Surface	4	110	3795	16	747	0.412	8.06	7.9	20.282917°S68.888778°W
MM4-1S	MM4	Lysis-resistants	Microbial mat	Pond_4	Highly structured. Submerged. Pink area	Surface	1.56	1.56	3795	16	747	0.412	8.06	7.9	20.282917°S68.888778°W
MM5-1	MM5	Total	Microbial mat	Pond_4	Highly structured. Submerged. Pink area	Surface	4	168	3796	16	1386	0.738	9.4	7.89	20.283056°S68.888778°W
MM5-1S	MM5	Lysis-resistants	Microbial mat	Pond_4	Highly structured. Submerged. Pink area	Surface	0.706	0.706	3796	16	1544	0.738	9.4	7.89	20.283056°S68.888778°W
MM6-1	MM6	Total	Microbial mat	Pond_5	Submerged. Brown	Surface	4	114	3803	16	1459	0.738	9.4	7.89	20.282861°S68.888778°W
MM6-1S	MM6	Lysis-resistants	Microbial mat	Pond_5	Submerged. Brown	Surface	0.2	0.2	3803	16	1459	0.738	9.4	7.89	20.282861°S68.888778°W
MM7-1	MM7	Total	Microbial mat	Pond_6	Submerged	Surface	4	110	3789	16	1142	0.738	9.4	7.89	20.282917°S68.888778°W
MM7-1S	MM7	Lysis-resistants	Microbial mat	Pond_6	Submerged	Surface	1.54	1.54	3789	16	1142	0.738	9.4	7.89	20.282917°S68.888778°W
MM8-1	MM8	Total	Microbial mat	Pond_7	Highly structured. Several layers	Surface	4	195	3799	16	8322	4.229	11.3	9.74	20.282889°S68.888778°W
MM8-1S	MM8	Lysis-resistants	Microbial mat	Pond_7	Highly structured. Several layers	Surface	4	6.18	3799	16	8322	4.229	11.3	9.74	20.282889°S68.888778°W
MM9-1	MM9	Total	Microbial mat	Pond_8	Reddish mat not highly structured. Submerged	Surface	4	17.5	3790	16	3788	2.029	21.3	9.31	20.283389°S68.888778°W
MM9-1S	MM9	Lysis-resistants	Microbial mat	Pond_8	Reddish mat not highly structured. Submerged	Surface	4	4.18	3790	16	3788	2.029	21.3	9.31	20.283389°S68.888778°W
Salar1-1-1	S111	Total	Lake sediment	Sample_1	Gray and liquid	1	4	112	3791	2	78.04	53.69	17.1	8.32	20.285444°S68.888778°W
Salar1-1-1S	S111	Lysis-resistants	Lake sediment	Sample_1	Gray and liquid	1	1.04	1.04	3791	2	78.04	53.69	17.1	8.32	20.285444°S68.888778°W
Salar1-2-1	S121	Total	Lake sediment	Sample_1	Gray and liquid	2	4	118	3791	2	78.04	53.69	17.1	8.32	20.285444°S68.888778°W
Salar1-2-1S	S121	Lysis-resistants	Lake sediment	Sample_1	Gray and liquid	2	1.34	1.34	3791	2	78.04	53.69	17.1	8.32	20.285444°S68.888778°W
Salar1-3-1	S131	Total	Lake sediment	Sample_1	Gray and liquid	3	4	102	3791	2	78.04	53.69	17.1	8.32	20.285444°S68.888778°W
Salar1-3-1S	S131	Lysis-resistants	Lake sediment	Sample_1	Gray and liquid	3	0.648	0.648	3791	2	78.04	53.69	17.1	8.32	20.285444°S68.888778°W
Salar1-4-1	S141	Total	Lake sediment	Sample_1	Gray and liquid	4	4	51.4	3791	2	78.04	53.69	17.1	8.32	20.285444°S68.888778°W
Salar1-4-1S	S141	Lysis-resistants	Lake sediment	Sample_1	Gray and liquid	4	0.918	0.918	3791	2	78.04	53.69	17.1	8.32	20.285444°S68.888778°W
Salar2-1-1	S211	Total	Lake sediment	Sample_2	Dark gray	1	4	212	3787	5	78.04	53.69	17.1	8.32	20.285194°S68.888778°W
Salar2-1-1S	S211	Lysis-resistants	Lake sediment	Sample_2	Dark gray	1	1.88	1.88	3787	5	78.04	53.69	17.1	8.32	20.285194°S68.888778°W
Salar2-2-1	S221	Total	Lake sediment	Sample_2	Dark gray	2	4	136	3787	5	78.04	53.69	17.1	8.32	20.285194°S68.888778°W
Salar2-2-1S	S221	Lysis-resistants	Lake sediment	Sample_2	Dark gray	2	2.18	2.18	3787	5	78.04	53.69	17.1	8.32	20.285194°S68.888778°W
Salar3-1-1	S311	Total	Lake sediment	Sample_3	4m from delta	4	4	74	3786	4	78.04	53.69	17.1	8.32	20.284611°S68.888778°W
Salar3-1-1S	S311	Lysis-resistants	Lake sediment	Sample_3	4m from delta	4	0.678	0.678	3786	4	78.04	53.69	17.1	8.32	20.284611°S68.888778°W
Salar3-2-1	S321	Total	Lake sediment	Sample_3	4m from delta	3	4	27.8	3786	4	78.04	53.69	17.1	8.32	20.284611°S68.888778°W
Salar3-2-1S	S321	Lysis-resistants	Lake sediment	Sample_3	4m from delta	3	0.776	0.776	3786	4	78.04	53.69	17.1	8.32	20.284611°S68.888778°W
Salar3-3-1	S331	Total	Lake sediment	Sample_3	4m from delta	2	4	118	3786	4	78.04	53.69	17.1	8.32	20.284611°S68.888778°W
Salar3-3-1S	S331	Lysis-resistants	Lake sediment	Sample_3	4m from delta	2	0.772	0.772	3786	4	78.04	53.69	17.1	8.32	20.284611°S68.888778°W
Salar3-4-1	S341	Total	Lake sediment	Sample_3	4m from delta	1	4	91.2	3786	4	78.04	53.69	17.1	8.32	20.284611°S68.888778°W
Salar3-4-1S	S341	Lysis-resistants	Lake sediment	Sample_3	4m from delta	1	1.94	1.94	3786	4	78.04	53.69	17.1	8.32	20.284611°S68.888778°W
Salar4-1-1	S411	Total	Lake sediment	Sample_4	Foul odour	1	4	85.6	3780	10	52450	34.19	12	8.43	20.283778°S68.888778°W
Salar4-1-1S	S411	Lysis-resistants	Lake sediment	Sample_4	Foul odour	1	0.274	0.274	3780	10	52450	34.19	12	8.43	20.283778°S68.888778°W
Salar4-2-1	S421	Total	Lake sediment	Sample_4	Foul odour	2	4	102	3780	10	52450	34.19	12	8.43	20.283778°S68.888778°W
Salar4-2-1S	S421	Lysis-resistants	Lake sediment	Sample_4	Foul odour	2	0.458	0.458	3780	10	52450	34.19	12	8.43	20.283778°S68.888778°W
Salar4-3-1	S431	Total	Lake sediment	Sample_4	Foul odour	3	4	100	3780	10	52450	34.19	12	8.43	20.283778°S68.888778°W
Salar4-3-1S	S431	Lysis-resistants	Lake sediment	Sample_4	Foul odour	3	0.88	0.88	3780	10	52450	34.19	12	8.43	20.283778°S68.888778°W
Salar5	S4	/	Lake sediment	Sample_5	No sediment could be collected	/	/	/	3789	13	52.48	34.17	7.3	8.43	20.283528°S68.888778°W
Salar6-1-1	S611	Total	Lake sediment	Sample_6	Strong foul odour	1	4	94.8	3791	15	50490	32.77	7.9	8.43	20.283444°S68.888778°W
Salar6-1-1S	S611	Lysis-resistants	Lake sediment	Sample_6	Strong foul odour	1	0.198	0.198	3791	15	50490	32.77	7.9	8.43	20.283444°S68.888778°W
Salar6-2-1	S621	Total	Lake sediment	Sample_6	Strong foul odour	2	4	70	3791	15	50490	32.77	7.9	8.43	20.283444°S68.888778°W
Salar6-2-1S	S621	Lysis-resistants	Lake sediment	Sample_6	Strong foul odour	2	0.606	0.606	3791	15	50490	32.77	7.9	8.43	20.283444°S68.888778°W
Salar6-3-1	S631	Total	Lake sediment	Sample_6	Strong foul odour	3	4	108	3791	15	50490	32.77	7.9	8.43	20.283444°S68.888778°W
Salar6-3-1S	S631	Lysis-resistants	Lake sediment	Sample_6	Strong foul odour	3	0.134	0.134	3791	15	50490	32.77	7.9	8.43	20.283444°S68.888778°W
Salar7-1-1	S711	Total	Lake sediment	Sample_7		4	4	114	3777	16	132	0.706	12	8.22	20.283472°S68.888778°W
Salar7-1-1S	S711	Lysis-resistants	Lake sediment	Sample_7		4	0.432	0.432	3777	16	132	0.706	12	8.22	20.283472°S68.888778°W
Salar7-2-1	S721	Total	Lake sediment	Sample_7		3	4	102	3777	16	132	0.706	12	8.22	20.283472°S68.888778°W
Salar7-2-1S	S721	Lysis-resistants	Lake sediment	Sample_7		3	0.36	0.36	3777	16	132	0.706	12	8.22	20.283472°S68.888778°W
Salar7-3-1	S731	Total	Lake sediment	Sample_7		2	4	63.6	3777	16	132	0.706	12	8.22	20.283472°S68.888778°W
Salar7-3-1S	S731	Lysis-resistants	Lake sediment	Sample_7		2	0.364	0.364	3777	16	132	0.706	12	8.22	20.283472°S68.888778°W
Salar7-4-1	S741	Total	Lake sediment	Sample_7		1	4	112	3777	16	132	0.706	12	8.22	20.283472°S68.888778°W
Salar7-4-1S	S741	Lysis-resistants	Lake sediment	Sample_7		1	0.658	0.658	3777	16	132	0.706	12	8.22	20.283472°S68.888778°W

Additional Table 2. Values of the DNA quantification after DNA extraction. DNA concentration in ng/ul.

Sample type	Fungus	Treatment	DNA (ng/ul)
asexual spores	Coprinopsis cinerea	lysis	0.222
asexual spores	Coprinopsis cinerea	lysis	< 0,05
asexual spores	Mortierella antarctica	lysis	0.120
asexual spores	Mortierella antarctica	lysis	< 0,05
asexual spores	Aspergillus niger	lysis	2.620
asexual spores	Aspergillus niger	lysis	0.126
sexual spores	Coprinopsis cinerea	direct	< 0,05
sexual spores	Coprinopsis cinerea	direct	< 0,05
yeast	Candida albicans	direct	4.420
yeast	Candida albicans	direct	2.700
asexual spores	Ulocladium alternata	direct	< 0,05
asexual spores	Ulocladium alternata	direct	< 0,05
asexual spores	Mortierella antarctica	direct	< 0,05
asexual spores	Mortierella antarctica	direct	< 0,05
asexual spores	Ulocladium alternata	lysis	0.126
asexual spores	Ulocladium alternata	lysis	< 0,05
sexual spores	Coprinopsis cinerea	lysis	< 0,05
sexual spores	Coprinopsis cinerea	lysis	0.154
asexual spores	Coprinopsis cinerea	direct	< 0,05
asexual spores	Coprinopsis cinerea	direct	< 0,05
yeast	Candida albicans	lysis	< 0,05
yeast	Candida albicans	lysis	0.172
asexual spores	Aspergillus niger	direct	< 0,05
asexual spores	Aspergillus niger	direct	< 0,05
mycelium	Ulocladium alternata	direct	< 0,05
mycelium	Ulocladium alternata	direct	< 0,05
mycelium	Ulocladium alternata	lysis	< 0,05
mycelium	Ulocladium alternata	lysis	< 0,05
mycelium	Aspergillus niger	direct	< 0,05
mycelium	Aspergillus niger	direct	< 0,05
mycelium	Aspergillus niger	lysis	< 0,05
mycelium	Aspergillus niger	lysis	< 0,05
mycelium	Coprinopsis cinerea	direct	2.14
mycelium	Coprinopsis cinerea	direct	4.6
mycelium	Coprinopsis cinerea	lysis	< 0,05
mycelium	Coprinopsis cinerea	lysis	< 0,05
mycelium	Mortierella antarctica	direct	28.000
mycelium	Mortierella antarctica	direct	7.4
mycelium	Mortierella antarctica	lysis	< 0,05
mycelium	Mortierella antarctica	lysis	< 0,05

Chapter 4 — Multiple roads leading to Rome: unique morphology and chemistry of resistant cells in bacteria

Foreword:

This chapter will be submitted to the Access Microbiology journal. Here, we assessed the differences and similarities between bacterial spore models, endospores, exospores, myxospores, cysts, and akinetes. To this end, we analyzed their morphological characteristics through Cryo-EM and microscopy and their chemical composition with single cell Raman microspectroscopy. My personal contribution as first author was sample preparation, analysis and writing of the manuscript.

Multiple roads lead to Rome: unique morphology and chemistry of resting cells in bacteria

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Abstract

The production of specialized resting cells is a remarkable survival strategy developed by many organisms to withstand unfavorable environmental factors such as nutrient depletion or other changes in abiotic and biotic conditions. Five bacterial phyla are recognized to form specialized resting cells: *Firmicutes*, forming endospores*; Actinobacteria,* forming exospores*; Cyanobacteria,* forming akinetes*;* the δ-Proteobacterial order Myxococcales, forming myxospores; and Azotobacteraceae, forming cysts. All these specialized resting cells are characterized by a low to absent metabolic activity and higher resistance to environmental stress (desiccation, heat, starvation, etc.) as compared to vegetative cells. Given their similarity in function, we tested the hypothesis of the existence of a universal morpho-chemical marker for identifying these specialized resting cells. After the production of endospores, exospores, akinetes, and cysts in model organisms, cryo-electron microscopy was used to analyze the morphology of the resting cell compared to their respective vegetative cells. Resting cells only shared a thicker cell envelope, but no other morphological component was common to all of them. The chemical composition of the different specialized resting cells at the single cell level was also investigated using single-cell confocal Raman microspectroscopy. Our results show that the different specialized cells do not share a common chemical signature, but on the contrary, each group has a unique composition with a variable conservation of the chemical signal of the vegetative cells. Furthermore, this is to the best of our knowledge, the first across-species comparison of bacterial sporulation. Additionally, we present the identification of a marker for the isolation of endospores from environmental samples, as a proof of concept of the feasibility of isolating bacterial spores based on their unique chemical

signature using a Raman-activated cell sorting platform. Based on this across-species comparison and the current knowledge of genetic pathways inducing the formation of the resting cells in each group, we propose that the formation of specialized resting cells in bacteria did not evolve from a common ancestor. Instead, the formation of specialized resting cells is a convergent strategy with the same goal, the promotion of survival.

Introduction

Microorganisms are exposed to periodic cycles of nutrient exhaustion and different abiotic and biotic stresses that are unfavorable for growth and reproduction (1,2). An important survival strategy for many bacteria under these conditions is the transition into dormancy (3,4). There are two main strategies to transition into dormancy: the production of long-lived dormant persister cells that are resistant to antibiotics (5), and the production of specialized resistant cells called spores or cysts. The phyla recognized for the formation of specialized resistant cells are: Firmicutes, Actinomycetes, Cyanobacteria, and the δ-Proteobacterial order Myxococcales, and Azotobacteracea. The best studied specialized structures are dormant long-lived endospores produced by members of the Firmicutes. This phylum is predominantly conformed by Gram-positive bacteria, however it also includes two Gram-positive clades, the Negativicutes and the Halanaerobiales (6,7). Endospores are one of the most resistant specialized cells, being able to resist high temperatures (up to 100°C), ionizing radiation, chemical solvents, detergents, and also to stay dormant in the environment for long periods of time (8–10). In *Bacillus* species, endosporulation is induced by starvation. This process begins with the asymmetric division of the cell, where two cells of significantly different size are produced. Then the pre-spore (the smaller cell) is engulfed by the mother cell (larger cell). After engulfment the development of the cortex and the synthesis of the spore's coat take place. Finally, the mother cell lyses, releasing a mature spore (11,12) (Fig. 1A). In diderm Firmicutes such as *Acetonema longum* (Negativicutes class) the endosporulation process is the same as in monoderm Firmicutes. However, during the sporulation process, the inner membrane of the mother cell is inverted and transformed to become the outer membrane of the spore and after germination, the outer membrane of the cell (13,14). After endospores, the second most studied type of spore are exospores, which are

produced by members of the Actinobacteria phylum. Actinobacteria are Gram-positive bacteria, some of which evoke fungi due to their mycelial morphology. The production of exospores in Actinobacteria is also triggered by nutrient depletion (15,16). After sporulation is triggered, non-branching aerial hyphae are produced from the colony surface. Then the hyphae initiate synchronous cell division to produce many identical spores and finally, the mature spores are released into the environment (Fig. 1B). Mature exospores produced by *Streptomyces* are more resistant to desiccation, low temperature and osmotic changes than vegetative cells (17). However, they are less resistant to heat and desiccation than endospores (18).

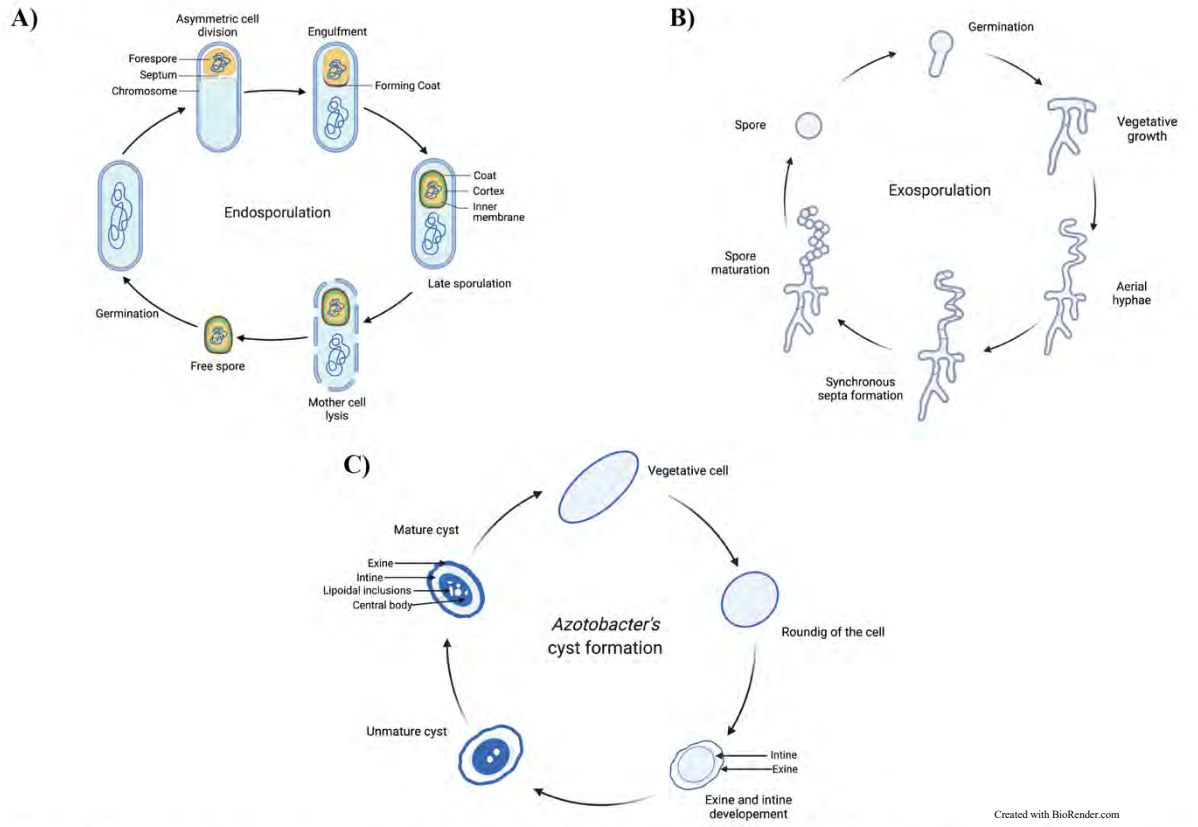

Figure 1. Diagram of the formation of **A)** Endospores produced by some members of the Firmicutes phyla, **B)** Exospores produced by some members of the Actinobacteria phyla, **C)** Cyst produced by some members of the Azotobacteracea order.

In comparison to Firmicutes and Actinobacteria, *Azotobacter, Myxococcus* and Cyanobacteria are less studied spore formers. In the case of *Azotobacter*, most of the research done on the physiology and morphology of *Azotobacter* cysts (specialized resistant cells) was done in the 70's and 80's, while in recent years the research in *Azotobacter* has mainly focused on nitrogen fixation and its application in agriculture (19–21). Thus, little has been done to better understand the encystment process and

its genetic components. *Azotobacter* are Gram-negative, nitrogen fixing bacteria belonging to the Pseudomonadacea family and characterized by the production of cysts, which are resistant to desiccation. These cyst have been shown to survive in dry soil for more than 10 years (22,23). In laboratory conditions, the production of cysts takes places in the late stationary phase of a culture, or it is induced by the addition of n-butanol or β-hydroxybutyrate as a carbon source. The process starts by the rounding up of the cell and then the exine (a bark-like coat) starts to form loosely around the cells. Finally, the cyst matures between the 3rd and 5th day. At this point, the mature cyst presents a thick layered exine, a small central body with lipoidal inclusions and a fibrous structure between the exine and central body called the intine, that makes more than half of the volume of the cysts (24–26) (Fig. 1C).

Like *Azotobacter, Myxococcus* are Gram-negative bacteria belonging to the Proteobacteria phyla. *Myxococcus* has a unique strategy to form resistant dormant cells called myxospores. The formation of myxospores starts when cultures reach high density and encounter starvation conditions. The rod-shape vegetative cells glide to aggregation centers, to later merge and form large mounds, which then lead to the formation of fruiting bodies. Finally some cells inside the fruiting body will differentiate into dormant coccus-shaped myxospores, while the peripheral rod-shape cells remain as dormant cells (27,28) (Fig. 2A). Myxospores are not as resistant as endospores, but they show higher resistance to temperature, desiccation, UV, and sonic vibration than the corresponding vegetative cells (29). This unique multicellular strategy to form spores has been more investigated in comparison to *Azotobacter* encystment, but many genetic markers and mechanisms that lead to the production of myxospores are still unknown (30–32).

In the Cyanobacteria phyla, only two orders are known to form spore-like cells: the Nostocales and Stigonematales. They are also known by the formation of heterocysts, which are nitrogen fixing specialized cells. The spore-like cells formed by Cyanobacteria are called akinetes and are characterized by being dormant, non-motile cells with thick walls (33–35). The formation of akinetes allows these cyanobacteria to dominate many water bodies, as they have the ability to survive harsh environmental conditions and grow back when conditions become favorable. As for the other spores, nutrient depletion triggers the formation of akinetes. However, Perez et al., 2016 showed that the specific triggers might be species dependent. For example, for *Anabaena variabilis,* the trigger is low light, while for *Nostoc punctiforme* it is

phosphate starvation. After receiving the signal, cell differentiation starts by the accumulation of glycogen, cyanophycin, and lipid droplets, as well as by the reduction of metabolic activity until the mature akinete is formed. Mature akinetes are bigger and have a thicker wall than the vegetative cells. In addition, one important characteristic of akinetes is that their density is higher than water, thus they are able to sink to the bottom where they can survive for several months or decades until conditions improve (33,34,37,38) (Fig. 2B).

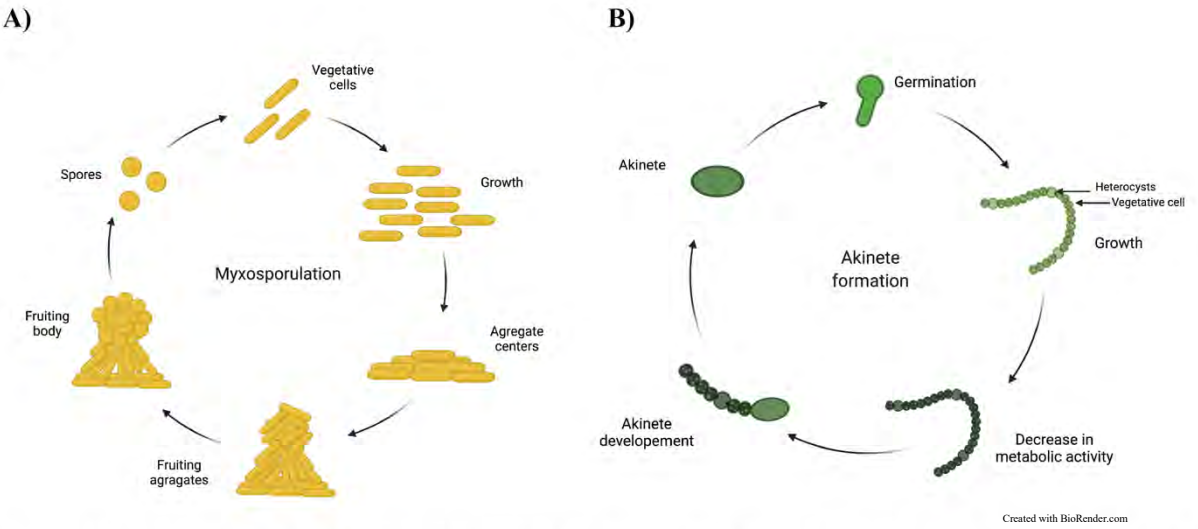

Figure 2. Diagram of the formation of **A)** Myxospores produced by some members of the Myxococcales order, **B)** Akinetes produced by some members of the Cyanobacteria phyla.

Even though the strategies used to form specialized resting cells in Firmicutes, Actinobacteria*, Azotobacter, Myxococcus,* and Cyanobacteria differ significantly, the resulting cells have in all cases a similar function. This has led some authors to suggest that formation of a spore-like cell is an ancestral character in bacteria. It has been previously hypothesized that a form of endosporulation might have been a trait in a common ancestor of bacteria that led to the origin of the outer membrane, and thus to the origin of Gram-negative bacteria (13,39–42). One of the reasons for this hypothesis is the key step of engulfment in which a monoderm (Gram-positive pre-spore cell) acquires a second temporary “spore” membrane (diderm) from the mother cell. The spore membrane is lost upon germination in monoderm species, but in the case of Gram-negative spore-forming Firmicutes such as *Acetonema longum*, it is retained and remodeled to constitute the outer membrane of the vegetative cell (39). If such a common sporulating ancestor existed, then extant spore-like cells might still

share similar features. Therefore, we tested the hypothesis of the existence of a universal morpho-chemical marker to identify these specialized resting cells. Strains representative of the five spore types were investigated: *Bacillus* spp. (endospores), *Streptomyces* spp. (exospores), *Azotobacter chroococcum* (cysts), *Myxococcus xanthus* (myxospores), and *Anabaena cylindrica* (akinetes). In the attempt to find a universal marker for the identification and isolation of resistant cells (hereafter referred to as "spores"), we assessed the morphology of both types of cells (vegetative and spore) using optical microscopy and cryo-electron microscopy (Cryo-EM). By using Cryo-EM instead of traditional electron microscopy, the native cell structure is preserved as the steps of dehydration and fixation are not carried out, and the production of artifacts is minimized. In addition, we assessed the chemical composition of the vegetative cells and spores using single-cell confocal Raman microspectroscopy. This single cell approach allowed us to overcome the limitation of asynchronous sporulation of a bacterial culture and only assess the chemical composition of vegetative or spore cells. Our results show that although spores of the different model bacteria present a thicker cell envelope, no other morphological characteristic could be identified as universal marker for sporulation. Similar results were found for the chemical composition. No shared chemical marker could be identified among different spore types, but specific markers were found to discriminate between vegetative cells and spores from the same genus/spore type. Additionally, as a proof of concept, we identified an endospore-specific chemical marker that could be used for sorting and isolation of endospores from environmental samples.

Material and Methods

1. Bacterial strains

The following bacterial strains were used: for the analysis of endospores — *Bacillus subtilis* (NEU16, Neuchâtel University culture collection), *Bacillus sphaericus* (NEU1003, Neuchâtel University culture collection), *Bacillus thuringiensis* (NEU1070/DSM350); exospores — *Streptomyces violaceoruber* (NEU1225/DSM 40783), *Streptomyces avermitilis* (NEU1226/DSM46492); myxospores — *Myxococcus xanthus* (Serengeti 01, Chinhaya 20 and Indiana MC3.5.9C15, culture collection from the Department of Environmental Systems Science at ETHZ); cysts —

Azotobacter chroococcum (NEU1159/DSM2289; akinetes — *Anabaena cylindrica* (PCC7122) (Table 1).

2. Sample preparation

2.1. Vegetative cell cultures

Vegetative cells were prepared as follows: *B. subtilis*, *B. sphaericus*, *B. thuringiensis*, *S. violaceoruber*, and *S. avermitilis* were cultured in 15 ml of liquid sterile Nutrient Broth (NB) (Art. Nr. AE92.2, Carl Roth, Karlsruhe, Germany) under shaking at 120 rpm at 30°C and were collected after 12-18 h of incubation. *A. chroococcum* was cultured in 15 ml of sterile liquid Burk's media (43) under shaking at 120 rpm at 30°C and were collected after 24 h of incubation. The strains of *M. xanthus* were cultured in 15 ml of sterile liquid Casitone-Tris complex medium (CTT) (44) under shaking at 120 rpm at 30°C and were collected after 12-18 h of incubation. *A. cylindrica* was cultured in 20 ml of sterile nitrogen free BG11 (45) in a 16 h light, 8 h darkness cycle, shaking at 70 rpm at RT and the cells were collected after 36 h of incubation (Table 1).

Table 1. Bacterial strains used for each analysis and the culture conditions used for the production of vegetative and spore cells. Analysis: Microscopy (M), Cyo-Electron Microscopy (CM), Raman spectroscopy (R)

Strain	Analysis	Vegetative cells			Spores			
		Media	Temperature (°C)	Time (hrs)	Type	Media	Temperature (°C)	Time (days)
Bacillus subtilis (NEU16)	M, CM, R	Nutrient Broth	30	12 to 18	Endospore	Nutrient Agar	30	3
Bacillus thuringiensis (NEU1070/DSM350)	R	Nutrient Broth	30	12 to 18	Endospore	Nutrient Agar	30	3
Bacillus sphaericus (NEU 1003)	R	Nutrient Broth	30	12 to 18	Endospore	Nutrient Agar	30	3
Streptomyces avermitilis (NEU1226/DSM46492)	R	Nutrient Broth	30	12 to 18	Exospore	Nutrient Agar	30	3 to 5
Streptomyces violaceoruber (NEU1225/DSM 40783)	M, CM, R	Nutrient Broth	30	12 to 18	Exospore	Nutrient Agar	30	3 to 5
Myxococcus xanthus (Serengeti 01)	M, CM, R	liquid CTT	30	12 to 18	Myxospore	¼ CTT (1.5% agar)	30	3 to 5
Myxococcus xanthus (Chinhaya 20)	R	liquid CTT	30	12 to 18	Myxospore	¼ CTT (1.5% agar)	30	3 to 5
Myxococcus xanthus (Indiana MC3.5.9C15)	R	liquid CTT	30	12 to 18	Myxospore	¼ CTT (1.5% agar)	30	3 to 5
Azotobacter chroococcum (NEU1159/DSM2289)	M, CM, R	Burk's media	30	24	Cysts	Burk's media ⁻sucrose +0.2%n-butanol	30	3 to 5
Anabaena cylindrica (PCC7122)	M, CM, R	BG11 ⁻N	RT	36	Akinete	BG11 ⁻N	4 in the dark	10 to 14

2.2. Spore induction and collection

The spores of *B. subtilis*, *B. sphaericus*, *B. thuringiensis*, *S. violaceoruber*, and *S. avermitilis* were produced by inoculating Petri dishes of Nutrient Agar (NA) (Art. Nr. AE92.2, Carl Roth, Karlsruhe, Germany) and incubating at 30°C for 2-3 days for endospores and 3-5 days for exospores. The endospores of *B. subtilis*, *B. sphaericus*, and *B. thuringiensis* were collected with the loop from the surface of the agar and

resuspended in 2 ml of sterile 0.2 M glycerol solution. The exospores of *S. violaceoruber* and *S. avermitilis* were collected by adding 100 sterile glass beads (4-mm diameter) into the surface of the culture and shaking for 30 sec. To recover the exospores that have been adhered to the beads, 5 ml of TX buffer (0.05M tris(hydroxymethyl)aminomethane, 0.001% (vol/vol) Triton X-100, pH 7.3) were then added and then the fluid was collected into a 15-ml Falcon tube. This process was repeated twice to maximize the collection of all spores. The buffer containing the spores was then centrifuged at 10,000 x g for 10 min, then the supernatant was removed, and the spores were resuspended in 2 ml of sterile 0.2 M glycerol solution. The cysts of *A. chroococcum* were produced as follows. Approximately 20 ml of vegetative cells were produced as indicated above in Burk's media. After 12 h the media was centrifugated at 3,000 x g and the supernatant was discarded. The pellet was then resuspended in approximately 20 ml of Burk's sucrose free liquid media with 0.2% of n-butanol and was incubated at room temperature for 5 to 7 days. The myxospores from *M. xanthus* strains were produced by inoculating densely on Petri dishes of ¼ CTT (CTT media with ¼ of the original Casitone) solid medium and incubating at 30°C for 5 to 7 days. ¼ CTT media was used instead of TPM media to obtain a higher number of spores. The myxospores were then collected with a loop from the agar surface and re-suspended in 2 ml of sterile physiological water (0.9% NaCl dH2O). Akinetes of *A. cylindrica* were produced by placing approximately 20 ml of a stationary culture of *A. cylindrica* covered with aluminum at 4°C for 10-14 days (Table 1).

3. Optical microscopy

All optical microscopy was performed in an Upright Leica DM4 B Microscope (Leica, Wetzlar, Germany) and the images were generated with a Leica Microscope Camara DFC7000T (Leica, Wetzlar, Germany). To obtain better images, an agar pad was used to observe the cells. For this a 1.5% agar was prepared and approximately 750 µl of it were added onto a glass slide. While still warm a cover slip was place on top of the agar. Once the agar was solid, the cover slip was removed and 1 µl of the vegetative culture was pipetted on top. In the case of the spores, of *B. subtilis*, *S. violaceoruber*, and *M. xanthus* they were collected from the agar plate and resuspended in 1 ml of physiological water (0.9%NaCl) before microscopy. While for spores of *A. cylindrica*,

a 1 µl drop of the culture was pipetted onto the agar pad. Furthermore, to improve the identification of the *A. chroococcum* cysts, 100 µl of the spore culture were collected in a 1.5 ml Eppendorf and mixed with 100 µl of "cyst stain" prepared as described before (46). The mix was let to rest for 10 min before it was used for microscopy, where a 1 µl drop was pipetted onto the agar pad.

4. Cryo-EM

4.1. Sample preparation and imaging

The vegetative cells and spores from *B. subtilis*, *S. violaceoruber*, *A. chroococcum*, *M. xanthus*, and *A. cylindrica,* were prepared as described above — culture volumes were doubled or tripled to obtain a higher biomass. This samples were prepared for the electron microscopy by high-pressure freezing (47). The samples were resuspended in PBS supplemented with 30% dextran (D1662 from Sigma-Aldrich, St. Louis, Missouri, USA). They were then cooled under high-pressure (2000 bar) to liquid nitrogen temperature (-196° C) within milliseconds using an EM-PACT2 machine (Leica Microsystems, Wetzlar, Germany). These conditions prevent the formation of ice crystals, which can damage cellular structures. The frozen samples were then cut into thin slices (50 nm) in a cryo-ultramicrotome UC6 FC6 (Leica Microsystems, Wetzlar, Germany) and placed on a R3.5-1 holey carbon EM grid (Quantifoil, Großlöbichau, Germany). Samples prepared in this way were then loaded into a Tecnai F20 transmission electron microscope (ThermoFisher Scientific, Waltham, Massachusetts, USA) on a cryo-holder model 626 (Gatan, Pleasanton, California, USA) keeping the sample temperature low (-180°C) and analyzed by the low-dose method at 200 kV acceleration voltage.

Table 2. Bacterial strains used for the establishment of a endospore threshold and the culture conditions used for the production of vegetative and spore cells.

Strain	Vegetative cells			Spores			
	Media	Temperature (°C)	Time (hrs)	Type	Media	Temperature (°C)	Time (days)
Bacillus subtilis (NEU16)	Nutrient Broth	30	12 to 18	Endospore	Nutrient Agar	30	3
Bacillus thuringiensis (NEU1070/DSM350)	Nutrient Broth	30	12 to 18	Endospore	Nutrient Agar	30	3
Bacillus sphaericus (NEU 1003)	Nutrient Broth	30	12 to 18	Endospore	Nutrient Agar	30	3
Paenibacillus marcerans (NEU1004)	Nutrient Broth	30	12 to 18	Endospore	Nutrient Agar	30	3
Paenibacillus alvei (NEU1291)	Nutrient Broth	30	12 to 18	Endospore	Nutrient Agar	30	3
Bacillus cereus (SPO95)	Nutrient Broth	30	12 to 18	Endospore	Nutrient Agar	30	3
Bacillus weihenstephanensis (SPO146)	Nutrient Broth	30	12 to 18	Endospore	Nutrient Agar	30	3
Bacillus cereus (SPO91)	Nutrient Broth	30	12 to 18	Endospore	Nutrient Agar	30	3
Bacillus sp. (SPO122)	Nutrient Broth	30	12 to 18	Endospore	Nutrient Agar	30	3
Streptomyces avermitilis (NEU1226/DSM46492)	Nutrient Broth	30	12 to 18	Exospore	Nutrient Agar	30	3 to 5
Streptomyces violaceoruber (NEU1225/DSM40783)	Nutrient Broth	30	12 to 18	Exospore	Nutrient Agar	30	3 to 5
Actinomycetes sp. (NEU70)	Nutrient Broth	30	12 to 18	Exospore	Nutrient Agar	30	3 to 5
Arthrobacter globiformis (NEU1124/DSM20124)	Nutrient Broth	30	12 to 18	//	//	//	//
Arthrobacter nicotianae (NEU1123/20123)	Nutrient Broth	30	12 to 18	//	//	//	//
Azotobacter chroococcum (NEU1159/DSM2289)	Burk's media	30	24	Cysts	Burk's media ⌀sucrose +0.2%n-butanol	30	3 to 5
Anabaena cylindrica (PCC7122)	BG11 ⌀N	RT	36	Akinete	BG11 ⌀N	4 in the dark	10 to 14

4.2. Image analysis

The Cryo-EM images were analyzed using the software Fiji version 2.3.0/1.53f (48). For each species, representative high-quality images were selected to analyze the cell envelope density profile of both vegetative and spore cells. Using the line tool, a perpendicular line with a 23-pixel width was drawn across the cell envelope to reduce the noise. From this line, the density profile was plotted for each image. In addition, the width of the cell wall was measured 5 times in different areas of the cell for each image to obtain an average width.

5. Single-cell Raman microspectroscopy

A confocal Raman microspectroscope (LabRAM HR Evolution, Horiba Scientific, France) was used. This system is based on an upright microscope (Olympus BXFM, Olympus, Shinjuku, Tokyo, Japan) that is integrated with components for Raman measurements, including a 532 nm neodymium-yttrium aluminum garnet (Nd:YAG) laser (Cobolt Samba^{TM}, Hübner Photonics GmbH, Kassel, Germany); a 600 lines/mm diffraction grating (blazed at 500 nm); a 100× dry objective (Olympus MPlan N, numerical aperture (NA) = 0.9); a confocal pinhole with 100 µm; and a back-illuminated deep depleted charge-coupled device (CCD) detector (1,024×256 pixels, pixel size: 26×26 µm; Horiba Scientific).

A two µl drop containing a sample of either vegetative cells or spores for each strain was put on an aluminum-coated slide (AI136; EMF Corporation, Rochester, New York, USA) and dried at 30°C for 15 min. The sample was dipped into a 50-ml falcon tube (for 1–2 sec) filled with 0.2 M glycerol solution to remove the residual traces of the medium. The sample was then dried using air blowing and the Raman spectra of single cells were measured. A 10-mW laser was focused on a single cell and a spectral window of 800–3,300 cm^{-1} (3.5 cm^{-1} resolution) was measured with a 10 sec. exposure time. For *A. cylindrica* PCC7122 that generates strong Raman signals (resonance Raman scattering due to the presence of carotenoids (49)), a 0.3 mW laser power and a 1 sec. exposure time were used. For each sample 15 to 20 individual cells were measured, and a representative Raman spectrum normalized with respect to the maximum and minimum intensities (I - I_{min} / I_{max} - I_{min}) is displayed. No further data processing (smoothing or baseline subtraction) was conducted. The manipulations

performed during the analysis are not known to alter the chemical composition or structure of the cells (50).

6. Identification of an endospore-specific marker

Nine endospore forming bacteria were used to establish an endospore-specific chemical marker. Those were compared with other spore formers and asporogenic bacteria. The endospore former bacteria used were *B. subtilis* (NEU16, Neuchâtel University culture collection), *B. thuringiensis* (NEU1070/DSM350), *B. sphaericus* (NEU1003, Neuchâtel University culture collection), *Paenibacillus marcerans* (NEU1004), *Paenibacillus alvei* (NEU1291, Neuchâtel University culture collection), *Bacillus cereus* (SPO95, Neuchâtel University culture collection), *Bacillus weihenstephanensis* (SPO146, Neuchâtel University culture collection), *Bacillus cereus* (SPO91, Neuchâtel University culture collection), and *Bacillus* sp. (SPO122, Neuchâtel University culture collection). Other spore forming bacteria used for comparison. Those were: *S. violaceoruber* (NEU1225/DSM 40783), *S. avermitilis* (NEU1226/DSM46492), *Actinomycetes* sp. (NEU70, Neuchâtel University culture collection), *A. chroococcum* (NEU1159/DSM2289; *A. cylindrica* (PCC7122), and the asporogenic bacteria *Arthrobacter nicotianae* (NEU1123/20123) and *Arthrobacter globiformis* (NEU1124/DSM20124). The growth conditions for each strain are presented in Table 2. The method used for the sample preparation for the vegetative cells and spores are presented in point 2.1 and 2.2 of Material and Methods section.

Table 3. Cell envelope width measurements of the different bacterial cells in vegetative (VEG) or spore (SPO) form. Measurement replicates are shown as M1 to M5. The measurements are shown in nm

Bacteria	Cell type	M1	M2	M3	M4	M5	Average length (nm)
B. subtilis	VEG	44	42	43	42	41	42.4
	SPO	175	183	187	164	194	180.6
S. violaceoruber	VEG	25	25	25	23	24	24.4
	SPO	106	106	109	81	94	99.2
M. xanthus	VEG	31	32	33	29	32	31.4
	SPO	239	240	233	233	217	232.4
A. cylindrica	VEG	61	62	61	60	61	61
	SPO	204	205	204	211	208	206.4

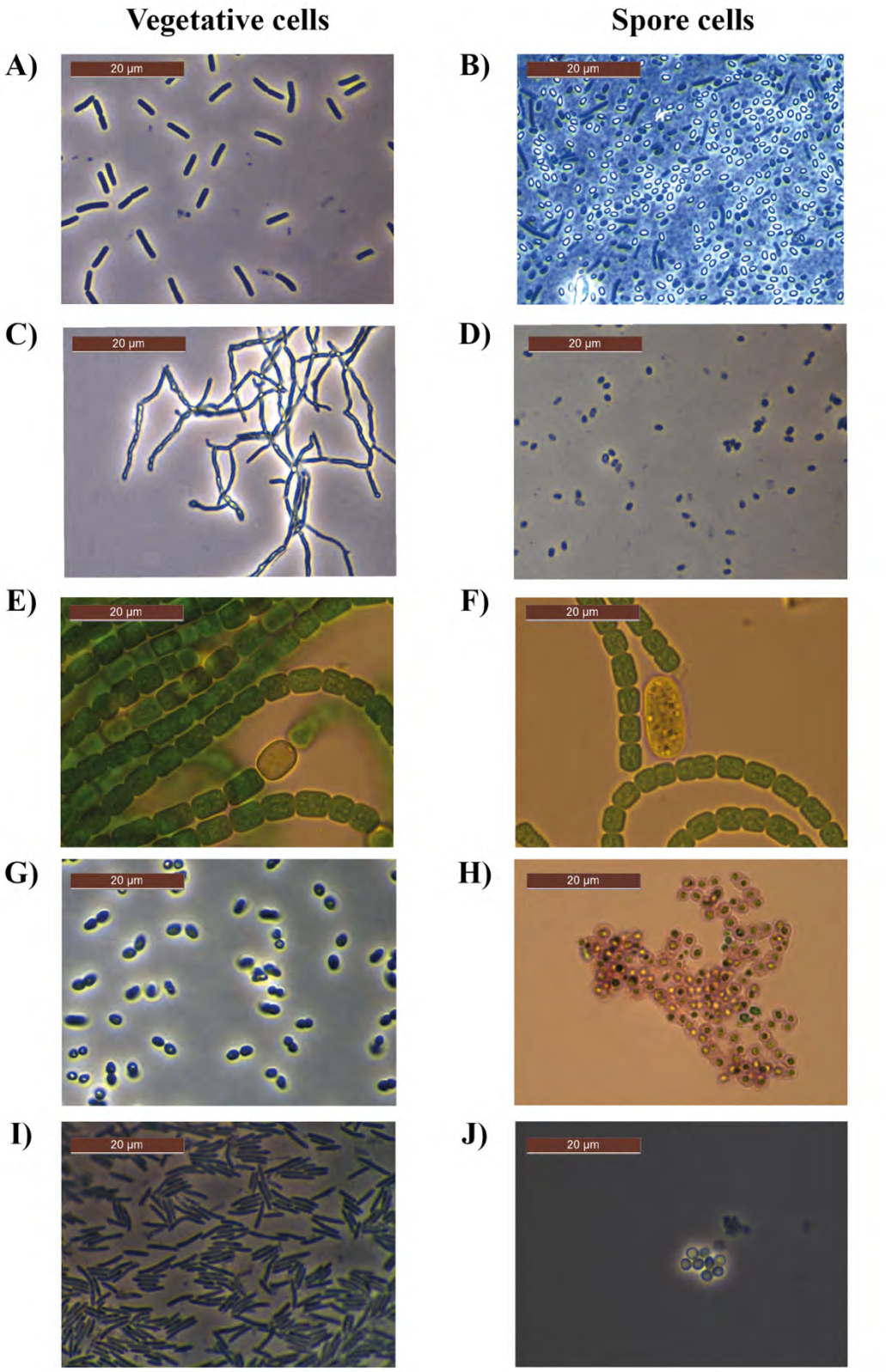

Figure 3. Microscopic images of vegetative cells and spores of **A)** Rod-shaped vegetative cells of *B. subtilis*, observed in phase contrast mode, **B)** Phase bright spore cells of *B. subtilis,* observed in phase contrast mode, **C)** Mycelium-like vegetative cells of *S. violaceoruber*, observed in phase contrast mode, **D)** Mature spore cells of *S. violaceoruber*, observed in phase contrast mode, **E)** Vegetative cells chain with a round/yellow heterocyst of *A. cylindrica*, observed in brightfield mode, **F)** Big Akinete of *A. cylindrica*, surrounded by a chain of vegetative cells, observed in brightfield mode, **G)** Vegetative cells of *A. chroococcum*, observed in phase contrast mode, **H)** Mature cysts of *A. chroococcum*, stained with "cyst stain" and observed in brightfield mode, **I)** Elongated rod shaped vegetative cells of *M. xanthus*, observed in brightfield mode, **J)** Light refracting, mature spore cells of *M. xanthus*, observed in brightfield mode. The bar on the left top corner indicates the scale.

6.1. Single-cell Raman microspectroscopy in liquid

In this case, the cells analyzed were suspended in liquid, to make the pipeline of analysis compatible with a fluid-based sorting capability. The same confocal Raman microspectroscope (LabRAM HR Evolution, Horiba Scientific, France), laser, confocal pinhole, and detector as indicated above were used. In this case, the system was based on an inverted microscope (Nikon Ti-E). A 300 lines/mm diffraction grating (blazed at 600 nm) and a 63 × water-immersion objective (Nikon SR Plan Apo IR 60XC WI, NA = 1.27) were installed. Additionally, optical tweezers that are formed using a 1,064-nm laser were used to immobilize cells during the Raman measurement. For these measurements, a sample of either vegetative cells or spores was re-suspended in 0.2 M glycerol solution and 5 µl drop of the sample was put in a microchamber. The sample was sandwiched by two glass coverslips (150-µm thickness) and additional two coverslips were placed between these coverslips as a spacer, thereby providing the still fluid microchamber with the thickness of 150 µm. Single cells were captured using optical tweezers (formed using a 1,064-nm laser) and their Raman spectra were measured. A spectral window of 400–3300 cm^{-1} were measured with a 100-mW laser power and a 1-sec exposure time. For *A. cylindrica* PCC7122, a 3-mW laser power and a 1 sec. exposure time were used. For each sample 5 to 26 individual cells were measured.

Results

1. Morphology

The vegetative cells of the model microorganisms underwent a very conspicuous morphological transformation during the formation of spores (Fig. 3). The spores belonging to *B. subtilis*, *S. violaceoruber*, *A. chroococcum*, and *M. xanthus* showed a significant reduction in cellular size (Fig. 3 B, D, H, and J) and rounding up of the cells, while the contrary was observed in spores of *A. cylindrica* (Fig. 3F). The spores of *A. cylindrica* were about three times larger than the vegetative cells. Furthermore, when observed in the phase contrast mode, the spores of *B. subtilis* and *M. xanthus* refracted light (i.e., cells look white) (Fig. 3B and J). This light refracting characteristic could also be observed in spores and vegetative cells of *A. cylindrica* (data not shown), whereas no light refraction was observed in the spores of *S. violaceoruber* and *A. chroococcum*. Cryo EM images were obtained for vegetative cells and spores from *B.*

subtilis, *S. violaceoruber*, *M. xanthus* and *A. cylindrica* (Fig. 4). Cryo-EM images of *Azotobacter* spores could not be obtained due to very low spore concentration and water crystallization. Vegetative cells of *B. subtilis* and *S. violaceoruber* (Fig. 4A, B; left side) presented an inner cellular membrane, a thick peptidoglycan layer, and a granulated cytoplasm. The vegetative cells of *M. xanthus* and *A. cylindrica* (Fig. 4C, D; left side) presented an inner and an outer membrane, a granulated cytoplasm, and in the case of the *A. cylindrica*, the cytoplasm included curved lines, which correspond to the membrane of thylakoids. The cytoplasm of the spores of *B. subtilis*, *S. violaceoruber*, *M. xanthus,* and *A. cylindrica* (Fig. 4A, B, C and D; right side) showed in all the cases a denser and smoother composition, and the cell envelope was thicker.

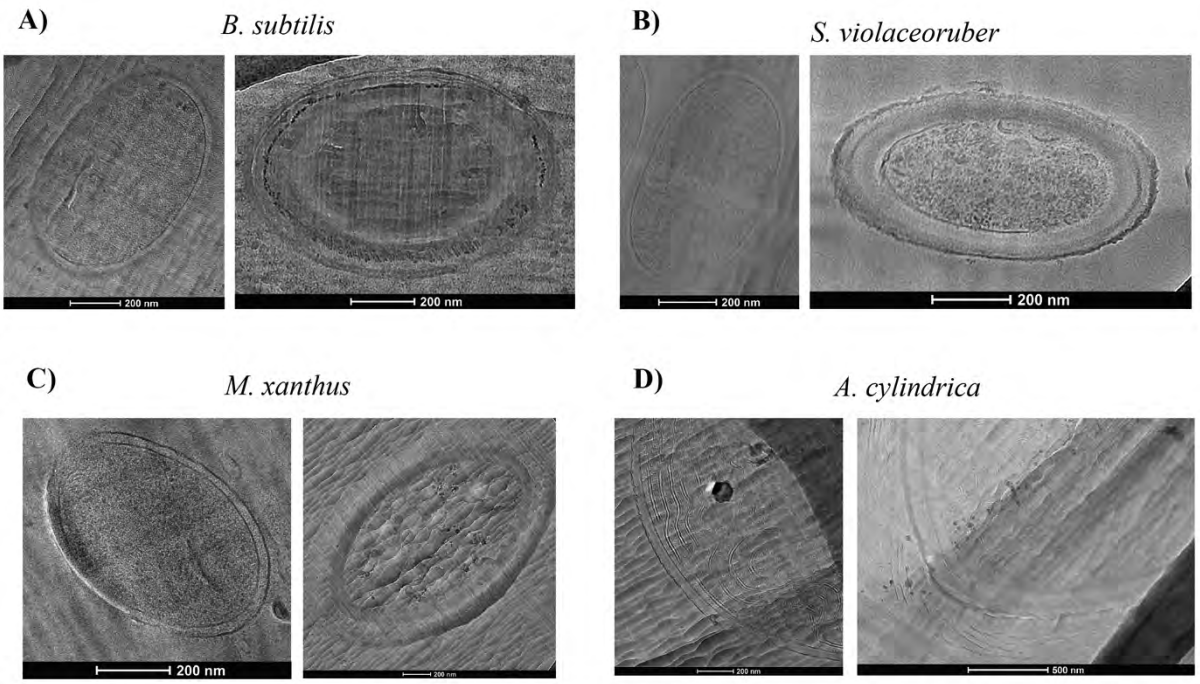

Figure 4. Cryo-Electron microscopy of vegetative cells (left side) and spores (right side) of **A)** *B. subtilis*, **B)** *S. violaceoruber,* **C)** *M. xanthus*, and **D)** *A. cylindrica*. The bar on the bottom of each images shows the scale.

To further assess the changes in the cell envelope, we measured the cell envelope width (Table 3) and its density profile. The characteristic cell envelope of Gram-positive bacteria could be observed in *B. subtilis* and *S. violaceoruber* (Fig. 5A and B; up). The envelope was composed of the cell membrane (CM) and an inner wall zone (IWZ). Additionally, a low gray value layer could be identified, which has been previously denominated as Outer Wall Zone (OWZ) (51,52). In the case of *M. xanthus* and *A. cylindrica* (Fig. 5C and D; up), both Gram-negative bacteria, one could observe

not only the CM and a thin Intermembrane spacer (IMS) layer (which included the peptidoglycan and periplasm), but also the presence of an outer membrane (OM). When comparing the vegetative cell and endospore of *B. subtilis* (Fig. 5A), a few differences could be observed. First, the enlargement of the cell envelope from 42.4 nm in the vegetative cell to 180.6 nm in the endospore. Second, the endospore presented after the CM two more layers, the cortex (CX), and a laminated spore coat (SC). These changes in the cell envelope were also reflected in the density profile. The density profile of the spore's cortex showed in average lower gray values, than the IWZ of the vegetative cell. In addition, the spore's profile also showed the SC surrounding the CX. In the case of *S. violaceoruber* (Fig. 5B) we also observed an enlargement of the cell envelope from 24.4 nm in the vegetative cell to 99.2 nm in the exospore. A change in the structure of the cell envelope was also detected. After the CM a thick spore wall (SW) was present and was surrounded by the SC. The density profile of the *S. violaceoruber*'s SW showed two different high gray value regions (SW.2 and SW.4) separated by low gray value regions (SW.1, SW.3). The SW was then followed by a low gray value region, which corresponded to the SC.

The Gram-negative cell envelope of the vegetative cells of *M. xanthus* went from 31.4 nm in width to 232.4 nm in the spores, thus showing an enlargement of the cell envelope. Furthermore, the spore showed both the presence of an CM and the Intermediate Coat (IC) surrounding a thick CX layer. In addition, the spore also presented a Surface Coat (SuC) that surrounded the IC (Fig. 5C) (53). These morphological changes could also be observed when comparing the density profile of the vegetative cell and the spore of *M. xanthus*. The density profile of the CX and IC showed higher variation in the spore than the IMS in the vegetative cell, additionally the SuC had a higher gray value than that of the OM in the vegetative cell.

The typical structure of a Gram-negative cell envelope was also detected in the vegetative cell of *A. cylindrica* (Fig. 5D). Furthermore, the akinete showed a thicker cell envelope (206.4 nm) in comparison to the vegetative cell (61 nm). In addition to the enlargement of the cell envelope, changes in structure of the cell envelope were observed in the akinete. After the CM, two layers were present, the here denominated layer 1 (L1) and possibly the mucilaginous layer (ML). These layers seemed to be separated by a thin laminated layer (LL), which presented a lower gray value. The most exterior layer was a low-gray value, which has been previously denominated as

electron-dense layer (EDL) (36). After the EDL, several dense lines were observed surrounding the cells, however these lines were not present in other images.

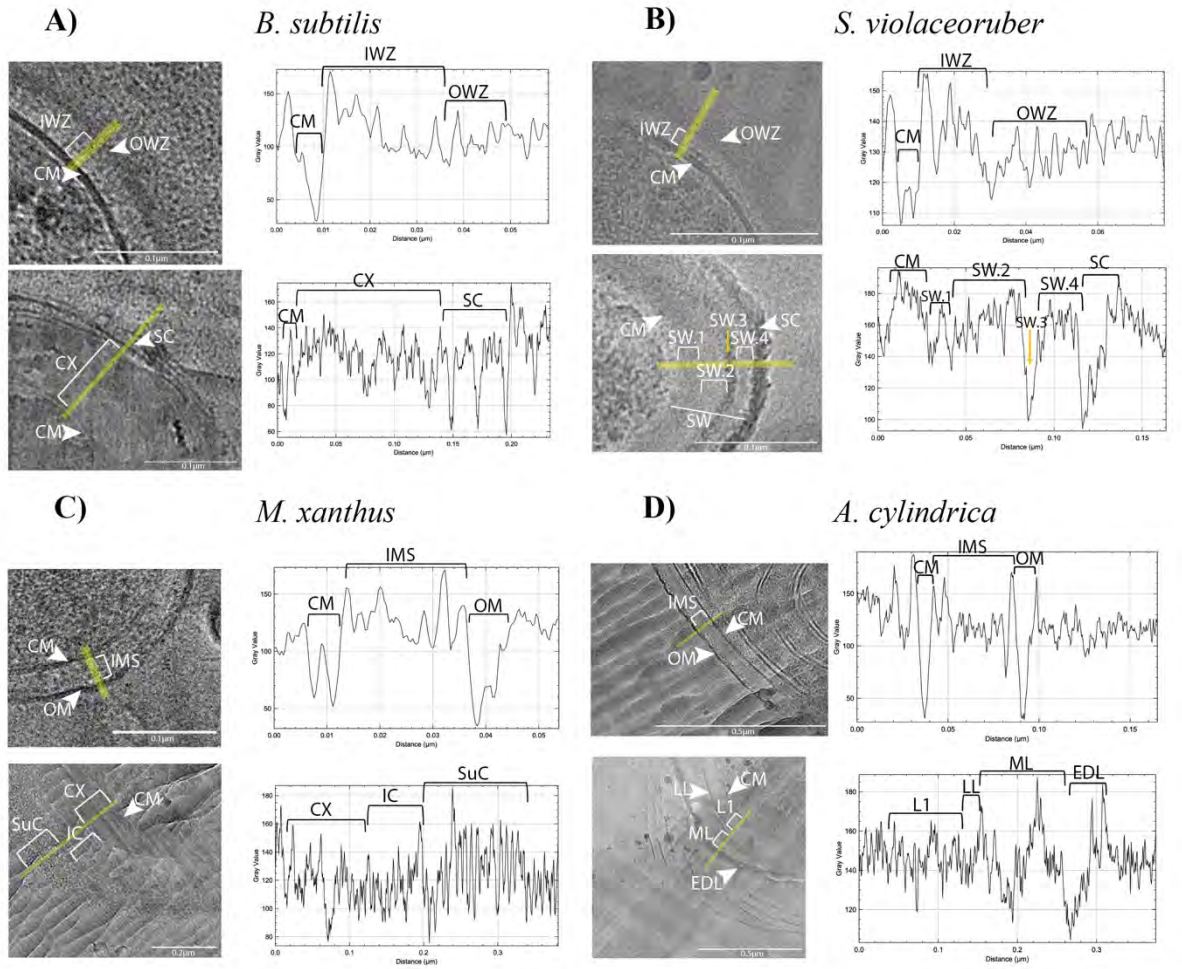

Figure 5. Cryo-Electron microscopy of the cell envelope of vegetative cells (up) and spores (down) and corresponding density profile of **A)** *B. subtilis,* **B)** *S. violaceoruber*, **C)** *M. xanthus*, and **D)** *A. cylindrica*. The density profile was always measured perpendicular to the knife lines (except for the spore of *B. subtilis*) and corresponds to the drawn yellow line on the cell envelope. The width of the line was 23 to reduce the noise. The different sections of the vegetative cells and spore's envelope were indicated: Inner Wall Zone (IWZ), Cortex (CX), Cell Membrane (CM), Outer Wall Zone (OWZ), Spore Coat (SC), Spore Wall (SW), Spore Wall section 1 (SW.1), Spore Wall section 2 (SW.2), Spore Wall section 3 (SW.3), Spore Wall section 4 (SW.4), Intermembrane Spacer (IMS), Outer Membrane (OM), Layer 1 (L1), Mucilaginous Layer (ML), Laminated Layer (LL), Electron-dense Layer (EDL).

2. Chemical composition

We assessed the chemical composition of individual vegetative cells and spores using single cell Raman spectroscopy. In Figure 6 a representative spectra per bacteria is shown for the vegetative cells and spores of *B. subtilis*, *S. violaceoruber*, *A. chroococcum*, *M. xanthus* and, *A. cylindrica* (the analysis of additional strains can be found in Supplementary Figure 1). When comparing the spectra belonging to the vegetative cells and spores of *B. subtilis* (Fig. 6A), a peak around the 2,900 cm^{-1} was

observed in both spectra. This peak usually corresponds to a CH_3 or CH_2 bond. A clear difference between the two spectra of *B. subtilis* was the intensity in the peaks belonging to Calcium Dipicolinic Acid (CaDPA; 1,017, 1,395, and 1,443 cm^{-1}), which were only present in the spores. This chemical, CaDPA, is a well-known component of endospores (54,55). In the vegetative cell's spectra of *S. violaceoruber* (Fig. 6B) the CH_3/CH_2 bond was also found, but it was not present in the spore's spectra. Furthermore, the spore spectra of *S. violaceoruber* showed a different topography to the one of the vegetative cells, presenting mainly two peaks, one at 1,342 cm^{-1} and one at 1,586 cm^{-1}. The vegetative cells of *M. xanthus*'s (Fig. 6C) presented the CH_3/CH_2 bond peak, which was also present in the spore's spectra. Three peaks (1,120, 1,149 and 1,550 cm^{-1}) in the spore spectra had a distinctive intensity as compared to the vegetative cell. The Raman spectra of the vegetative cell and spore of *A. chroococcum* (Fig. 6D) presented a similar topography, where the carbon peak around 2,900 cm^{-1} could be clearly appreciated in both spectra. Nonetheless, the spore's spectra presented three different peaks (830, 1,150 and 1,350 cm^{-1}) with increased intensity, in comparison to the vegetative cell's spectra. Finally, the spore and vegetative spectra of *A. cylindrica* (Fig. 6E), showed the same topography, except for a peak at the 2,295 cm^{-1} in the vegetative spectra, which was not present in the spore spectra. Similarities between spectra from different species were found among *B. subtilis*, *M. xanthus,* and *A. chroococcum* (Fig. 6A, C and D). The CH_3/CH_2 bond peak (2,900 cm^{-1}) was found in both the spectra of vegetative cells and spores. In the case of *S. violaceoruber* (Fig. 6B) this peak was only present the vegetative cell. On the other hand, the CH_3/CH_2 peak was not detected in either of the spectra of *A. cylindrica* (Fig. 6E). Moreover, significant differences were observed between the spore spectra of the different bacteria. When comparing the spore spectra of the different species*,* no conserved region was found, but with the exception of *A. cylindrica,* unique differences between the vegetative cell and its corresponding spore spectra were detected. These regions were the peaks at 1,017, 1,395, and 1,443 cm^{-1} for endospores, the peaks at 1,345 and 1,586 cm^{-1} for exospores, the peaks at 1,120, 1,149, and 1,505 cm^{-1} for myxospores, and the peaks at 830, 1,150, and 1,350 cm^{-1} for cysts. All these peaks were unique to each spore type (Fig. 6).

3. Identification of an endospore-specific Raman marker

We measured the Raman spectra of endospores and vegetative cells from fourteen different endospore-producing bacterial strains and two asporogenic bacterial strains with the aim of identifying an endospore-specific Raman marker. The measures were conducted in liquid to make the pipeline of analysis compatible with a fluid-based sorting capability. The average Raman spectra of the vegetative cells and spores for each bacterium are presented in Supplementary Figure 2. The peak at around 2,900 (CH_3/CH_2 bond) cm^{-1} was present in all spectra (Supplementary Figure 2). This peak is characteristic of biological samples. Furthermore, all the vegetative cell's spectra presented a similar flatten topography, in comparison the spore's spectra that presented pronounced peaks. In the endospore spectra, the characteristic CaDPA peaks were observed at 1,017, 1,395, 1,446, 1,572 cm^{-1}. None of these peaks was found in either the vegetative cells, exospore, myxospores, cysts or in akinetes.

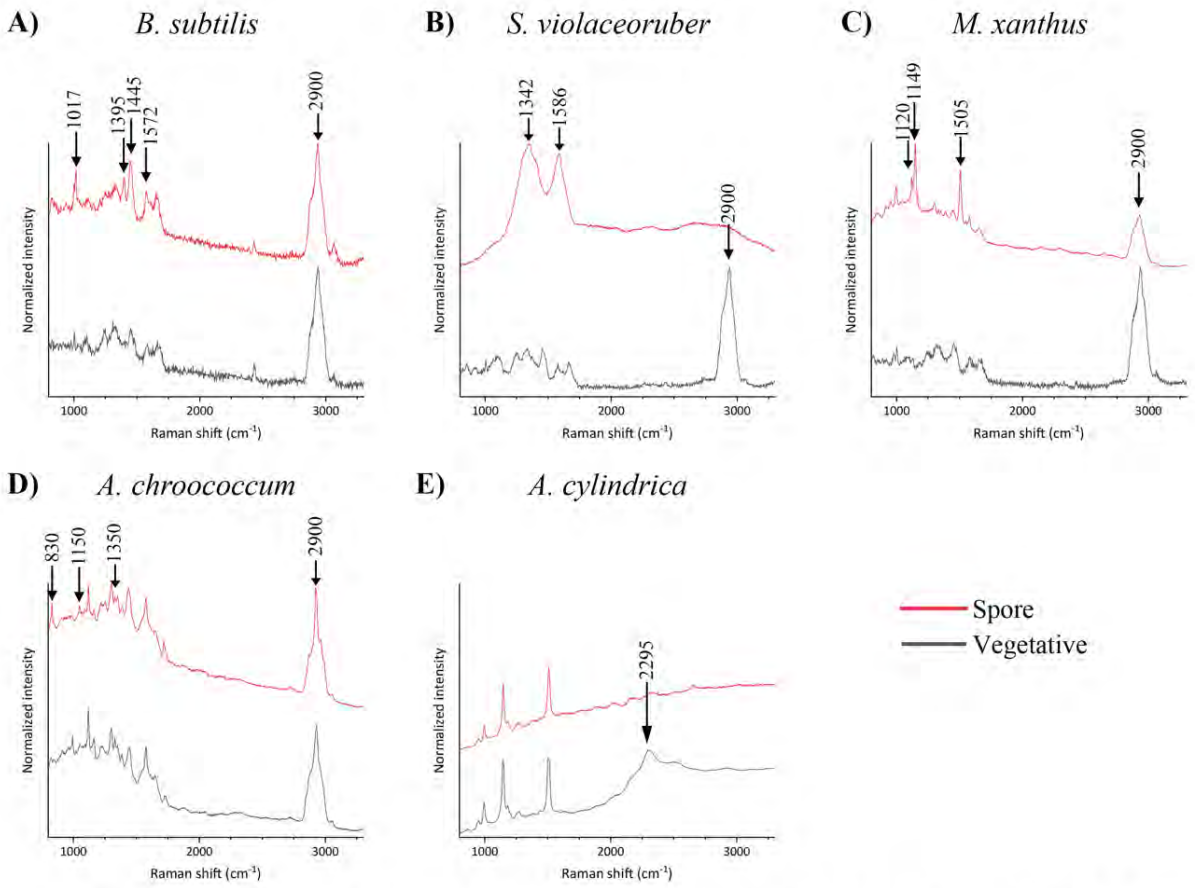

Figure 6. Representative Raman spectra of a vegetative cell or spore of **A)** *B. subtilis*, **B)** *S. violaceoruber*, **C)** *M. xanthus*, **D)** *A. chroococcum*, and **E)** *A. cylindrica*. The arrows indicate specific peaks which are unique either to the vegetative cell or spore and the values are in cm-1. For each sample 15 to 20 individual cells were measured, and a representative Raman spectrum normalized with respect to the maximum and minimum intensities (I - Imin / Imax - Imin) is displayed. No further data processing (smoothing or baseline subtraction) was conducted.

Although the CaDPA peaks were present in all endospore's spectra, their intensity varied between the different spores, as well as among individual cells. This is clearly exemplified in Supplementary Figure 2 A and B, showing that the intensity of the spore's spectra in *B. subtilis* (NEU16), was more than 3 times higher than the one of *B. thuringiensis* (NEU1070). Moreover, in *B. thuringiensis* (NEU1070) and *P. marcerans* (NEU1004) (Supplementary Figure 2 B and C respectively), both the vegetative and spore spectra presented similar intensities, except for the CaDPA peaks that showed an increase in intensity in the spore's spectra.

The single cell Raman sorting platform requires an established threshold to first discriminate bacterial cells (Pc) from other debris in the environmental samples, and a second threshold to identify endospores from vegetative cells (P_{CaDPA}). To discriminate cells from debris, the peak at 1'650 cm^{-1} and the one at 2,900 cm^{-1} (CH_3/CH_2 bond) were initially considered. However, the 2,900 cm^{-1} peak is highly variable between different cells and, as it is located at the end of the spectra, is more susceptible to being altered by background noise. For these reasons, the cell discriminating threshold was established using the peak at 1'650 cm^{-1} (region from 1'620 to 1'670 cm^{-1}) (Fig. 7A). Even though this region does not correspond to a specific molecular bond, it was specific for cells and did not present high variation depending on the experimental conditions. The ratio between the peak of the cell and that of the fluid (integrated intensity at 1'620 to 1'670 cm^{-1}) were calculated for each measurement. Once the ratio was calculated for each cell, the cell threshold (Pc) value was established at 1, to capture as many cells as possible (Fig. 7B). Any particle or cell with a ratio lower than one would be directly sorted to the waste, while any cell with a ratio value higher than 1 would be further analyzed to identify if the cell corresponds to an endospore or a vegetative cell. The endospore threshold was established by calculating the ratio between the integrated intensity of the CaDPA peak at 1'395 cm^{-1} (CaDPA) and that of the "cell peak" at around 1'650 cm^{-1} (Fig. 7C). This ratio was lower for all vegetative cells, as the CaDPA peak is absent. For endospores the ratio was higher than one given that the CaDPA peak presents higher intensity than the peak at 1'650 cm^{-1} (Fig. 7D). As a conservative measure to ensure the isolation of endospores only, the endospore threshold (P_{CaDPA}) was established at 1.1. Any cell with a ratio lower than 1.1 would be directly sorted to the vegetative compartment, while any cell with a ratio value higher than 1.1 sorted to the endospore compartment.

Discussion

Even though the production of the different types of spores (endospores, exospores, cysts, myxospores, and akinetes) follows a distinctive pathway, all of them have a common function: promoting long-term survival. In addition, previous studies had suggested the potential ancestral character of sporulation in bacteria (39). This could suggest a common ancestry to the formation of specialized resting cells that could be traced back by similarities in the structure or chemical composition of spores. To test this hypothesis, the morphological characteristics of the model sporulating bacteria *B. subtilis*, *S. violaceoruber*, *M. xanthus, A*. *cylindrica,* and *A. chroococcum* were assessed by optical microscopy and cryo-EM. When comparing the vegetative cells and spores produced by these organisms, the only shared characteristic that could be identified was the enlargement of the cell envelope in spores. Moreover, although the reduction in cell size is characteristic of endospores, exospores, myxospores, and cysts, this was not the case for akinetes. Akinetes showed increased sizes, compared to vegetative cells. The increased size observed in akinetes is a recognized characteristic of this type of resistant structure (34,37). Moreover, although the Cryo-EM images revealed highly significant morphological plasticity and structural changes in the cell envelope, common structural components in all spore types were absent. In addition, morphological plasticity is not an exclusive strategy of sporulating bacteria, as many bacteria use it to survive harsh environmental conditions (56,57).

In a similar way, the chemical analysis of the bacterial model organisms with single cell Raman spectroscopy showed that no conserved chemical marker is shared among the different spores compared. Instead, unique differences between the vegetative cell and its corresponding spore spectra were detected. The two peaks observed in the Raman spectra of *S. violaceoruber* at 1,342 and 1,586 cm^{-1} are both amino acid signals, which might correspond to L-Glutamate and L-Phenylalanine, respectively (58,59). Moreover, although *Streptomyces* spores are known to contain trehalose, no signal for this chemical was found (60,61). Many other organisms aside *Streptomyces* are known to accumulate this sugar, as it is known to aid in the resistance to desiccation (62,63). The absence of the trehalose signal in our study might be due to background noise originated from the pigments produced by *Streptomyces.* In contrast, in the case of *M. xanthus's* spore spectra, two of the peaks (1,120 and 1,149 cm^{-1}), which are significantly different from the vegetative cell spectra are part of the trehalose Raman signal (58). As in the case of *Streptomyces,*

Myxococcus's spores are known to accumulate this compound (64,65). In a similar way, trehalose is also known to be accumulated in the cysts of *Azotobacter* (26,66), and accordingly, the trehalose peak at 830 cm^{-1} (58) is one of the Raman markers in *A. chroococcum's* spore spectra. Contrary to *S. violaceoruber*, *M. xanthus,* and *A. chroococcum,* the vegetative and spore spectra of *A*. *cylindrica*'s, did not show unique signals that could be used for the identification of either cell type. The only difference among the *A*. *cylindrica*'s spectra was the increase in intensity in the spore's spectra in comparison to the vegetative cell's one and the disappearance of the peak at 2,295 cm^{-1} in the spore's spectra.

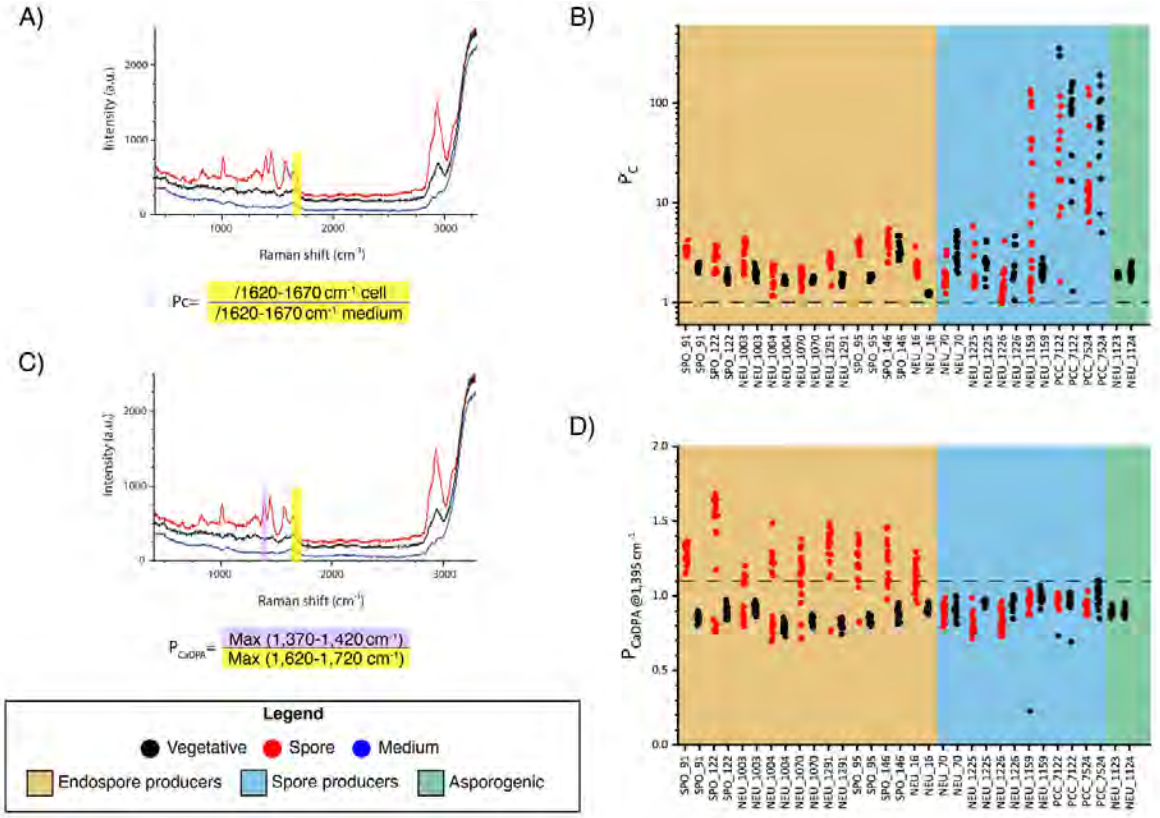

Figure 7. Establishment of the endospore-specific Raman marker. **A)** Standardized Raman spectra of a representative endospore forming bacteria (SPO91—*B. cereus*) in spore (red) and vegetative cell (black) and the background medium in which the cells are suspended (blue). Highlighted in yellow is the selected region (1,620 to 1,670 cm-1) to calculate the cell threshold (Pc). Under the plot, the formula used to calculate the Pc, this value was calculated by dividing the integrated intensity of the 1,620 to 1,670 cm-1 region of the measured cell (vegetative cell, spore, or debris) by the integrated intensity of the 1,620 to 1,670 cm-1 region of the medium. **B)** Plot of the Pc values (y axis) from individual vegetative cells (black) and spores (red) of endospore forming bacteria (orange), spore forming bacteria (blue) and asporogenic bacteria (green). In the x axis the strains ID are indicated, and the dashed line indicates the established cell threshold. **C)** Standardized Raman spectra of a representative endospore forming bacteria (SPO91—*B. cereus*) in spore (red) and vegetative cell (black) and the background medium in which the cells are suspended (blue). Highlighted in purple (1,370 to 1,420 cm-1) and yellow (1,620 to 1,720cm-1) are the two selected regions to calculate the endospore threshold (PCaDPA). Under the plot, the formula used to calculate the PCaDPA, this value was calculated by dividing the maximum intensity of the 1,370 to 1,420 cm-1 region of the measured cell (vegetative cell or spore) by the maximum intensity of the 1,620 to 1,670 cm-1 region of the measured cell. **D)** Plot of the PCaDPA values (y axis) from individual vegetative cells (black) and spores (red) of endospore forming bacteria (orange), spore forming bacteria (blue) and asporogenic bacteria (green). In the x axis the strains ID are indicated, and the dashed line indicates the established endospore threshold.

In the case of *B. subtilis*'s spores (and other endospore-formers), they presented a high concentration of CaDPA. The signal of CaDPA was not found in the vegetative

cell's spectra. This chemical (i.e., CaDPA) is a well-known component of endospores that has been shown to have an important role in the resistance and gemination of endospores (9,55,67,68). This striking chemical difference between vegetative cells and endospores was exploited here as a proof of concept of the feasibility of identifying individual spore-specific Raman markers to isolate endospores from environmental samples. Indeed, identification of CaDPA using Raman spectroscopy has been used to detect the presence of endospores in pure cultures and powder samples (69,70). Also, CaDPA has been extensively used in the past for the quantification and detection of endospores. For instance, quantification of endospores has often been done through the detection of CaDPA using HPLC (high performance liquid chromatography) or Tb-DPA (i.e., DPA chelated with Tb) photoluminescence, after its release by autoclaving, bead beating or by inducing germination (71–73). However, these methods infer abundance assuming a standard concentration of CaDPA/spore, even though this concentration is known to vary between strains (72,74). Another molecular marker used to identify and quantify endospores is the gene encoding the regulatory protein Spo0A (75,76). However the quantification of the *spoA* requires specialized DNA extraction methods to break the highly lysis-resistant endospores (77–79). All the methods indicated above allow detection and quantification, but not isolation. The markers developed in our study offer a nondestructive approach that enables not only the identification of single cell endospores, but also further isolation when combined, for instance, with the Raman-activated microbial cell sorting (RACS) platform developed by Lee et al., 2019. This platform combines single-cell Raman microspectroscopy with microfluidics to sort cells according to a previously established threshold, with a throughput of approximately 500 cell/h. This approach would provide a wealth of information in environments in which endospores are known to be highly prevalent, such as the gut human microbiome (81–83) or the deep biosphere (71,84,85). This can also be an important tool in environmental forecasting, for instance, to allow studying the prevalence of antibiotic resistance genes associated to endospores though time in sediments (86,87).

In conclusion our results in the morphological and chemical composition of sporulating bacterial models shows the absence of chemical or morphological universal markers for spores. The information available on genetic markers, also corroborates the non-universality of sporulation. Indeed, individual sporulation genetical markers have been found for Firmicutes (88,89), but in the case of Actinobacteria, Cyanobacteria,

Myxococcus and *Azotobacter*, the high genetic variation among species (18,90,91) or the complexity of the sporulation process (26,28,92) has made the identification of general molecular markers for exospores, akinetes, myxospores and/or cysts impossible. Thus the hypothesis of Tocheva et al., 2016 suggesting a last common sporulating bacterial ancestor is not supported by these results. Furthermore, our results and the fact that the ability to form spores is present in widely distant taxonomic clades (93), suggests that the ability to form spores might have emerged independently many times in bacteria. Furthermore, our results are supported by the results obtained by Megrian *et al.* 2020 and Taib *et al.* 2020, which reject the hypothesis of a common sporulating ancestor as the origin of the diderm cell (94,95). Here we also presented the identification of an endospore-specific Raman marker as a proof of concept of the feasibility of identifying individual spore markers for other spore types (i.e., endospores, exospores, akinetes, myxospores, and cysts). These markers could open the door to future investigations on the isolation and characterization of the underexplored bacterial seed bank. Finally, the term “spore” is generally associated to endospores and thus the characteristics expected from a newly discovered bacteria spore, are those of endospores. However, here we showed that endospores, exospores, myxospores, akynetes, and cysts do not share morphological or chemical similarities. Hence, it is conceivable that other bacterial spore-like cells will differ in morphology, chemical composition, and mechanism of formation from those in known spore-formers. Nonetheless, characteristics such as low metabolic activity, enlargement of the cell wall, increased resistance to temperature, dessication and/or lysis compared to the vegetative cell could be used as parameters to identify new spores or spore-like cells.

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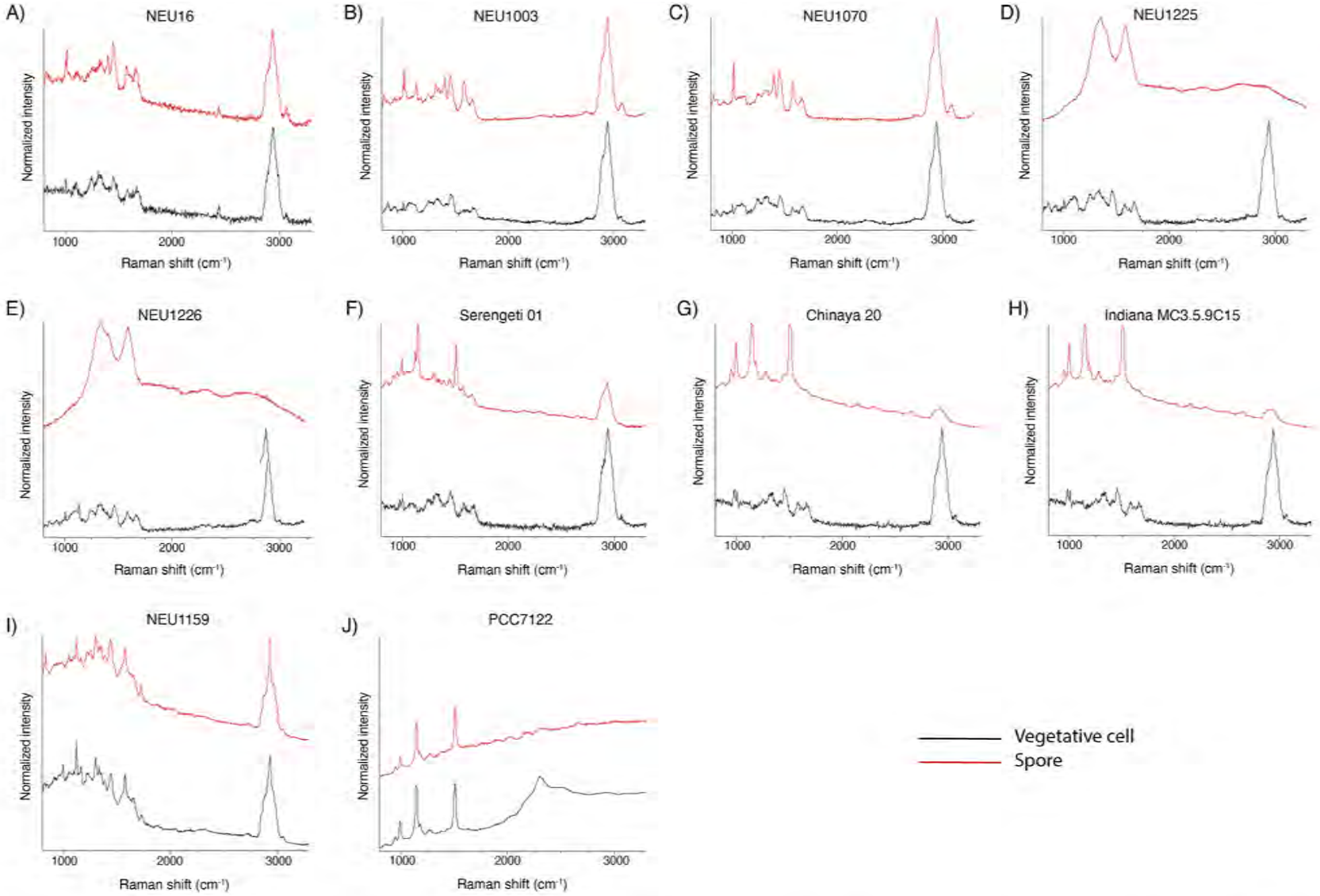
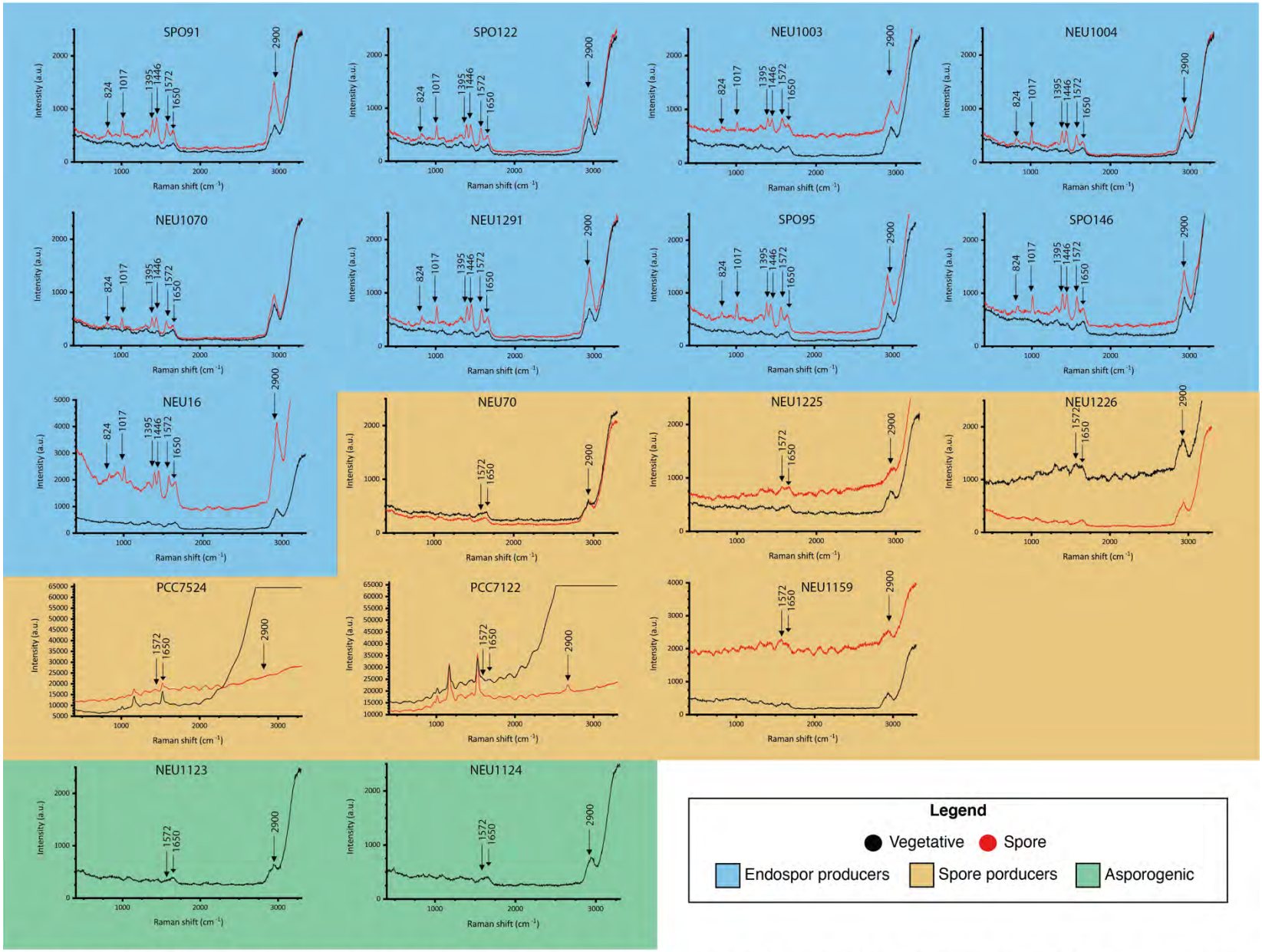
Supplementary Figures



Supplementary Figure 1. Representative Raman spectra of a vegetative cell (black) or spore (red) of **A)** *Bacillus subtilis*, **B)** *Bacillus sphaericus*, **C)** *Bacillus thuringiensis*, **D)** *Streptomyces violaceoruber*, **E)** *Streptomyces avermitilis*, **F-H)** *Myxococcus xanthus*, **I)** *Azotobacter chroococcum*, **J)** *Anabaena cylindrica*. For each sample 15 to 20 individual cells were measured, and a representative Raman spectrum normalized with respect to the maximum and minimum intensities (I - Imin / Imax - Imin) is displayed. No further data processing (smoothing or baseline subtraction) was conducted.



Supplementary Figure 2. Representative Raman spectra of a vegetative cell (black) or spore (red) of endospore forming bacteria (blue), spore forming bacteria (orange) and asporogenic bacteria (green). The cells were suspended in a 0.2 M glycerol solution. For each sample 15 to 20 individual cells were measured, and a representative Raman spectrum normalized with respect to the maximum and minimum intensities (I - Imin / Imax - Imin) is displayed. No further data processing (smoothing or baseline subtraction) was conducted.

Chapter 5 — General Discussion

General discussion

Dormancy and the formation of specialized resistant structures as a survival and dispersion strategy can be found in all branches of the tree of life and microorganisms are not an exception. Microorganisms have developed a high diversity of specialized resistant structures. In the case of bacteria, the formation of persisters, cysts, spores, and spore-like structures, and in the case of fungi, the formation of spores, sclerotia and zoospores have been studied. Meanwhile, in archaea, although some indication of dormancy and the formation of persisters has been found, this ability in archaea has not yet been recognized. Furthermore, the important influence of microbial seed banks in community evolution and in the preservation of genetical and phenotypical diversity has been previously highlighted (Joergensen & Wichern, 2018; Jones & Lennon, 2010; Shoemaker & Lennon, 2018; Sorensen & Shade, 2020). Nonetheless, many metagenomic studies assessing microbial diversity do not distinguish the extracellular DNA and/or dormant microbial fraction from the active fraction (Carini et al., 2017, 2020). On the other hand, some methods have been developed to assess the active and inactive microbial diversity by targeting RNA instead of DNA or by using dyes that bind to extracellular DNA (Menni et al., 2018; Salgar-Chaparro & Machuca, 2019). However, these methods only distinguish between death and live cells. Other methods evaluate the bacterial endospores abundance by quantifying the concentration of CaDPA (Rattray et al., 2021; Wörmer et al., 2019) or the abundance and diversity of fungal spore diversity, though spore washing or spore traps (Abrego et al., 2018; Banchi et al., 2018; Júnior et al., 2019; Soka & Ritchie, 2018). However, these methods only target endospores in bacteria or only mycorrhizal and/or air-suspended fungal spores, thus overlooking a big fraction of the microbial dormant or resistant structures.

The goal of the present dissertation was to develop a method that would enable the assessment of microbial resistant structures in the environment. To this end, a method previously developed for the isolation of endospores was evaluated for its use in the assessment of the diversity of lysis-resistant microbial cells (bacteria, archaea, and fungi). Alongside, the universality of bacterial sporulation was evaluated in an attempt to find a spore marker that could be used in the isolation of bacterial spores from environmental samples.

1. Lysis-resistance as a proxy to assess microbial dormancy and sporulation in environmental samples

In Chapters 2 and 3, we evaluated the performance, advantages, and limitations of the "Endospore enrichment method" developed by Wunderlin et al., 2016 (here on referred as "lysis-resistant enrichment method"), for the assessment of the environmental diversity of spore and spore-like cells of bacteria, archaea (Chapter 2), and fungi (Chapter 3). This enrichment method uses lysis-resistance as a proxy for specialized resistant structures such as bacterial and fungal spores, cysts, or sclerotia, among others. The samples analyzed were collected at the Salar de Huasco, located in the Chilean Altiplano. This polyextreme environment was selected as the formation of dormant cells has been shown to affect the fitness and ecological distribution of bacteria (Mutlu et al., 2020) and polyextreme environments might be more selective for microorganisms able to produce specialized resistant structures (Filippidou et al., 2016). The application of the lysis-resistant enrichment method in environmental samples yielded different results for each domain assessed. In Chapter 2, the lysis-resistant diversity of bacterial and archaeal cells was evaluated. The lysis-resistant enrichment method previously validated for the isolation and enrichment of bacterial endospores, also enriched bacterial phyla known to form other types of spores, such as Actinomycetes (exospores), Proteobacteria (cysts and myxospores), and Cyanobacteria (akinetes). Furthermore, asporogenic genera such as *Nitriliruptor* (Sorokin et al., 2017) and *Enterococcus* (Byappanahalli et al., 2012) were also enriched. The enrichment of these, and of other asporogenic genera, suggests that a highly resistant cell form might be responsible for enhancing their survival to the lysis treatment and probably their survival in the environment. Moreover, the bacterial lysis-resistant fraction (DNA after the lysis-resistant enrichment method) was distinct in comparison to the total community (direct DNA extraction). This shows that lysis-resistance is restricted to a fraction of the bacterial community. In contrast, lysis-resistance in archaea seems to be widely spread as there was no clear grouping of the lysis-resistant fraction relative to the total fraction. Nonetheless, some genera were enriched after the lysis-resistant enrichment method, such as *Natrinema* and *Halarchaeum*. The enrichment of such genera might reflect the existence of cyst-like or other types of resistant archaeal cells in the environment. However, some constrains in the assessment of lysis-resistance in archaea were, first the method has not been validated for archaea, and thus the results obtained should be interpreted

with caution. Nevertheless, the same sample was used to assess both groups (i.e., bacteria and archaea), and it is unlikely that archaeal DNA released during the physical and chemical lysis could withstand better the DNase digestion step than bacterial DNA (Miettinen et al., 2019; Sorensen et al., 2021), thus contamination by DNA from archaea that do not withstand lysis but are highly abundant is unlikely. Second, the large fraction of unidentified Amplicon Sequence Variant (ASVs) complicates the assessment of the effect of the lysis-resistant method.

In Chapter 3, the effect of the lysis-resistant enrichment method was assessed in the fungal community. First, the validation of the method was done with pure cultures of mycelium and with fungal resistant structures representing different life cycles and different types of specialized resistant structures (sexual and asexual spores and non-melanized yeast). Here we compared the quantity of DNA obtained from the mycelium and resistant structures by direct DNA extraction from that obtained after the application of the lysis-resistant enrichment method, followed by DNA extraction. The results showed that spore DNA was only obtained after the lysis-resistant enrichment method while no mycelial DNA was recovered after the lysis-resistant enrichment method. In the case of yeast, only a small fraction of DNA was recovered after the lysis-resistant enrichment method, compared to that obtained after the direct DNA extraction. Similar results were observed in the environmental fungal community. In contrast to bacteria, lysis-resistance was not restricted to some clades (Higgins & Dworkin, 2012; Julien et al., 2000; Kaplan-Levy et al., 2010; Kramer & Socolofsky, 1970; Sexton & Tocheva, 2020), but the total community and lysis-resistance fraction showed high similarity in their composition. However, differences were still observed, for example, the phylum Rozellomycota was enriched in the total community, while the Chytridiomycota was enriched in the lysis-resistant community. The differentiated enrichment of these phyla (i.e., resistance to lysis) is probably a result of their distinct cell structure. Members of the Rozellomycota phylum lack chitin in its parasitic stage and lack a cell-wall in their zoospores (Corsaro et al., 2014; James & Berbee, 2011). In contrast, members of the Chytridiomycota have chitin-based cell walls (McConnaughey, 2014; Money, 2016). Similarly, the enrichment of the Basidiomycota class, Lecanoromycetes (lichenized fungi) in the lake sediments is probably due to increased lysis-resistance of the fungal spores and/or propagules, as the identified genera are not aquatic lichens (Caloplaca and Acarospora). On the other hand, the

most diverse phyla, Ascomycota and Basidiomycota, were neither enriched in the total community, nor in the lysis-resistant fraction, thus showing equal abundance in either fraction. However, when looking at the class level the enrichment of certain classes was observed. These results reflect the complexity and diversity of fungi, as members of both phyla are diverse and are represented by yeasts to filamentous fungi with highly complex macroscopic fruiting bodies (Naranjo-Ortiz & Gabaldón, 2019).

Although the use of culture independent method such as the one used in Chapter 2 and 3 allows the molecular assessment of the lysis-resistant cells of a microbial community, it also presents a few setbacks. First, it is a destructive method, thus further analysis on the cells of interests is impossible. Second, enrichment after the lysis treatment does not constitute strong enough evidence to prove the existence of alternative resistant structures. Third, the lysis-resistant enrichment method is time consuming, and as any metagenomic study, it is susceptible to contamination during the extraction steps. Lastly, although the validation of the lysis-resistant enrichment method for the isolation of bacterial and fungal spores can be done, this is not possible in the case of archaea, as little information is available on archaeal dormancy and as until now there is no evidence of archaeal spore formation. Nonetheless exploring the diversity of lysis-resistant cells in the environment did yield interesting results. In the case of bacteria, not only known spore formers were enriched after the lysis treatment but also some asporogenic bacteria. Moreover, in bacteria, lysis-resistance seemed to be restricted to a fraction of the population. In archaea, the resistance to lysis was more broadly distributed than in bacteria. The enrichment of some archaeal genera indicates that some archaeal cells are as resistant to lysis as bacterial endospores. Similar to the results observed in archaea, fungal lysis-resistance was widely distributed. This might be the result not only of the great variety of resistant structures produced by fungi, but also of their complex and diverse life cycles.

To better understand the production of lysis-resistant cells in the environment, new methods that enable the identification of lysis-resistant cells without the limitations of culturing and that also allow further analysis of the cells (e.g., microscopy, resistant tests) are still needed. To this end, we set to find a universal marker for the isolation of bacterial spores and spore-like cells (Chapter 4).

2. The non-universality of bacterial sporulation

Even though the strategies used to form specialized resting cells in Firmicutes, Actinobacteria*, Azotobacter, Myxococcus* and Cyanobacteria differ significantly, the resulting cells shared some common features (such as having a low metabolic activity) and serve the same function, survival. This similarities have led to hypothesis suggesting that a form of endosporulation might have been a trait in a common ancestor of bacteria that led to the origin of the outer membrane, and thus to the origin of Gram-negative bacteria (Errington, 2013; Tocheva et al., 2016; Vollmer, 2012; Xavier et al., 2021). If this hypothesis is true, then we could hypothesize that the derived spores might still share common characteristics. In the attempt to find a universal spore marker, in Chapter 4, we systematically assessed the morphological characteristics (though cryo-electron microscopy -Cryo-EM and optical microscopy) and the chemical characteristics (though Raman microspectroscopy) of vegetative cells and spores of model sporulating bacteria: *Bacillus, Streptomyces, Myxococcus xanthus, Azotobacter choroococcum,* and *Anabaena cylindric*. From this analysis, the only shared morphological characteristic among all spores was the enlargement of the cell wall compared to their corresponding vegetative cells. Indeed, all spores presented morphological changes from the original vegetative cell, such as the number and structure of the cell envelope layers, however these changes were not similar in different groups. Moreover, the spores did not share any chemical feature that was unique to the spores and not shared with the vegetative cells. Nevertheless, we could identify unique chemical markers to distinguish each spore type (endospore, exospore, myxospore, cysts, and akinete) from its vegetative cell. For example, the signature (1,017, 1,395, and 1,443 cm^{-1}) of Calcium Dipicolinic Acid (Ca-DPA) was unique to endospores. Likewise, exospores presented two peaks (1,342 and 1,586 cm^{-1}) that might correspond to L-Glutamate and L-Phenylalanine, respectively (De Gelder et al., 2007; Verrier et al., 2004). Furthermore, for myxospores and azotobacter cysts, peaks belonging to the trehalose spectra were unique to the spore's spectra (De Gelder et al., 2007). In myxospores, the peaks at 1,120 and 1,149 cm^{-1} were unique, while in azotobacter the peak at 830 cm^{-1} was unique to the cyst's spectra. For cyanobacterial vegetative cells and akinetes the Raman spectra only differed in the absence of the peak at 2,295 cm^{-1} in the spore's spectra.

Similarly, to the best of our knowledge, no shared genetic marker has yet been found for the production of spores, but unique genetic makers have been identified for each

spore type. In Firmicutes, the DNA binding protein Spo0A is recognized as a key component of the stress activated gene expression machinery and thus of endosporulation (Molle et al., 2003; Ramos-Silva et al., 2019). Nonetheless the specific triggers and receptors that contribute to phosphorylation of Spo0A are not conserved even between all endospore formers. In addition, many studies have shown that there is a high variation in the genes involved in endosporulation (Galperin et al., 2012, 2021, 2022; Paredes-Sabja et al., 2011; Stragier, 2002) and genes previously thought to be essential for endosporulation have been found not to be essential in some sporulating bacteria or to have orthologues in non-sporulating bacteria (Onyenwoke et al., 2004; Rigden & Galperin, 2008). Likewise, when comparing the genetic pathways of sporulation in Firmicutes and Actinobacteria, it is clear that they differ greatly. In the case of *Streptomyces,* sporulation is regulated by levels of the nucleotide second messenger c-di-GMP (Gallagher et al., 2020; Haist et al., 2020), and although many of the regulatory networks controlling sporulation are conserver in Actinobacteria, the genes linked to the regulons are not conserved (Beskrovnaya et al., 2021). Moreover, the genes regulating myxospore production, are not found in either Firmicutes or in Actinobacteria and they show high variation among members of the Myxococcales order (Goldman et al., 2007; Huntley et al., 2011; Sarwar & Garza, 2016). Similarly, the regulators and genes involved in the formation of *Azotobacter's* cysts and Cyanobacteria's akinetes, differ from each other and from all other spores (Argueta et al., 2006; Argueta & Summers, 2005; León & Espín, 2008; Moreno et al., 2018; Qiu et al., 2020; Segura et al., 2020; Zhou & Wolk, 2002). Thus, to our knowledge no genetic marker is shared among the sporulating models studied here. In summary, the absence of a chemical, morphological or genetical universal marker for spores, does not support the hypothesis of Tocheva et al., 2016, suggesting a last common sporulating bacterial ancestor. On the contrary, our results and the fact that the ability to form spores is present in widely distant taxonomic clades (Shimkets & Brun, 2014), suggest that the ability to form spores emerged independently many times in bacteria.

3. Single cell Raman operated sorting platform

Although there was no morphological, chemical or genetical marker that could be applied for the identification and isolation of bacterial spores from a mix community, in

Chapter 4 we identified individual marker that could be used to isolate endospores, exospores, cysts, and akinetes. Additionally, we assessed and showed the feasibility of using these markers to isolate spores by establishing an endospore-specific Raman marker. To this end, pure cultures of sporulating (spores and vegetative cells) and asporogenic bacteria (vegetative cells) were assessed by Raman microspectroscopy. From these measurements, two thresholds were established to be used in combination with a Raman-activated microbial cell sorting (RACS) platform (Lee et al., 2019). The first threshold, the peak at 1'650 cm^{-1}, distinguishes bacterial cells from other debris that might be present in the sample. The second threshold distinguishes endospores from any other spore or vegetative cells. For this the ratio between the peak at 1'650 cm^{-1} and one of the CaDPA peaks (1'395 cm^{-1}) was selected (as other bacterial spores or vegetative cells lack CaDPA). With these two thresholds, endospores could be isolated with the RACS platform from environmental samples. In a similar way, other thresholds could be established to identify exospores, myxospores, cysts and akinetes from environmental samples.

The RACS platform provides a few advantages in comparison to the lysis-enrichment method. First, the environmental sample after the separation of the biomass can be directly loaded to the microfluidic device without further treatment. Second, the sorted spores and vegetative cells can be further analyzed as the Raman does not damage the cells. Third, the platform is automated, and the production of the microfluidic RACS device is cheap and fast. Finally, by reducing the manipulation steps also the risk of contamination is reduced. However, this method also presents a few setbacks, first the need of a Raman microspectroscope that is not available for every laboratory. Second, although the throughput is relatively high (up to 200–500 cells h^{-1}), the more markers that are used, the lower the throughput would be. Finally, this method only allows the isolation of spores containing one of the established markers, while spore-like cells that do not share any of these markers would be sorted with all other vegetative cells. Thus, limiting our ability to isolate unknown spore-like structures.

4. Conclusions and outlook

Through the systematic evaluation of the effect of the lysis-resistant enrichment method in bacteria, archaea, and fungi, we gathered key information on the abundance of resistant cell in each domain. First, in bacteria, lysis-resistance is

restricted to a fraction of the community, however it is not restricted to only well described sporulating bacteria. In fact, bacteria classified as asporogenic were enriched in the lysis-resistance fraction, thus indicating lysis-resistance similar to that of endospores. Second, contrary to bacteria, lysis resistance in archaea was more broadly distributed, which could be the result of highly resistant vegetative cells or the presence of spore-like archaeal cells. However, until now, no evidence of specialized resistant archaeal structures has been found, thus further research needs to be done to understand the characteristics and distribution of lysis-resistance in archaea. Third, in fungi, lysis-resistance was also broadly distributed, which was expected as the production of specialized resistant structures is a general characteristic of the domain. Additionally, the complex life cycles of fungi could also explain the broad distribution of lysis-resistance and different fungal structures (hyphae, fruiting body, yeast, lichen, spore, conidia, zoospore, etc.) of the same strain could have different resistance to lysis. Fourth, although metagenomic studies have revealed a high diversity of microorganisms in the environment, the high proportion of unidentified sequences make the interpretation of the results complicated, especially in the archaeal and fungal domain that have been less studied than bacteria. The proportion of unidentified sequences is bigger in less studied environments, such as extreme environments like the Salar de Huasco. Unveiling the identity and function of the unclassified microbial fraction has been the focus of many studies (Bernard et al., 2018; Jiao et al., 2021; Lloyd et al., 2018; Vollmers et al., 2022) but until now, no clear answer has been found.

In relation to bacterial sporulation, the term “spore” is generally associated to endospores and thus the characteristics expected from a newly discovered bacteria spore, are those of endospores. However, in Chapter 4 we showed that endospores, exospores, myxospores, akinetes, and cysts do not share morphological or chemical similarities. Hence, it is conceivable that other bacterial spore-like cells will differ in morphology, chemical composition, and formation from known spores. Nonetheless, characteristics such as low metabolic activity, enlargement of the cell wall, increased resistance to temperature, dissection and/or lysis compared to the vegetative cell could be used as parameters to identify new spores or spore-like cells. Furthermore, the absence of a shared chemical or morphological characteristic, makes the identification of a universal spore sorting parameter difficult. Alternatively, we suggest the use of individual chemical markers for the isolation of each spore type and

presented as a proof of concept the establishment of an endospore-specific Raman marker. Regrettably due to the overbooking of the Raman facilities and the lack of time, we were not able to test the sorting platform to isolate endospores from a mix community. Thus, before running environmental samples in the RACS platform a validation test with synthetic mix communities should be performed to assess the accuracy of the marker. Furthermore, the thresholds for all other types of spores aside endospores should be calculated and then validated using synthetic mix communities. Once the markers have been validated a few challenges arise, first, how to optimize the automated sorting to increase the through put, as having six markers (cell, endospore, exospore, myxospore, cyst, and akinete) can reduce it. Second, due to the low biomass that can be expected after the sorting, obtaining enough genetical material to sequence could be a problem. Although single cell sequencing has improved, and the technology could be used to overcome the low cell biomass constrain, the high resistance to lysis of the bacterial cells still represents a challenge. And finally, even when these two limitations (low biomass and bacterial spore lysis) could be overcome, the high proportion of unidentified sequences would still limit the amount of information that could be gathered.

In summary, in this dissertation we showed that lysis-resistance in bacteria is not limited to the known sporulating models and that bacterial spores do not share morphological, chemical or genetical markers. Thus, it could be hypothesized that other spore-like cells also do not share any of these characteristics, but only their low metabolic activity and increased lysis-resistance compared to the vegetative cells. We also showed that lysis-resistance similar to that of endospores is widely distributed in archaea. Nonetheless, lysis-resistance, dormancy and the possible production of specialized cells need to be further investigated in archaea. As well, we showed that the application of the lysis-resistant enrichment method in fungi does not only target fungal spores but probably other specializes resistant structures such as yeast, lichen propagules and/or sclerotia. Furthermore, we show that bacteria spores do not share morphological or chemical characteristics, but they are unique. Alongside, we presented individual Raman-spore markers that could be used to isolate bacterial spore cells from environmental samples. However, this method would not be able to isolate bacterial spore-like cells that do not share the individual “spore markers”. A similar method could not be used for fungal spores, as their diversity is much higher

than that of bacterial spores, thus increasing the number of markers that would be needed to target all fungal spores. This research has moved us a step closer to understanding the diversity of the microbial seed bank and has open multiple questions such as:

- Is there a higher diversity of bacterial spores than that recognized to date? We have shown through the application of the lysis-resistant enrichment treatment that asporogenic bacteria show resistance to lysis similar to that of endospores, and this would suggest that diversity of spore-like cells is indeed higher.
- Should the term spore be broadened? As previously mentioned, the term “spore” is often used to refer to endospores. However, as presented in this dissertation, all model bacterial spores differ in their formation, morphology, chemical composition, genetical triggers, and their resistance to different stressors. Thus, using only one of these models as reference for sporulation would be biased. On the other hand, a general characteristic of all model spores is a modification of their shape, enlargement of the cell wall and increased resistance, as compared to their vegetative state.
- Are the lysis-resistant cells in archaea similar to bacterial spores? The enrichment in the lysis-resistant fraction of some archaeal genus presented in Chapter 2 indicates that some archaeal cells are as resistant to lysis as bacterial spores. However, we do not have microscopic evidence of their structure. Further investigation is needed to understand lysis-resistance as well as dormancy in archaea.
- Are fungal metagenomic studies missing a fraction of the fungal diversity as spores seem to be resistant to normal DNA extraction? From the validation of the lysis-resistant enrichment method in fungi we learned that fungal spore DNA is only obtained after the application of the harsh lysis treatment and almost no DNA is obtained from direct DNA extraction. Thus, it is possible that normal DNA extraction methods are biased, underestimating the abundance of fungal spores.

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