# Comparison of proteolytic system secreted in Dermatophytes and *Aspergillus fumigatus*, used as a reference

Thèse de doctorat ès Sciences de la vie (PhD)
Présentée à l'Université de Neuchâtel par :

**Dev Sriranganadane**

Diplômé en Biologie et Biotechnologies, spécialité Protéomique et Physicochimie du vivant, de l'Université des Sciences et Technologies de Lille.

**Accepté sur proposition du Jury**

Prof. Jean-Marc Neuhaus (Directeur de these)
Prof. Michel Monod (co-Directeur de thèse)
Dr. Daniel Job (Rapporteur)
Dr. Manfredo Quadroni (Rapporteur)

**Neuchâtel 2011**

Université de Neuchâtel

FACULTE DES SCIENCES
Secrétariat-Décanat de la faculté
Rue Emile-Argand 11
CH-2000 Neuchâtel

# IMPRIMATUR POUR LA THESE

## Comparison of proteolytic system secreted in Dermatophytes and *Aspergillus fumigatus* used as a reference

**Dev Sriram SRIRANGANADANE**

---

UNIVERSITE DE NEUCHATEL

FACULTE DES SCIENCES

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

MM. Jean-Marc Neuhaus (directeur de thèse), D. Job,
M. Monod (co-directeur de thèse, Université de Lausanne)
et M. Quadroni (Université de Lausanne)

autorise l'impression de la présente thèse.

Neuchâtel, le 19 mai 2011

Le doyen :
P. Kropf

**« A un gustazo un trancazo »**

**El cubaneo**

# Remerciements

Tout d'abord je souhaite remercier les institutions du Centre Hospitalier Universitaire du canton de Vaud (CHUV) et de l'Université de Neuchâtel (UNINE) pour m'avoir permis de réaliser cette thèse en Biologie.

Un grand merci aux membres du jury dont :

- Le directeur de ma thèse, le Pr. Neuhaus qui m'a permis d'effectuer cette thèse à l'UNINE.
- Le Pr. Job qui m'a permis d'avoir un nouveau regard sur ces extraordinaires champignons que sont les *Aspergillus*.
- Mon co-directeur de thése et mon chef de labo, le Pr. Monod qui a dirigé tout mon travail et qui m'a permis d'arriver au bout de ce doctorat.
- Et enfin toute l'équipe du PAF (Protein Analysis Facility) dirigé par le Dr. Manfredo et aidé de Patrice, Jachen et Alexandra et qui m'ont coaché tout au long de mes analyses de spectrométrie de masse.

Je remercie également toutes les techniciennes du laboratoire de Mycologie, Mme Salamin Karine, Mme Fratti Marina, Mme Bontems Olympia ainsi que Mlle Verrier Julie, la nouvelle doctorante, pour leur bonne humeur et leur aide dans mes différentes expériences de biologie moléculaire.

Je remercie aussi toutes les personnes de l'étage de la dermatologie, comprenant les doctorants et post-doctorants du laboratoire de biologie cutanée et de neurodermatologie : Christophe, Dianelyse, Stéphane, Magda, Christine, Marcel et Lyda; le Dr. Lurati et le Dr. Spring ; ainsi qu'Isabelle et Messod du service de chirurgie plastique et reconstructive pour leur franche camaraderie et les brainstormings scientifiques. Sans eux, cette thèse aurait été beaucoup plus difficile.

Et enfin, merci à tous ceux qui, de près ou de loin, m'ont permis de murir humainement et scientifiquement afin de poursuivre ma route sur ce long sentier qu'est la recherche.

Un grand Merci à tous!!!!

# Table of contents

# Summary

Dermatophytes are highly specialized pathogenic fungi which grow exclusively in the *stratum corneum*, nails or hair and are the most common agents of superficial mycoses. In a medium containing keratin as the sole nitrogen source they secrete a set of endo- and exoproteases able to digest keratin into amino acids and short peptides to be assimilated. Proteases secreted by dermatophytes are similar to those of *Aspergillus* spp. Therefore, *Aspergillus fumigatus* was used as a model to investigate the different steps of protein degradation in acidic and neutral environments by fungi such as dermatohytes.

During growth in a protein medium at neutral pH, *Aspergillus fumigatus* secretes neutral and alkaline endoproteases, an X-prolyl peptidase (DppIV) and leucine aminopeptidases (Laps) which are non-specific monoaminopeptidases. Laps cannot remove any amino acids from a peptide with a N-terminal X-Pro sequence. However, large peptides generated from protein digestion by endoproteolysis can be further digested into amino acids and X-pro dipeptides by the synergistic action of Laps and DppIV. We have shown that *A. fumigatus* secretes a distinct set of proteases at acidic pH which includes an aspartic endoprotease of the pepsin family (Pep1), a novel glutamic protease, AfuGprA, homologous to *Aspergillus niger* aspergillopepsin II, tripeptidyl-peptidases of the sedolisin family (SedB and SedD) and a novel prolylpeptidase, AfuS28.

The importance of AfuGprA in protein digestion was evaluated by deletion of its encoding gene in *A. fumigatus* wild type D141 and in a *pep*Δ mutant. We have shown that either *A. fumigatus* Pep or AfuGprA is necessary for fungal growth in protein medium at acidic pHIn conclusion, Pep and AfuGprA constitute a pair of endoproteases active at acidic pH in analogy to *A. fumigatus* alkaline protease (Alp) and metalloprotease I (Mep), where at least one of these enzymes is necessary for fungal growth in protein medium at neutral pH.

We have shown that Seds and AfuS28 synergistically digest large peptides generated by exoprotease activity into amino acids, di- and tripeptides. Seds degrade peptides from their N-terminus into tripeptides, however Pro in P1 and P’1 position

acts as a stop sequence. In a complementary manner, X-X-Pro and X-X-X-Pro sequences can be removed by AfuS28 thus allowing Seds further sequential proteolysis. In conclusion, both alkaline and acidic sets of proteases contain exoprotease activity capable of cleaving after proline residues not bypassed by other exoproteases.

In a third part of this thesis we have tested the ability of two dermatophyte species, *Microsporum canis* and *Arthroderma benhamiae*, to grow in a protein medium that promotes secretion of proteases. We have shown that at neutral and acidic pH, dermatophytes secreted different proteases. Our investigation revealed new dermatophyte secreted proteases homologous to those secreted by *A. fumigatus* and suggests common basic mechanisms for extracellular protein digestion in dermatophytes and in *Aspergillus* spp. at acidic and neutral pH.

# Résumé

Les dermatophytes sont des champignons pathogènes qui se développent dans le stratum corneum de la peau, les ongles et les cheveux et sont la cause du plus grand nombre des mycoses cutanées. En culture dans un milieu ne contenant que de la kératine, ces champignons sécrètent de nombreuses protéases pour digérer cette source de protéine en acides aminés et petits peptides qui sont utilisés comme nutriments. Les protéases sécrétées par les dermatophytes sont similaires à celles sécrétées par les espèces du genre *Aspergillus*. C'est pourquoi, le champignon *Aspergillus fumigatus* a été utilisé dans ce travail pour étudier les différentes étapes de dégradation des protéines par des champignons tels que les dermatophytes à pH acide et à pH neutre.

Lors de la première étape de ce travail, nous avons montré que deux différents ensembles de protéases étaient sécrétés par *Aspergillus fumigatus* à pH 4.0 et à pH 7.0. A pH 7.0, cet ensemble comprend une subtilisine et une métalloprotéase qui sont des endoprotéases, des aminopeptidases non spécifiques (Laps pour leucine aminopeptidases) et une dipeptidylpeptidase IV (DppIV) qui est une X-prolyl peptidase. Il était connu que des peptides générés par une activité endoprotéolytique sur des grandes protéines pouvaient être digérés ensuite synergiquement par les Laps et la DppIV. Lors de ce processus les Laps ôtent les acides aminés un par un depuis l'extrémité N-terminale d'un peptide jusqu'à une séquence X-Pro où les laps s'arrêtent. Toutefois, les séquences X-Pro sont enlevées par la DppIV qui génère ainsi un nouveau substrat pour les Laps. A pH 4.0, l'ensemble des protéases sécrétées par *Aspergillus fumigatus* comprenait une pepsine, une protéase inconnue de la classe des glutamique-protéases (appelée ici AfuGprA), des sédolisines (SED) qui avaient été caractérisées comme étant des tripeptidyl-peptidases non spécifiques, et une nouvelle protéase de la famille S28 (appelée ici AfuS28). Il a été montré dans ce travail que des grands peptides pouvaient être digérés à pH acide par les activités synergiques des Seds et AfuS28. Lors de ce processus les Seds ôtent les acides aminés trois par trois depuis l'extrémité N-terminale d'un peptide jusqu'à une proline en position 3 ou 4. Toutefois, les séquences d'arrêt X-XX-Pro et X-X-XX-Pro sont enlevées par l'activité d'AfuS28 qui génère ainsi un substrat dégradable par les Seds. En conclusion, chacun des

deux ensembles des protéases sécrétées par *Aspergillus fumigatus* comprenait des aminopeptidases non spécifiques butant sur des résidus Pro, et une prolyl peptidase. L'activité de cette dernière enzyme génère un nouveau substrat pour les aminopeptidases non spécifiques.

La deuxième étape a consisté en l'étude d'AfuGprA et son importance dans l'activité endoprotéolytique d'*Aspergillus fumigatus.* Nous avons montré que soit Pep soit AfuGprA était nécessaire pour la croissance du champignon à pH acide dans un milieu contenant des protéines non dégradées comme seul nutriment.

Et enfin, la dernière partie de cette thèse s'est focalisée sur l'identification des protéases sécrétées par deux espèces de dermatophytes, *Microsporum canis* et *Arthroderma benhamiae* dans un milieu ne contenant que des protéines. Comme chez *Aspergillus fumigatus*, ces deux dermatophytes sécrètent à pH 4.0 et à pH 7.0 un ensemble particulier de protéases. Nombres de ces protéases ne sont pas connues chez ces dernières, mais sont des orthologues probables de protéases caractérisées chez *Aspergillus fumigatus.* C'est la première fois que des protéases acides sont identifiées à pH acide chez ces champignons. Ces investigations suggèrent des mécanismes communs de dégradation des protéines chez les *Aspergillus* et chez les dermatophytes.

# Abbreviations

7-amido-4-methylcoumarine (AMC)

Acetonitryle (AcN)

Alkaline serine peptidase (Alp)

Carboxypeptidase (Cp)

Collision induced dissociation (CID)

Diethylaminoethyl (DEAE)

Dimethylsulfoxid (DMSO)

Dipeptidylpeptidase (Dpp)

Gene coding for trypsinlike proteolytic activity (PrtT)

High performance liquid chromatography (HPLC)

Internal transcripted spacer (ITS)

Isopropyl thiogalactoside (IPTG)

Leucine aminopeptidase (Lap)

Linear trap quadripole (LTQ)

Liquid chromatography (LC)

Mass spectrometry (MS)

Natrium Deoxycholate (Doc)

Neutral protease or deuterolisin (NP)

Oligopeptide transporter (OPT)

Pepsin (Pep)

p-Nitroanilide (p-NA)

p-nitroanilide (p-Na)

Polymerase chain reaction (PCR)

Proteins containing A Disintegrin and A Metallaprotease domain (ADAMs)

Proton dependant oligopeptide transporter (POT)

Sedolisin (Sed)

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

Subtilisin (Sub)

Tandem mass spectrometry (MS/MS)

Trichloroacetic acid (TCA)

Yeast nitrogen base (YNB)

# Introduction

# General properties of fungi

The kingdom of Fungi contains many organisms such as yeasts, dermatophytes, *Penicillium* spp., morels and gilled mushrooms. It includes more than 100’000 species. Although it’s difficult to give a brief and strict definition of fungi, they are characterized by the following properties.

- Fungi are eukaryotes.
- They are heterotrophic organisms (they use only organic carbon source for their nutrition and do not possess chlorophyll).
- Fungi spread as colonies of isolated cells (yeasts) or mycelium. A mycelium is a multicellular filament (or hypha) which branches to form a network. Each fungus cell contains one or several haploid or diploid nuclei.
- Fungal cells are delimited by a β 1-3 and β 1-6 glucan wall and chitin.
- Depending on the species and conditions, fungi reproduce both sexually and asexually often resulting in the production of spores which often are wind-disseminated. Spores are generated on hyphae or in micro or macroscopic sporangia which show a limited tissue differentiation. The asexual and sexual forms in the same species are morphologically very different. The asexual forms of fungi are called anamorphs and the sexual forms are called teleomorphs. The term of “holomorph” is used to designate the total organism and a fungal colony where an ananmorph and the teleomorph coexist. A holomorph can give rise to an exclusive anamorph species by loss of the teleomorph during evolution. The term of “synanamorph” is used to designate two different anamorphs of the same species.

Fungi are considered as a separate kingdom of living organisms at the same level as the animals (zoobionta), the plants (chlorobionta, which regroups green algae, mosses and vascular plants), the red algae (rhodobionta), the kingdom including brown algae, diatoms and oomyceta (heterokontobionta), and protists which include protozoa (amibia, ciliates, euglena, dinoflagellates and myxomycetes).

# Classification of fungi

Classification of the fungi was based on the synthesis of phenotypic, morphologic, cellular and biochemical data. Analysis of genomic and protein sequences allowed to reassess and correct the classification of numerous groups. The fungi were divided in 5 main classes based on their reproduction mode and the shape of their sporangia.

- The Chitridiomycetes producing spores with one or several flagella and including 500 to 600 species.
- The Zygomycetes representing 1000 species
- The Ascomycetes forming sexual spores internally produced in a bag called an ascus. The Ascomycetes are the largest taxonomic group and include edible fungi such as morels and truffles.
- The Basidiomycetes reproducing sexually via external spores which are formed on a special club-shaped cell called basidium. The basidia are generally carried by a basidiocarp. Most of basidiomycetes are gilled mushrooms and polypores. Many basidiomycetes interact with roots plant forming mycorrhizae.
- The Deuteryomycetes or fungi imperfecti (more than 30’000 species) lacking sexual spores or reproducing by asexual spores’ formation only, whose anamorphs form were only known.

The most important changes in actual classification concern the groups of chytridiomycetes and zygomycetes. These groups were shown to be polyphyletic and are now abandoned (Hibbett, Binder *et al.*, 2007). The ascomycetes and basidiomycetes are now considered forming a subkingdom, the Dikarya (Fig 1, Hibbett, Binder *et al.*, 2007).

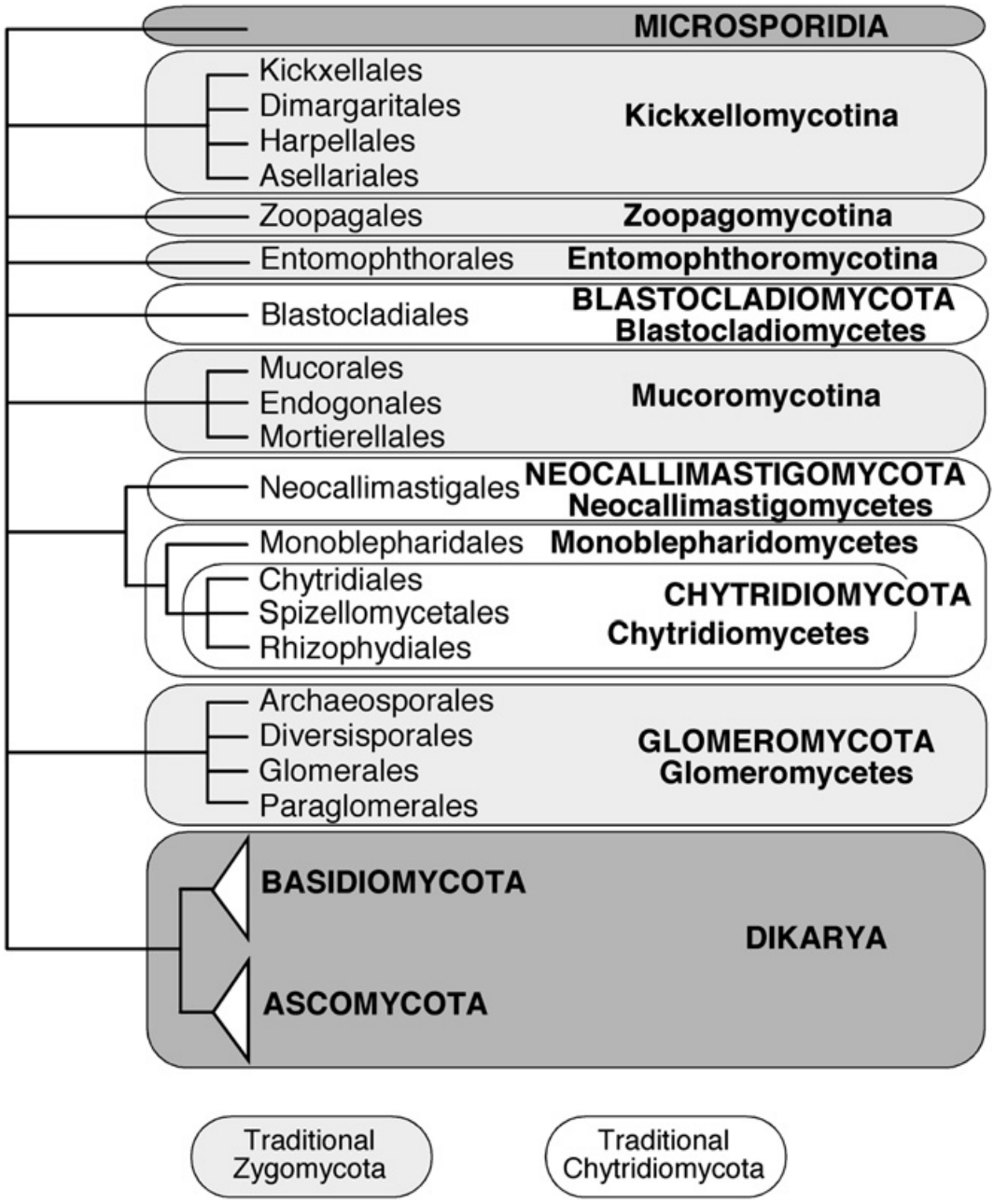



**Fig. 1:** Phylogeny and classification of Fungi. Basal Fungi and Dikarya. Branch lengths are not proportional to genetic distances.

## The genus *Aspergillus*

The Aspergillus are fungi characterized by a mycelium made of thin septate branching hyphae, from which the conidiophore broaden at the apex into an elliptical, hemispherical, or globose vesicle. This vesicle is surrounded by specialized cells called phialides which produce conidia (mitospores) by successive buddings (Fig. 2). The genus *Aspergillus* belongs to the order of *Eurotiales* and family of *Trichocomaceae* in the Ascomycetes. The genus *Aspergillus* was originally described

and illustrated in the 1860s by Fresenius, who worked with lung material from birds dying from aspergillosis (Fresenius, 1863).

The monograph of Raper and Fenell (1965) made authority till revisions or changes were made with the contribution of DNA sequencing data. 150 taxa based on morphology are described in this monograph. More than 40 new species descriptions have been published since then and were listed by Geiser *et al.* 2007. ITS sequence were used to delineate species, however these sequences were not enough discriminative. They show little or no variation between otherwise known and easily distinguished species. To date, solid phylogenetic species recognition generally requires genomic sequence analysis of more variable loci such as β-tubulin and actin (Samson *et al.*, 2007).

## *Aspergillus fumigatus*

The species *Aspergillus fumigatus* belongs to the *Fumigati* subgenera. The members of this taxon are important because they play a significant role as human pathogens and as food contaminants. *A. fumigatus* is a widespread saprophyte typically found in dust, soil and decaying organic matter such as compost heaps, where it plays an essential role in carbon and nitrogen recycling. Colonies of the fungus produce from conidiophores grey-green 2-3µm conidia that readily become airborne. *A. fumigatus* is an opportunistic fungus which is the causative agent of a number of diseases in humans, including allergic bronchopulmonary aspergillosis, aspergilloma and invasive aspergillosis (Fig. 2)

Recently, sexual reproduction in *A. fumigatus* was demonstrated (Szewczyk and Krappmann, 2010). Sexual mating was found between two strains with distinct mating-type idiomorphs MAT-1 and MAT-2. Production of cleistothecia containing unitunicate asci was observed.

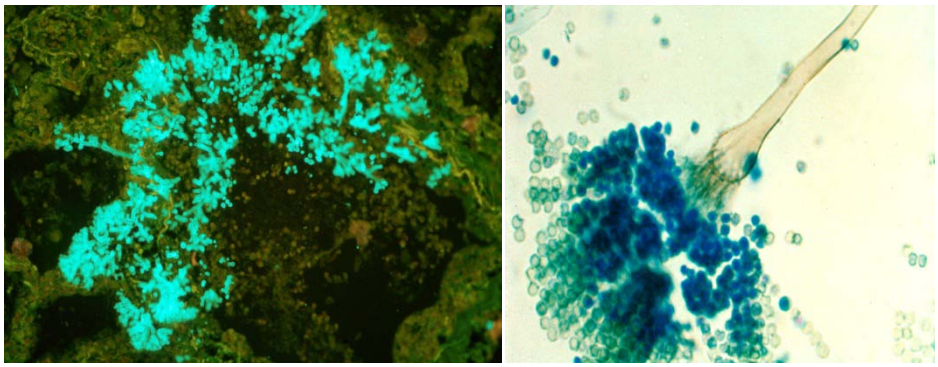


Fig. 2: Invasine aspergillosis (lung section observed by fluorescence microscopy (Monod et al., 1989) and *Aspergillus fumigatus* conidial head.

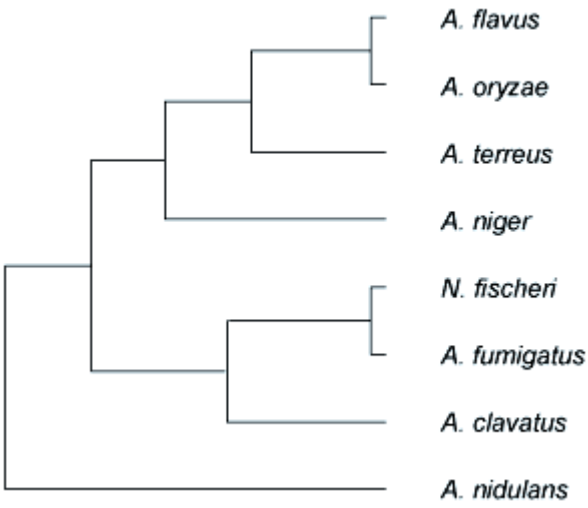



Fig. 3: Phylogenetic relation of *A. fumigatus* with other sequenced *Aspergillus* (from http://www.broadinstitute.org)

# The dermatophytes

Dermatophytes groups highly specialized pathogenic fungi which are the most common agents of superficial mycoses (Kwon-Chung *et al.*, 1992; Weitzman and Summerbell, 1995; Monod *et al.*, 2002). These fungi grow in the mammals' *stratum corneum*, nails or hair utilising them as sole nitrogen and carbon source. Dermatophytoses vary depending on the causative agent and the body site affected. The disease is described with the word "tinea" followed by a term for the particular infected body site.

Dermatophytes are ascomycete fungi, but only anamorphs (or asexual forms) are isolated from infected patient, animals or soil. Dermatophyte anamorphs are

classified in three genera, *Trichophyton*, *Microsporum*, and *Epidermophyton*. *Microsprum* species make spindle-shaped thick-walled macroconidia (Fig. 4). In *Epidermophyton* only one validated dermatophyte species (*Epidermophyton floccosum*) has been described.


Fig. 4: *Microsporum canis* observed by microscopy and in culture on Sabouraud agar.

Dermatophyte species are recognized and classified as antropophilic, zoophilic, or geophilic, depending on their major reservoir in nature (humans, animals, and soil, respectively). Zoophilic dermatophytes may result in zoonoses when humans are exposed to these organisms, and dermatophytosis is considered to be one of the most common zoonotic diseases. The majority of zoonotic dermatophytoses are caused by *Microsporum canis* (generally transmitted by cats and dogs), *Trichophyton verrucosum* (generally transmitted by cattle), *Arthroderma vanbreuseghemii* (generally transmitted by cats and dogs) and *Arthroderma benhamiae* (generally transmitted by guinea-pigs). In humans, the anthropophilic species (e.g. *Trichophyton rubrum*, *Trichophyton interdigitale*, and *Trichophyton tonsurans*) tend to be associated with more chronic infections which are less inflammatory. In contrast, zoophilic dermatophytes generally cause highly inflamed lesions in humans. Geophilic species (*Microsporum gypseum, Trichophyton ajelloi*), like zoophilic species, cause inflamed lesions.

In cases of highly inflammatory tinea corporis, tinea faciei and tinea capitis in humans, it is important to identify with certainty the precise etiologic agent and to examine pets as the possible source of infection in order to prevent the reoccurrence of new infections, especially in children, and to use the best therapeutic approach.

Dermatophyte teleomorphs (or sexual forms) have been classified in the *Arthroderma* genus in the Ascomycotina subphylum. Teleomorphs of *Microsporum* species were previously classified in the *Nannizzia* genus but this has now been

shown to be congeneric with *Arthroderma* (Weitzman *et al*. 1986). The dermatophytes are heterothallic fungi, that is conjugation for sexual reproduction is possible only through the interaction of individual isolates of different mating type (designated as either '+' or '−'). In a number of zoophilic species like in anthropophilic species sexual reproduction has not been observed. In some species clinical isolates tend to be of a single mating type as shown in other pathogenic fungi such as *Cryptococcus neoformans* (Idnurm *et al.* 2005). For example a bias for one particular mating type was observed in *M. canis*, *A. benhamiae* and *A. vanbreuseghemii*.

Dermatophytes classification is complex and was the object of numerous controversies. Nowadays, classification is principally based on molecular and especially ITS regions sequences (internal transcripted spacer) data from the ribosomal DNA (Gräser *et al.,* 2008).

## *Aspergillus* and dermatophytes genomes

*A. fumigatus, A. oryzae and A. nidulans* are the three first species of Aspergilli which have been sequenced (Nierman *et al.,* 2005.; Machida *et al.*, 2005; Galagan *et al.*, 2005) followed by several other species. To date, seven dermatophyte genomes are sequenced. The genomes of *T. rubrum*, *M. canis*, *M. gypseum*, *T. tonsurans* and *T. equinum* were recently sequenced by the BROAD Institute and are now available in the NCBI data base as well as Aspergilli. The genome of an *A. benhamiae* strain isolated from a patient in Lausanne (Burmester *et al.,* 2011) and that of *T. verrucosum* were sequenced at the Hans Knoell Institute in Jena (Germany). The dermatophyte genomes (24,000 kb) are accessible on-line on the website (http://www.broadinstitute.org).

# Fungus nutrition

Only small molecules such as amino acid, short peptides or mono and di-glycoside can be used by fungi as nutrients for growing *in vivo.* Large and complex molecules (e.g. polymers such as polysaccharides and proteins) must be broken down into smaller molecules which can then be absorbed. This degradation takes place outside the fungal cell or hypha and is achieved by enzymes which are released through the fungal wall. Since water is essential for the diffusion of extracellular enzymes and nutrients across the fungal wall and plasma membrane, actively growing fungi are usually restricted to relative moist (or humid) environment.

Yeasts and filamentous fungi as well as bacteria, specialised cells of plants and animals express membrane proteins (transporters) for uptake small molecules such as amino acids and short peptides. In addition to amino acid permeases, *A. fumigatus* possesses seven transporters of the proton-dependent oligopeptide transporter (POT) family and eight transporters of the oligopeptide transporter (OPT) family (see Transporter data base at www.membranetransport.org/). Well characterized members of the POT family are the di- and tripeptide transporters Ptr2 from *S. cerevisiae* and *Candida albicans* (Perry *et al.*, 1994, Basrai *et al.*, 1995). POT transporters are found in species from all taxonomic kingdoms. OPT transporters are limited to fungi and plants allow the uptake of tetra- and pentapeptides, and were recently the object of intensive investigation in *S. cerevisiae*, *C. albicans* and *Arabidopsis thaliana* (Hauser *et al.*, 2001; Lubkowitz *et al.*, 1997; Reuss and Morschhäuser, 2006; Koh *et al.*, 2002).

Dermatophytes, as well as Aspergillii, secrete important endo- end exoprotease activities involved in the virulence and in keratin degradation. The ability of fungi to degrade proteins (of the skin, nail and hair for dermatophyte) requires the use of specific combinations of proteases which will be the object of the present thesis.

# Fungal Secreted proteases

## Proteases

The term protease is synonymous with peptidase, proteolytic enzyme and peptide hydrolase. The proteases include all enzymes that catalyse the cleavage of the peptide bonds (CO-NH) of proteins, digesting these proteins into peptides or free amino acids. The classification and the nomenclature of proteases can be found together with information about them in the Handbook of proteolytic enzymes (Barrett *et al.*, 2nd ed, Elsevier, 2004) and in the MEROPS database accessible on the website (http://merops.sanger.ac.uk). The proteases are initially classified following their mode of action and their active sites: aspartic, cystein, glutamic, metallo, serine and threonine proteases as well as proteases with unknown catalytic mechanism are recognized. Each protease is then assigned to a family that is a set of homologous enzymes.

The proteases can be further divided into endoproteases (or endopeptidases) and exoproteases (or exopeptidases). The endoproteases cleave peptide bonds internally within a polypeptide. The term oligopeptidase is used to refer to endopeptidases that act only on substrates smaller than proteins. The exopeptidases cleave peptide bonds only at the N- or C-terminus of the polypeptide chains.

## Secreted proteases

Most eukaryotic and prokaryotic secreted proteins are synthesized as precursors with a hydrophobic N-terminal extension of 15-30 amino acids, known as the prepeptide or signal peptide (Blobel and Dobberstein, 1975). This signal peptide is necessary for entering the secretory pathway by enabling transport of the protein across the membrane of the endoplasmic reticulum (Pfeffer and Rothman, 1987), where it is subsequently cleaved by a signal peptidase (Milstein *et al.*, 1972). Secretion into the environment may not necessarily be an obligate feature of proteases made with a signal peptide, as several of these proteases are vacuolar, and remain intracellular. Other proteases with a signal peptide may be attached to the cell via a transmembrane domain or by a glycophosphatidylinositol (GPI)-anchor.

Many, but not all, secreted proteases are synthesized as precursors in a preproprotein form. The propeptide (30 to 250 amino acids in length), which is an intermediate sequence between the signal sequence and the N-terminus of the mature enzyme, has been found to be essential and specific in assisting the correct folding and secretion (Fabre *et al.*, 1991; Eder and Fersht, 1995; Fukuda *et al.*, 1996). Upon completion of folding, the propeptide is removed by an autoproteolytic or an exogenous proteolytic reaction to generate the active enzyme (Togni *et al.*, 1996; Newport and Agabian, 1997; Marie-Claire *et al.*, 1998; Zaugg *et al.*, 2008).

**Fungal endoproteases**

Fungal secreted endoproteases are aspartic protease of the pepsin family (A1 family), metalloprotease (M35 and M36 family) (Monod *et al.*, 1993), and serine proteases, most of the subtilisin family (S8) referred as alkalines proteases in Aspergillus spp. (Reichard *et al.*, 1990, Monod *et al.*, 1991).

Aspartic proteases have two aspartate residues involved in catalysis of peptide substrates. In general, they have are optimally active at acidic pH. Nearly all known aspartyl proteases are inhibited by pepstatin. They have a two-domain structure where each domain contains a catalytic Asp residue, with an extended active site cleft localized between the two lobes of the molecule. One lobe has probably evolved from the other through a gene duplication event in the distant past. In modern-day enzymes, although the three-dimensional structures are very similar, the amino acid sequences are more divergent, except for the catalytic site motif, which is very conserved. The presence and position of two disulfide bridges are other conserved features of most fungal aspartic proteases (Monod *et al.*, 2010).

Metalloproteases are proteolytic enzymes whose catalytic mechanism involves an atom of metal. Most fungal metalloproteases are zinc-dependent. Fungal secreted metallo-endoproteases are prevalently from the M35 and M36 family. These proteases (several other still hypothetical) belong to the same clan (MA), which gathers proteases with the specific HEXXH motif. More precisely, the HEXXH motif is found in a sequence Xaa-Xbb-Xcc-His-Glu-Xbb-Xbb-His-Xbb-Xdd where Xaa is hydrophobic or Thr, Xbb is uncharged, Xcc is any amino acid except Pro, and Xdd is hydrophobic (Jongeneel *et al.*, 1989; Barett *et al.*, 2004). The two His residues together with a third residue towards the C-terminus of the protein are zinc ligands.

The third residue varies depending on the family. Chelators inactivate MA proteases by removal the ion $Zn^{++}$. However, for exemple the *A. fumigatus* thermolysin Mep (M36 family) and the deuterolisin (M35 family) of *A. oryzae* can be reactivated by the addition of $Zn^{++}$ or $Co^{++}$ (Markaryan *et al.*, 1994; Doi *et al.*, 2003). The Glu residue in the HEXXH motif has a catalytic function. With the exception of the specific signature of the MA clan, members of a given MA family have no or low amino acid sequence similarity to members of another MA family.

Serine proteases or serine endopeptidases are proteases in which one of the amino acids in the active site of the enzyme is serine. They are found in both single-cell and complex organisms, in both eukaryotes and prokaryotes. In evolutionary history, serine proteases were originally digestive enzymes. In mammals, they evolved by gene duplication to serve functions in blood clotting, the immune system, and inflammation. The major clans of serine endoproteases found in humans include the chymotrypsin-like proteases and the subtilisin-like proteases. Fungal secreted serine endoproteases belong to the S8 family (subtilisins), the S1 family (trypsins) and S53 family (sedolysins).

Among fungal serine proteases in *A. fumigatus*, two subtilisins (Alp1 and Alp2, S8 family) and one endoprotease of the sedolisin family (SedA) have been characterized. The subtilisin Alp1 (formerly Alp) was isolated and characterized in the nineties by several groups (Reichard *et al.*, 1990; Monod *et al.*, 1991; Jaton-Ogay *et al.*, 1992; Larcher *et al.*, 1992; Frosco *et al.*, 1992, Kolattukudy *et al.*, 1993). This enzyme has a molecular mass of 33 kDa, and cleaves elastin as well as collagen. Alp1, like other subtilisins, very efficiently cleaves the synthetic substrate N-Suc-Ala-Ala-Pro-Phe-*p*-nitroanilide (pNA) (Larcher *et al.*, 1992) by releasing pNA. A second subtilisin (Alp2), which is vacuolar, was also found in the cell wall fraction of *A. fumigatus* like Pep2 (Reichard *et al.*, 2000). Alp2 is the orthologue of the cerevisin or *S. cerevisiae* proteinase B (Moehle *et al.*, 1987), and *A. niger* PepC (Frederick *et al.*, 1993). Alp1 and Alp2, like other secreted fungal and bacterial subtilisins are synthesized as preproproteins with a propeptide of approximately 120 amino acids.

The *A. fumigatus* endoprotease SedA (Reichard *et al.*, 2006) which belongs to the S53 family (sedolisin) is the orthologue of aorsin from *A. oryzae* (Lee *et al.*, 2003). Only a 25 kDa degradation product of SedA, but no full-length translation

product, has so far been detected in *A. fumigatus* culture supernatants (Reichard *et al.*, 2006).

Trypsins and chymotrypsins were identified and characterized in *Fusarium* Spp. and *Metarhizium anisopliae*, but not in *Aspergillus* spp. and dermatophyte species (refs).

**Fungal exopeptidases**

Fungal secreted exopeptidases are aminopeptidases [leucine aminopeptidases (Lap1 and Lap2), tripeptidyl peptidases (Tpps of the sedolisin family) and dipeptidyl peptidases (DppIV and DppV)], and carboxypeptidases.

- Fungal aminopeptidases

Leucine aminopeptidases were so called because of their preference for leucine-7-4-methylcoumarin (Leu-AMC) as a substrate. However, these enzymes are able to remove any amino acid from the N-terminus of a peptide provided that a proline is not in second position. Lap1 and Lap2 are metalloproteases which are active between pH 6.5 and 10.5 with a broad optimum peak between pH 7.0 and 9.0 (Monod *et al.*, 2005). Lap1 structurally belongs to the M28E subfamily as do *Vibrio* and *Aeromonas* leucyl aminopeptidases. Lap2 belongs to the M28A subfamily like the vacuolar protease Y from *Saccaromyces cerevisiae and the Streptomyces griseus* secreted aminopeptidase. Members of the M28A and M28E subfamilies share low sequence similarity, though the amino acid sequences between the two zinc binding sites are conserved.

Dipeptidyl peptidases (Dpp) are serine proteases members of the S9 family (Monod M. Et al., 2005) with a catalytic triad composed of serine, aspartic acid and histidine. Dpps are glycoproteins of approximately 90 kDa with about 10 kDa N-linked carbohydrates. Two Dpp have been isolated from *A. fumigatus* culture supernatants and characterized at both the biochemical and molecular level (Beauvais *et al.*, 1997a, Beauvais *et al.*, 1997b). The first released mainly X-Ala but also His-Ser and Ser-Tyr dipeptides from the N-terminal extremity of peptides (Beauvais *et al.*, 1997a). This protein was identified as one of the two major antigens used in the serodiagnosis of aspergillosis (Biguet *et al.*, 1967). It was wrongly named “chymotrypsic antigen” because of its ability to degrade N-acetyl-phenylalanine

naphtyl ester (NAPNE), a chromogenic substrate also for chymotrypsin. However, due to its distinctive biochemical properties, a new class of dipeptidyl-peptidases (DPPV) was created. The second dipeptidyl-peptidase released X-Pro and to a lesser extent X-Ala dipeptides from the N-termini of peptides and belongs to class IV of dipeptidyl-peptidases (DPPIV) (Beauvais *et al.*, 1997b). Similar enzymes in *A. oryzae* were subsequently discovered and raised interest due to their importance in protein degradation during koji fermentation (Doumas *et al.*, 1998). Amino acids and tripeptides are not removed from substrates by Dpps.

Fungal secreted tripeptidyl peptidases are serine proteases which are members of the S53 family (Sedolisins) (Reichard *et al.*, 2006). These enzymes were called sedolisins because the amino acid residues S, E and D in their catalytic triad. The specificities are broad, but there is an indication of preferences for hydrophobic residues in the P1 and P2 positions. Tpps of the sedolisin family are non specific aminopeptidases which are most active at acidic pH. The functions of the S53 peptidases in prokaryotic organisms are uncertain, although they probably contribute to the extracellular digestion of food proteins. Tripeptidyl-peptidase I in eukaryotes is a lysosomal enzyme (TPP1 human, Uniprot accession O14773).

- Fungal carboxypeptidases

Fungal secreted neutral carboxypeptidases are metalloproteases of the M14 family. *Trichophyton* and *Metarhizium anisopliae* metallocarboxypetidases were previously characterized and shown to be closely related to intestinal carboxypeptidase A. Fungal secreted acid carboxypeptidases are serine proteases of the S10 family. Such enzymes were characterized in *Aspergillus* spp., *Trichophyton rubrum* and *Fusarium* (Zaugg et al, 2008).

In general, the carboxypeptidases are classified by active site mechanism and/or by substrate preference. Carboxypeptidases that have a stronger preference for those amino acids containing aromatic or branched hydrocarbon chains are called carboxypeptidase A (A for aromatic/aliphatic). Carboxypeptidases that cleave positively charged amino acids (arginine, lysine) are called carboxypeptidase B (B for basic). The first studied were those involved in the digestion of food (pancreatic carboxypeptidases A1, A2, and B). A metallo-carboxypeptidase that cleaves a C-terminal glutamate from the peptide N-acetyl-L-aspartyl-L-glutamate is called

"glutamate carboxypeptidase". The serine carboxypeptidases that cleave the C-terminal residue from peptides containing the sequence -Pro-Xaa (Xaa is any amino acid on the C-terminus of a peptide) are called "prolyl carboxypeptidases".

# Thesis project

# Interests and Objectives of this work

Dermatophytes grow exclusively in the *stratum corneum*, nails or hair utilising them as sole nitrogen and carbon source. Similar to dermatophytes, *Aspergillus* spp. are able to utilize full length protein as a nutrient source using similar proteases.

Endo- and exoproteolytic activities are considered as virulence attributes in dermatophytes and Aspergilli. They are required to degrade protein substrates into amino acids or short peptides which can be used as nutrients for growing. The ability of fungi to degrade proteins (of the skin, nail and hair for dermatophyte) may require the use of specific combinations of proteases changing from one species to another and different from those used by the fungus to degrade proteins *in vitro* in a culture medium.

All investigated dermatophytes as well as *Aspergillus* spp. and many other fungal species secrete proteases *in vitro* when grown in a medium containing protein as sole nitrogen source. Fungal secreted proteases have been extensively studied using *Aspergillus oryzae* and *A. fumigatus.* In particular, a total of 111 proteases homologues are recorded in the MEROPS peptidase database for the *A. fumigatus* genome. Roughly, proteases constitute 1% of the total *A. fumigatus* proteins (about 10,000). A detailed analysis performed with all recorded proteases revealed that 46 possess a signal sequence. The distribution of the 14 families containing *A. fumigatus* secreted proteases varies among the organism kingdoms (Monod et al., 2008). Moreover inspection of *A. fumigatus* secreted proteases reveals that there are on one side alkaline or neutral proteases, and on the other side acidic proteases. Therefore, the existence of 2 different pathways of protein degradation can be postulated and had to be verified.

Not less than 20 genes encoding secreted endo- and exo-peptidases have been identified in dermatophytes (Table 1), and 15 encoded proteases were found to be secreted in vitro during growth of dermatophytes in a protein-containing medium (Giddey *et al.*, 2007). However, the functions and specificities of dermatophyte individual proteases which are involved in degradation of compact keratinized tissues are not known. Genome inspection reveals that dermatophytes contain 235 predicted protease-encoding genes, 87 of the deduced proteins possessing a secretion signal.

Table 1: Secreted endo-and exoproteases from dermatophytes

| Clan § | Protease (sub)family § | Genes | Proteases* | References |
|---|---|---|---|---|
| **Endoproteases** | | | | |
| MA(M) | M35 (Deuterolysins) | NPIIa *NPIIb* | NpIIA | Unpublished results |
| MA(E) | M36 (Fungalysins) | *MEP1 to MEP5* | Mep1; Mep3; Mep4; Mep5. | [Jousson et al., 2004b; Giddey et al., 2007] |
| SB | S8A (Subtilisins) | *SUB1* to *SUB7* | Sub2 to Sub7 | [Jousson et al., 2004a; Giddey et al., 2007] |
| **Exoproteases** | | | | |
| SC | S9B | DPPIV | DppIV | [Monod et al., 2005] |
| | S9C | DPPV | DppV | [Monod et al., 2005] |
| MH | M28B | *LAP1*; *LAP2* | Lap1, Lap2 | [Monod et al., 2005] |
| S10 | S10 | *SCPA*; *SCPB* | ScpA; ScpB | [Zaug et al., 2008] |
| M | M14 | *MCPA*; *MCPB* | McpA; McpB | [Zaug et al., 2008] |

§ Identification number in the MEROPS data base.
*Detected in Dermatophyte culture supernatants and/or obtained as recombinant proteins

At this state of our knowledge it was of interest to identify the different dermatophyte pathways in the process of degradation of compact keratinized tissues. In other words, which are the different essential steps and bottlenecks in the process of digestion of proteins into amino acids and short peptides. In order to answer to this question, we decided to use *A. fumigatus* as a model to determinate enzymes involved in the protein degradation mechanisms. This work is divided in three different parts:

- The analysis of the enzymatic processes by which *A. fumigatus* is capable to digest larges peptides of the skin generated by endoproteolytic activity into assimilable compounds (amino acids and oligopeptides) using exopeptidases (**Part 1**).

- The identification of *A. fumigatus* secreted proteases which are involved in the first step of protein degradation (from). The particular functions of a new glutamic protease, AfuGpA, and an aspartic protease, Pep1, were investigated (**Part 2**).
- The identification of different pathways of protein degradation in dermatophytes (*A. benhamiae* and *M. canis*) based on the knowledge acquired with *A. fumigatus* (**Part 3**).

# Projects preview

## Part 1

This first part shows that *Aspergillus fumigatus* grows well at neutral and acidic pH in a medium containing protein as the sole nitrogen source by secreting two different sets of proteases. Neutral pH favours the secretion of neutral and alkaline endoproteases, leucine aminopeptidase (Lap) which is non-specific monoaminopeptidase and an X-prolyl dipeptidase (DppIV). We have found that at acidic pH environment promotes the secretion of an aspartic endoprotease of pepsin family (Pep1) and tripeptidyl-peptidases of the sedolisin family (SedB and SedD). A novel prolyl peptidase, AfuS28, was secreted in both alkaline and acidic conditions. In previous studies Laps were shown to degrade peptides from their N-terminus until an X-Pro sequence acts as a stop signal. X-Pro sequences can be then removed by DppIV, which allows Laps access to the following residues. We have shown that at acidic pH Seds degrade large peptides from their N-terminus into tripeptides until Pro in P1 or P'1 position acts as a stop for these exopeptidases. However, X-X-Pro and X-X-X-Pro sequences can be removed by AfuS28 thus allowing Seds further sequential proteolysis. In conclusion, both alkaline and acidic sets of proteases contain exoprotease activity capable of cleaving after proline residues which cannot be removed during sequential digestion by non specific exopeptidases.

## Part 2

In the second part of this project we investigated the mechanisms by which full length proteins are digested into large peptides by endoproteases secreted by *A. fumigatus*. From part 1, we knew that in an acidic protein medium, the fungus secretes an aspartic endoprotease (Pep) as well as tripeptidyl-peptidases, a prolyl-peptidase and carboxypeptidases. A novel glutamic protease, AfuGprA homologous to *Aspergillus niger* aspergillopepsin II was discovered. We have shown that either *A. fumigatus* Pep or AfuGpA is necessary for fungal growth in protein medium at acidic pH. Exoproteolytic activity is therefore not sufficient for complete protein hydrolysis and fungal growth in a medium containing proteins as the sole nitrogen source. Pep and AfuGprA constitute a pair of endoproteases active at acidic pH analogous to *A. fumigatus* alkaline protease (Alp) and metalloprotease I (Mep), for which it was shown that at least one of the two enzymes is necessary for fungal growth in protein medium at neutral pH. Heterologous expression of AfuGprA in *Pichia pastoris* showed that the enzyme is synthesized as a preproprotein and that the propeptide is removed through an autoproteolytic reaction at acidic pH to generate the mature protease. In contrast to *A. niger* aspergillopepsin II, AfuGprA is a single chain protein and is structurally more similar to G1 proteases characterized in other non-*Aspergillus* fungi.

## Part 3

The last project is focused on dermatophytes proteolytic pathways. As explained in the introduction, dermatophytes infect healthy individuals and are exclusively found in the skin stratum corneum, nails, or hair.

*M. canis* and *A. benhamiae* were found to grow well in a protein medium at acidic as well as at neutral pH, accompanied by extracellular proteolysis. Shotgun mass spectrometry analysis of secreted protein revealed fundamentally different protease profiles during fungal growth in acidic versus neutral pH conditions. Most notably, novel dermatophyte secreted proteases were identified at acidic pH such as pepsins, sedolisins and acidic carboxypeptidases. We report for the first time the secretion of acidic proteases by dermatophytes. Analysis of the battery of secreted proteases suggests common basic mechanisms for extracellular protein digestion in dermatophytes and in *Aspergillus* spp. at acidic and neutral pH. However, in comparison to *A. fumigatus* more different proteases were secreted by dermatophytes at neutral as well as acidic pH. Families of genes encoding endoproteases of the subtilisin (S8), deuterolisin (M35) and fungalisin (M36) families have expanded in dermatophytes, and several members were found to be secreted. Therefore, the protein degradation pathways at acidic pH and that at neutral pH appear to be less strict than those in *A. fumigatus*.

Skin is a complex environment where pH varies according to many both endogenous and exogenous factors. Therefore, the large panel of acidic, neutral and alkaline proteases secreted by dermatophytes, including the newly recognized acidic ones, should trigger further investigations in order to define their role during skin infection.

# Part 1

## *Aspergillus* protein degradation pathways with different secreted protease sets at neutral and acidic pH.

**Sriranganadane, D.**, Waridel, P., Salamin, K., Reichard, U., Grouzmann, E., Neuhaus, J. M., Quadroni, M. & Monod, M. (2010). *J Proteome Res* 9, 3511-3519.

Key words: Secretome, *Aspergillus fumigatus*, sedolisin, prolylpeptidase, protein acidic hydrolysis, enzyme synergy, protein degradation mechanism.

# INTRODUCTION

*Aspergillus fumigatus* is an important opportunistic pathogen which is the main causative agent of invasive aspergillosis in neutropenic patients.[1] Under natural conditions in composts, this fungus plays an important role in the decomposition of organic material and in recycling environmental carbon and nitrogen. Like many other ascomycete fungi, *A. fumigatus* can grow in a medium containing protein as the sole nitrogen and carbon source.[2,3] This ability to grow in a protein medium depends on the synergistic action of secreted endo- and exoproteases since only amino acids and short peptides can be assimilated via membrane transporters. In contrast, large peptides cannot be directly used as nutrients. Accordingly, analysis of the *A. fumigatus* genome (www.tigr.org/tdb/e2k1/afu1) reveals that this fungus possesses seven transporters of the proton-dependent oligopeptide transporter (POT) family and eight transporters of the oligopeptide transporter (OPT) family in addition to amino acid permeases (see Transporter data-base at www.membranetransport.org/). Well characterized members of the POT family in fungi are the di- and tripeptide transporters Ptr2 from *Saccharomyces cerevisiae* and *Candida albicans*.[4,5] OPT transporters, which are limited to fungi and plants, allow the uptake of tetra- and pentapeptides .[6-9]

At neutral pH, *A. fumigatus* secretes two major endoproteases, an alkaline protease of the subtilisin family[2,3] (Alp1) and a metalloprotease of the fungalysin family[10,11] (Mep), leucine aminopeptidases[12] (Lap1 and Lap2) and a X-prolylpeptidase (DppIV).[13,14] A battery of ortholog proteases was found to be secreted by *Aspergillus oryzae*.[15-18] With this set of enzymes, large peptides generated from proteins by endoproteolysis can be further digested into amino acids and X-pro dipeptides by the synergistic action of leucine aminopeptidases and DppIV. Laps degrade peptides from their N-terminus until a X-Pro sequence acts as a stop signal. However, in a complementary manner, X-Pro sequences can be removed by DppIV, which allows Laps access to the following residues. Synergistic action of *A. oryzae* Lap and DppIV at pH 7.5 was found to digest a peptide consisting the sequence APGDRIYVHPF into amino acids, AP and HP di-peptides.[19]

Laps and DppIV are neutral proteases and are not active at acidic pH. However, *A. fumigatus* grows well in a protein medium at acidic pH as well as at

neutral and basic pH. This is indicative that other enzymes are expressed at lower pH and are able to digest complex proteins in acidic conditions. A fundamental objective of our research on fungal secreted proteolytic activity is to acquire a comprehensive view of the enzymes that allow the digestion of native proteins into free amino acids and short peptides under different environmental conditions. In the present study, we show that *A. fumigatus* secretes different sets of proteases at neutral and acidic pH, respectively. We have found that the set of proteases used by *A. fumigatus* at pH 3.5 includes an aspartic protease of the pepsin family (Pep1), tripeptidyl-peptidases of the S53 family[20] (SedB and SedD) and a novel prolylpeptidase of the S28 family called AfuS28A. We describe the different steps of protein digestion into assimilable amino acids and short peptides at acidic pH.

# MATERIALS AND METHODS

## Strains and plasmids.

*Aspergillus fumigatus* D141 (NRRL 6585; U.S. Department of Agriculture, Peoria, IL) was used in this study and all plasmid subcloning experiments were performed in *E. coli* XL1 blue using plasmid pKJ113.[21] *Pichia pastoris* GS115 (Invitrogen, Carlsbad, CA) was used to produce recombinant *A. fumigatus* peptidases.

## *Aspergillus fumigatus* growth media.

*Aspergillus fumigatus* was routinely grown on malt agar or in order to promote production of proteolytic activity at neutral pH, in liquid media containing protein as the sole nitrogen source (0.2% collagen).[3] The pH was approximately 7.0 and increased slightly to 7.5 during growth of the fungus. To promote production of proteolytic activity at acidic pH in collagen medium, 0.2% collagen was dissolved in 68 mM citrate buffer (pH 3.5). One liter flasks containing 200 ml of medium were inoculated with approximately $10^8$ spores and incubated for 70 h at 37°C on an orbital shaker at 200 rpm. The pH of the medium was measured at 30 h and at the end of the incubation and was found to remain constant.

## Recombinant protease production.

Recombinant *A. fumigatus* SedB was previously produced and purified from *P. pastoris* used as an expression system.[20] To construct *P. pastoris* strains producing AfuS28, amplified cDNA segments encoding N-terminal and C-terminal portions of the protein were obtained by PCR with a standard protocol[22,23] using homologous sense and antisense primers (P1 and P2, P3 and P4, respectively, supplementary Table 1) and 200 ng of DNA prepared from $10^6$ clones of a cDNA library as a template.[20] The primers P1 to P4 were derived from *A. fumigatus* sequence AFUA_1G01250. P5 was used instead of P4 as the antisense primer to obtain $His_6$-tagged AfuS28. The PCR products were digested with *Xho*I/*Sac*I and *Sac*I/*Bgl*II, respectively, and inserted end to end into pKJ113 digested with *Xho*I/*Bam*HI to generate the expression plasmids pAfuS28 and pAfuS28H-6. *Pichia pastoris* GS115

transformation with *Eco*RI linearized plasmid DNA and transformants were selected as previously described.[21]

For enzyme production, *P. pastoris* transformants were grown to near saturation ($OD_{600}$ = 10) at 30°C in 10 ml of glycerol-based yeast media (0.1 M potassium phosphate buffer at pH 6.0, containing 10 g/L yeast extract, 20 g/L peptone, 13 g/L yeast nitrogen base without amino acids (Becton-Dickinson, Sparks, MD), 10 ml/L glycerol and 40 mg/L biotin). Cells were harvested and resuspended in 2 ml (200 ml) of the same medium with 5ml/L methanol instead of glycerol and incubated for 2 days. Then, the culture supernatant was harvested after centrifugation (3000 × *g*, 4°C, 5 min).

Salts and small molecular weight solutes were removed from 2.5 ml of *P. pastoris* culture supernatant by passing through a PD10 column (Amersham Pharmacia, Dübendorf, Switzerland) with 20 mM citrate buffer (pH 6.0) before testing for proteolytic activity. The supernatant of *P. pastoris* GS115 grown under the same conditions was used as a negative control for comparison.

## Purification of heterologously produced AfuS28.

The secreted proteins from 250 ml of *P. pastoris* culture supernatant were concentrated by ultrafiltration to 6 ml using a Centricon Plus-70 (30 kDa cut-off) (Millipore, Volketswil, Switzerland). The $His_6$-tagged target protein was extracted with a Ni-NTA resin (Qiagen, Hilden, Germany) column with histidine elution buffer (50mM histidine in PBS 1X) as previously described.[24] Active fractions were pooled and concentrated by ultrafiltration using Amicon Ultra (Milipore 30000 kDa cut-off). Protein concentrations were measured by the method of Bradford using a commercial reagent (Bio-Rad).

In parallel, AfuS28 without $His_6$-tag was purified at 4°C as following: secreted proteins from 250 ml of *P. pastoris* culture supernatant were concentrated by ultrafiltration to 6 ml using a Centricon Plus-70 (30 kDa cut-off) (Millipore, Volketswil, Switzerland.) Thereafter, the concentrate was desalted with PD10 column (Amersham) and applied to a DEAE-Sepharose column which had previously been equilibrated with a 100 mM Na acetate buffer (pH 5.8). After washing the column with the same buffer, the recombinant protein was eluted with a 100 mM sodium acetate

buffer (pH 3.8). Enzymatic activity was tested with Ala-Ala-Pro-p-nitroanilide (Ala-Ala-Pro-pNA) as a substrate and active fractions were pooled.

## Quality control of purified AfuS28 (on silver stained SDS-PAGE gels).

To assess the degree of purity of AfuS28, eluted fractions were pooled and 5 µl aliquots were analysed by a SDS-PAGE and stained with silver nitrate according to Chevallet.[25]

## Antigen preparation for immunization of rabbits.

A 253 amino acid large peptide corresponding to the C-terminal part of AfuS28 was produced using plasmid pET-11aH6, a derivative of pET-11a made for $His_6$-tagged large peptide production.[20] Sense and antisense primers P6 and P7 (supplementary Table 1) were used to amplify DNA from plasmid pAfuS28 encoding heterologous AfuS28. The PCR products were digested with *Nco*I and *Bam*HI and cloned into the *Nco*I and *Bam*HI sites of pET-11aH6. The resulting plasmid was termed pAgAfuS28.

The corresponding heterologous $His_6$-tagged peptide was produced in *E. coli* BL21 transformed with pAgAfuS28. Cells were grown at 37°C to an $OD_{600}$ of 0.6 and $His_6$- tagged peptide expression was induced by adding IPTG to a 0.1 mM final concentration after which incubation was continued for an additional 4 h at 37°C. Cells were collected by centrifugation (4,500 × *g*, 4°C, 15 min), and the $His_6$- tagged peptides were extracted by lysis with guanidine hydrochloride buffer and Ni-NTA resin affinity (Qiagen, Hilden, Germany) columns according to the manufacturer. The column was washed with 0.1 M sodium phosphate buffer (pH 5.9) containing 8 M urea. Thereafter, antigen was eluted with the same buffer adjusted at pH 4.5. Rabbit antisera were made by Eurogentec (Liège, Belgium) by using the purified AfuS28 polypeptide chain as an antigen.

## Western blot analysis of native and recombinant AfuS28.

AfuS28 samples with or without prior N-glycosidase F digestion,[15] were analyzed by Western blotting of SDS-PAGE gels (12.5%). Western blots were

immunodeveloped using anti AfuS28 antiserum raised in rabbits, and alkaline phosphatase-conjugated goat anti-rabbit IgG (Bio-Rad, Hercules, CA).

## Proteolytic activities.

Endoproteolytic activities were measured with 50 µl *A. fumigatus* and *P. pastoris* culture supernatants and 50 µl of 0.2% resorufin-labeled casein at different pHs in sodium citrate buffer (50 mM final concentration; pH 2.0 to 7.0) in a total volume of 0.5 ml. After incubation at 37°C, the undigested substrate of the enzyme-substrate mix was precipitated with trichloroacetic acid (4% final concentration) and separated from the supernatant by centrifugation. Subsequently, 500 µl of Tris-HCl buffer (500 mM; pH 9.4) were added to the collected supernatant (neutralization step) and the $A_{574}$ of the mixture (1 ml) was measured. A blank was performed with 50 µl *P. pastoris* GS115 culture supernatant. For practical purposes, one milliunit of endoproteolytic activity was arbitrarily defined as producing an increase in absorbance of 0.001 per min in a proteolytic assay (1 ml) at optimal pH for activity. The assays were performed in triplicates.

Exoproteolytic activites were tested with synthetic substrates supplied by Genecust (Dunedange, Luxembourg). Stock solutions were prepared at 100 mM concentration and stored at –20°C. AP-pNA (Ala-Pro-p-nitroanilide), AA-pNA, FPA-pNA, AAP-pNA, AAAP-pNA and Suc-AAAP-pNA were dissolved in water/DMSO. The reaction mixture contained a concentration of 10 mM substrate and the enzyme preparation (between 0.1 to 1.0 µg per assay) in 50 µl of 100 mM acetate buffer at different pH values. After incubation at 37°C for 10 min, the reaction was terminated by adding 5 µl of glacial acetic acid and then 0.9 ml of water. The released pNA was measured by spectrophotometry at λ= 405nm. A control with substrate but without enzyme was carried out in parallel. The AfuS28 activities were expressed in mU (µmoles of released pNA/min) using Ala-Ala-Ala-Pro-pNA as a substrate.

The ability of AfuS28 to digest proline-rich peptides was investigated on neuropeptide Y 1-36 (NPY1-36, YPSKPDNPGEDAPAEDMARYYSALRHYINLITRQRY-NH2), NPY3-36 (SKPDNPGEDAPAEDMARYYSALRHYINLITRQRY-NH2) (Bachem), bradykinin (RPPGFSPFR) (Sigma, bradykinin acetate B3259-5MG), apelin-13 (QRPRLSHKGPMPF) (Bachem), dynorphin A (GGFLRRIRPKLKWDNQ, 2-17) (Bachem), and the peptide KDKRSFPFF (GeneCust). NPY1-36 and NPY3-36 were

dissolved in deionized water at 1.2 nmol/µl concentration. Bradykinin, apelin, dynorphin A and KDKRSFPFF were dissolved at 94 nmol/µl (100 µg/µl), 4 nmol/µl, 3 nmol/µl and 5.7 nmol/ µl, respectively. To measure by mass spectrometry the degradation of both NPY1-36 and NPY3-36, a solution containing 5 nmol of substrate with 0.02 nmol (1.5 mU) of AfuS28 in an acidic buffer (0.015% formic acid, pH4) was prepared. Degradation of bradykinin, apelin, dynorphin A and KDKRSFPFF by AfuS28 was performed with 0.3 nmol/µl of substrate as a final concentration. A control without AfuS28 was carried out in parallel. The enzymatic activity at 37°C was decreased at different times by adding 0.5% formic acid and stopped by freezing with liquid nitrogen. The solutions were diluted 5 to 10 fold in $H_2O$: acetonitrile 50:50 with 0.1 % formic acid and analyzed by mass spectrometry. They were infused in a LTQ-Orbitrap instrument (ThermoFisher, Bremen, Germany) via a TriVersa Nanomate (Advion Biosciences, Norwich, UK) system. Mass spectra were acquired in MS survey mode at a resolution of 60'000 (at 400 *m/z*) and accuracy better than 5ppm.

## Precipitation and separation of proteins from *A. fumigatus* culture supernatants by 1D- SDS-PAGE.

The mycelium was separated from culture medium by paper filtration (Miracloth Calbiochem). Thereafter, 50 ml of supernatant were centrifuged for 10 minutes at 5000 x *g* to remove debris, followed by a concentration step to 1 ml using a Centricon Plus-70 with a 10'000 Da cut-off. Concentrated media were precipitated as follows: 0.9 ml of 0.2% (w/v) sodium deoxycholate was mixed with 100 µl of concentrated medium and incubated for 10 min at room temperature. 100 µl of 6.1 N TCA was added to this mixture and gently shaken. The sample was incubated for 10 min at 4°C, and then centrifuged at 13000 rpm for 10 min to obtain a pellet. After removal of the supernatant, the pellet was washed twice with 100% acetone and dried.

For 1D-SDS-PAGE analysis, the pellet was dissolved in 20 µl of 20 mM Tris-HCl, pH 7.4 and mixed with SDS sample buffer. Proteins were separated on a 12% SDS polyacrylamide gel followed by staining with Coomassie brilliant blue R-250 (Bio-Rad). The total optical density in every lane was determined by densitometry and used to calibrate sample loadings onto a preparative gel. For protein digestion

(shotgun experiments), equal amounts of protein for every sample were subjected to limited electrophoretic separation on a 10% minigel, i.e. the migration was stopped after the front had moved by about 2.5 cm into the separating gel. At this time all bands up to 250 kDa of a prestained molecular weight marker had moved into the gel and were distinguishable. Gels were fixed for 10 min, partially stained with Coomassie Brilliant blue G (15 min) and then destained for 30 min. Every lane was cut into 4-5 sections beginning with high molecular weights.

## Digestion and MS analysis: shotgun MS experiments.

Proteins in 1D gel slices were digested with trypsin according to a described protocol.[26,27] Tryptic peptides were recovered in the supernatant of the digestion, dried by evaporation, reconstituted in 20 μl of $H_2O$: acetonitrile 97:3 with 0.1 % formic acid and analysed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Samples were loaded onto a trapping microcolumn ZORBAX 300SB C18 (5 mm x 300 μm ID, 5 μm, Agilent) in H2O:ACN 97:3 (v/v) + 0.1 % formic acid at a flow rate of 10 μl/min. After 5 min they were back-flush eluted and separated on a reversed-phase nanocolumn ZORBAX 300SB C18 column (75 μm ID x 15 cm, 3.5 μm, Agilent Technologies) with an Agilent 1100 nano HPLC system (Agilent Technologies). Solvents used for the mobile phase were 95:5 H2O:AcN (v/v) with 0.1 % formic acid (A) and 5:95 H2O:AcN (v/v) with 0.1 % formic acid (B). The flow rate was 300 nl/min and the gradient used lasted 90 min (5 min at 0 % of solvent B, from 0 to 25 % of B in 35 min, 25 to 50 % B in 15 min, 50 to 90 % in 5 min, 90 % B during 10 min, 90 to 0 % in 5 min and 15 min at 0 %). The chromatographic system was interfaced to a LTQ-Orbitrap mass spectrometer (Thermo Fisher Scientific) through a TriVersa Nanomate (Advion Biosciences). In data-dependent acquisition controlled by Xcalibur 2.0 software (Thermo Fisher), the four most intense precursor ions detected in the full MS survey performed in the Orbitrap (range 350-1500 *m/z*, resolution 60000 at *m/z* 400) were selected and fragmented. MS/MS was triggered by a minimum signal threshold of 10,000 counts, carried out at relative collision energy of 35 % and with isolation width of 4.0 amu. Only precursors with a charge higher than one were selected for CID fragmentation and fragment ions were analyzed in the linear trap. Spectra were acquired under automated gain control, with

maximal ion injection time of 100 ms. The *m/z* of fragmented precursors was then dynamically excluded, with a tolerance of 0.01 amu, from any selection during 120s.

## Database searching with MS data.

From *raw* files, MS/MS spectra were de-isotoped and exported as *mgf* files (Mascot Generic File, text format) using MascotDistiller 2.1.1 (Matrix Science, London, UK). MS/MS spectra were searched with Mascot (Matrix Science, London, UK; version 2.2.0) against the UNIPROT database (www.expasy.org) selected for Fungi assuming the digestion enzyme trypsin and one missed cleavage. The database release used was of April, 23th 2008 (5,939,836 sequences, Fungi: 358052 sequences). Mascot was searched with a fragment ion mass tolerance of 0.50 Da and a parent ion tolerance of 10.0 ppm. Iodoacetamide derivative of cysteine was specified in Mascot as a fixed modification. N-terminal acetylation of protein, deamidation of asparagine and glutamine, and oxidation of methionine were specified in Mascot as variable modifications.

## Criteria for protein identification.

Scaffold (version Scaffold_2_05_01, Proteome Software Inc., Portland, OR) was used to validate MS/MS based peptide and protein identifications. Peptide identifications were accepted if they could be established at greater than 90.0% probability as specified by the Peptide Prophet algorithm.[28] Protein identifications were accepted if they could be established at greater than 95.0% probability and contained at least 2 identified peptides. Protein probabilities were assigned by the Protein Prophet algorithm.[29] Proteins that contained similar peptides and could not be differentiated based on MS/MS analysis alone were grouped to satisfy the principles of parsimony.

# RESULTS.

## Comprehensive identification of proteins secreted by *A. fumigatus* at different pH values.

*Aspergillus fumigatus* grew well at 30°C in a medium containing 0.2% collagen protein as a sole carbon and nitrogen source at both pH 7.0 and pH 3.5. After two days of growth, clarification of the culture medium was observed. At this time, the amount of protein was 20-50 $\mu g \cdot ml^{-1}$ in culture supernatants at both pH values. Concomitantly, a substantial proteolytic activity was measured using resorufin-labelled casein as substrate (200-250, and 50-100 $mU \cdot ml^{-1}$ at pH 7.0 and pH 3.5, respectively). Substantial activities on APF-pNA, AAP-pNA and AAAP-pNA were also detected in culture supernatant. Activity on AP-pNA was detected in the culture supernatant at pH 7.0, but not at pH 3.5.

SDS-PAGEs of proteins precipitated from culture supernatants at pH 7.0 and pH 3.5 showed complex band patterns with major differences (Figure 1). In an attempt to map a maximum number of proteins secreted by *A. fumigatus* at different pH values, a systematic shotgun protein identification experiment was undertaken. After sample fractionation by limited 1D SDS-PAGE electrophoresis, five identical gel bands corresponding to different molecular weights were excised from every lane and proteins were in-gel digested. After peptide gel extraction, every fraction was analysed by LC-MS/MS on a LTQ-orbitrap mass spectrometer.

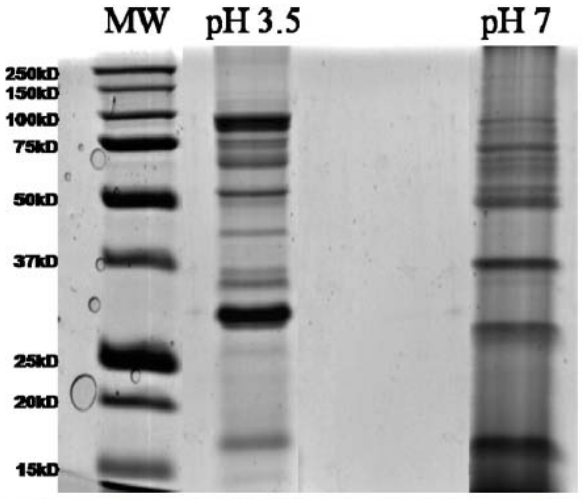



**Figure 1.** Ten percent SDS-PAGE stained with Coomassie blue of *Aspergillus fumigatus* secreted proteins at pH 3.5 and 7.

Lists of spectra for each lane were merged in order to obtain a dataset that was used to search a fungal sequence database (SPTrEMBl, 23.04.2008, Taxonomy Fungi: 358052 sequences, supplementary Tables 2, 3 and 4). Total numbers of MS/MS spectra assigned to peptides after database search were 3292 and 2352 for *A. fumigatus* samples at pH 7.0 and pH 3.5, respectively. Spectral counting coupled with redundant sampling of a mixture has been established as a reliable method to quantify protein abundances in shotgun experiments.[30] Here we have assumed that the number of matched spectra represents at least semi-quantitative estimates of the relative protein abundances between samples. The most abundant protein identified in the *A. fumigatus* sample at pH 7.0 was the dipeptidyl peptidase DppV (XP_755237) with 466 spectra corresponding to 38 unique peptide sequences. The most abundant protein identified in the *A. fumigatus* sample at pH 3.5 was a tripeptidyl peptidase called sedolisin B (SedB) with 315 spectra which correspond to 17 unique peptide sequences (supplementary Table 2). Extensive details on the results of identifications can be found in supplementary Tables 3 and 4, while all matched sequences are also reported in the supplementary materials. Overall, 171 different sequences were matched in the shotgun experiment.

As expected, proteases constituted a significant fraction of all identified proteins, with five endo- and 10 exoproteases (Table 1). Furthermore, proteases accounted for 30 to 40 % of total matched spectra in the shotgun analysis, a fact that highlights their quantitative dominance. These proteases fall into two only slightly overlapping groups corresponding to the acidic and neutral pH secretomes (Table1). Alkaline serine protease Alp1 (XP_751651), DppV (XP_755237) and leucine aminopeptidase Lap2 (XP_748386) were three major proteases exclusively secreted at pH 7.0. Aspartic endoprotease Pep1 (XP_753324), SedB (XP_746536) and serine carboxypeptidase Scp1 (XP_753901) were three major proteases specific to acidic growth medium (pH 3.5). A putative glutamic endoprotease (XP_748619) ortholog of *A. niger* aspergillopepsin II (XP_001388499), and SedD (XP_751432) were also found in culture supernatant at pH 3.5, but in an amount lower than those of the three aforementioned major acidic proteases. Only one putative serine protease of the S28 family, namely AfuS28, and homologous to a previously described *A. niger* prolyl endopeptidase[31] (XP_001392567), was secreted in similar amounts at both pH values (Table 1). A total of 100 identified proteins were identified as hydrolytic

enzymes. Other hydrolases detected were glycosidases (mannosidase, glutaminase, beta-1,3-endoglucanase), lipases and acid phosphatases. For nineteen sequences, no function could be assigned based on sequence similarity.

**Table 1.** Proteases Secreted by *A. fumigatuson* Media Containing Collagen at pH 3.5 and 7 during 70 h Growth under Shaking at 30 °C[a]

| family | identified proteins (enzymes massively secreted only) | as named in the text | accession number | molecular weight | number of spectra detected by mass spectrometry | |
|---|---|---|---|---|---|---|
| | | | | | *A. Fumigatus* pH 3.5 | *A. Fumigatus* pH 7 |
| S53 | Tripeptidyl-peptidase - *Aspergillus fumigatus* A1163 | SedB | B0YEL1 | 66 kDa | 315 | 0 |
| A1 | Aspartic endopeptidase Pep1 - *Aspergillus fumigatus* A1163 | Pep1 | B0Y1V8 | 42 kDa | 240 | 0 |
| S10 | Carboxypeptidase S1, putative - *Aspergillus fumigatus* A1163 | | B0Y1L0 | 54 kDa | 123 | 0 |
| S10 | Carboxypeptidase 5 - *Aspergillus fumigatus* (Sartorya fumigata) | | Q5VJG7 | 60 kDa | 45 | 0 |
| S10 | Carboxypeptidase 4 - *Aspergillus fumigatus* (Sartorya fumigata) | | Q5VJG8 | 58 kDa | 43 | 0 |
| S53 | Tripeptidyl peptidase A - *Aspergillus fumigatus* A1163 | SedD | B0Y502 | 64 kDa | 26 | 0 |
| S10 | Carboxypeptidase S1, putative - *Aspergillus fumigatus* A1163 | | B0YA52 | 61 kDa | 22 | 0 |
| G1 | Aspergillopepsin, putative - *Aspergillus fumigatus* A1163 | G1 | B0Y015 | 28 kDa | 23 | 0 |
| S28 | Serine peptidase, putative - *Aspergillus fumigatus* A1163 | AfuS28 | B0XT80 | 59 kDa | 57 | 45 |
| M36 | Elastinolytic metalloproteinase Mep - *Aspergillus fumigatus* A1163 | Mep | B0Y9E2 | 69 kDa | 0 | 7 |
| S8A | Autophagic serine protease Alp2 - *Aspergillus fischerianus* | Alp2 | A1DER5 | 53 kDa | 0 | 10 |
| S9 | Extracellular dipeptidyl-peptidase Dpp4 - *Aspergillus fumigatus* A1163 | DppIV | B0Y6C5 | 86 kDa | 0 | 54 |
| M28A | Aminopeptidase Y LAP2, putative - *Aspergillus fumigatus* A1163 | Lap2 | B0XX53 | 54 kDa | 0 | 133 |
| S8A | Alkaline serine protease Alp1 - *Aspergillus fumigatus* A1163 | Alp1 | B0Y708 | 42 kDa | 0 | 433 |
| S9 | Secreted dipeptidyl peptidase Dpp5 - *Aspergillus fumigatus* A1163 | DppV | B0XRV0 | 80 kDa | 13 | 466 |

[a] Numbers of matched spectra give a semiquantitative measure of protein amounts.

## Characterization of recombinant AfuS28

*Aspergillus fumigatus* Pep1, SedB and SedC secreted at pH 3.5 as well as Alp1, Mep, DppIV, Lap1 and Lap2 secreted at pH 7.0 were previously characterized as recombinant enzymes.[12-14,20,24,32] To learn more about the function of the new

serine protease AfuS28 and its importance in protein digestion, the enzyme was produced as a recombinant protein using *P. pastoris* as an expression system. A yield of 25 µg/ml culture supernatant was obtained. Fractions of the purified enzyme showed a single band on silver stained SDS-PAGE gel, attesting a high degree of purity (supplementary Figure 1). AfuS28 is a 65kDa glycoprotein with a carbohydrate content of about 20% (supplementary Figures 2 and 3). Recombinant AfuS28 had the same electrophoretic mobility than the native enzyme secreted by *A. fumigatus* in collagen medium.

Recombinant AfuS28 showed no detectable proteolytic activity using casein resorufin-labeled as a substrate but very efficiently released pNA when AAP-pNA, APP-pNA, AAAP-pNA and Suc-AAAP-pNA were used as substrates. AfuS28 was active between pH 3.0 and 9.0 with an optimum at pH 6.0. At optimal pH, AfuS28 activity was 1.5, 1.2, 1 and 0.2 mmol $min^{-1}$ $mg^{-1}$ using AAAP-pNA, Suc-AAAP-pNA, AAP-pNA and APP-pNA respectively. AfuS28 showed no activity on the DppIV substrates GP-pNA and AP-pNA, and on the sedolisin substrate APF-pNA.

The degradation of large proline-containing peptides by AfuS28 was analyzed by mass spectrometry. Digestion of bradykinin (RPPGFSPFR) resulted in RPP, FR, GFSPFR and PGFSPFR fragments. AfuS28 was also found to degrade NPY1-36 and NPY3-36 (YPSKPDNPGEDAPAEDMARYYSALRHYINLITRQRY-NH2 and (SKPDNPGEDAPAEDMARYYSALRHYINLITRQRY-NH2, respectively), cleaving after proline residues (supplementary Table 5). Measurement of the degradation kinetics of the amide form of NPY3-36 was accomplished. The reaction was monitored at different times from 0 to 15 min using 1.5 mU of enzyme at 37°C (Figure 2).

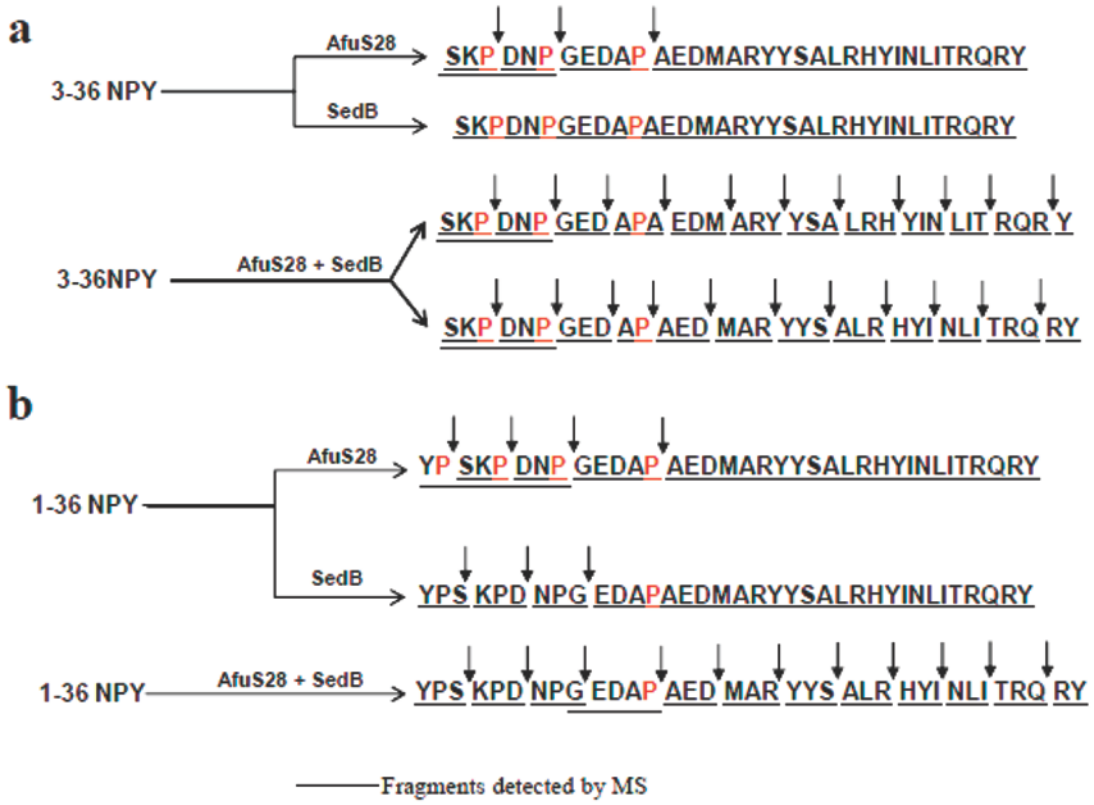
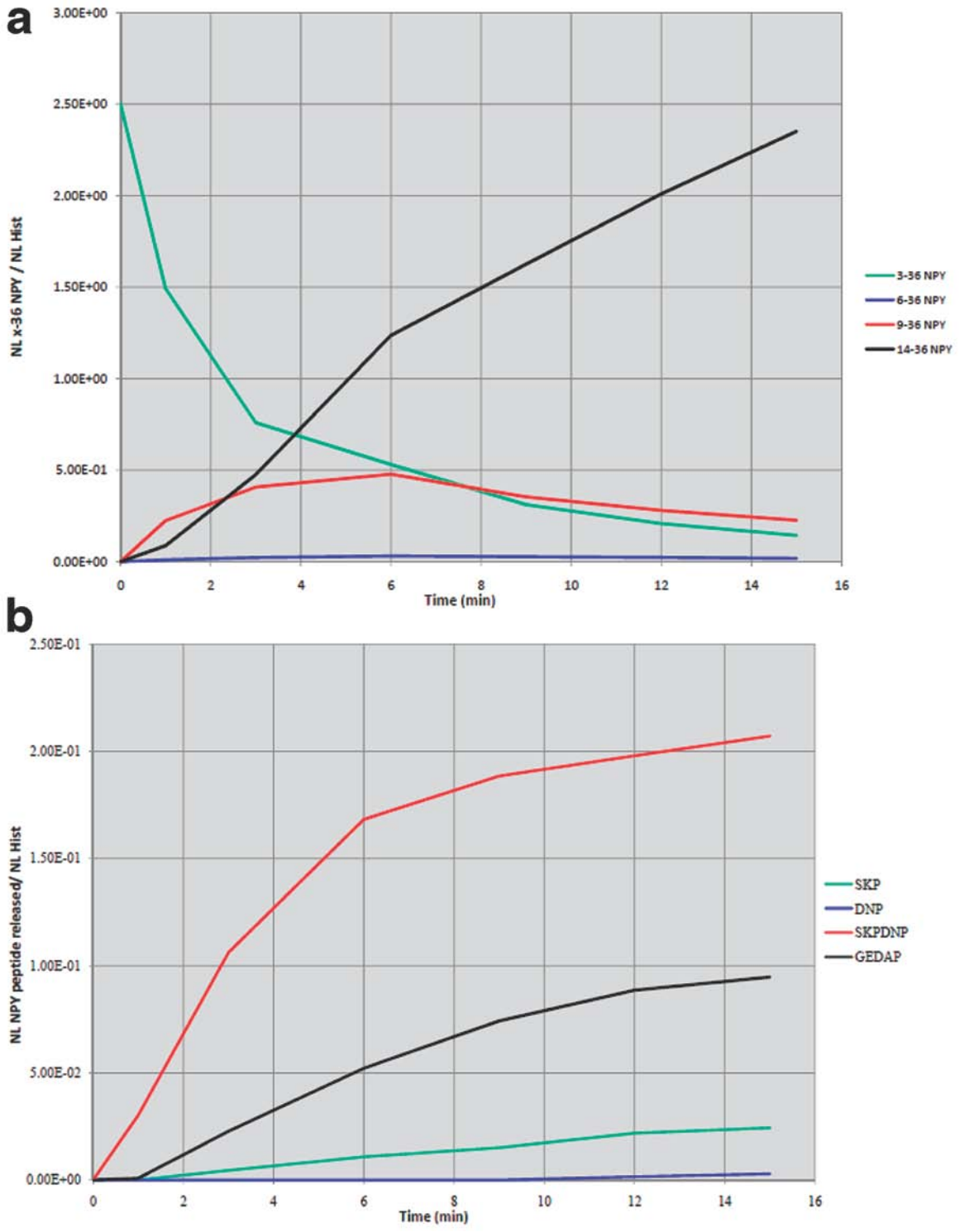



**Figure 2: NPY3-36 and NPY1-36 degradations by AfuS28 and SedB**

**a & b)** The reactional medium contains 4.8 nmol of NPY3-36 or 1-36, 0.02nmol of AfuS28 and/or 0.015nmol of SedB and 0.05 mmol of histidine in acidic buffer pH 4 (Formic acid ~0.0125 %) and was incubated at 37°C during 1h. Reaction was stopped by adding 0.5% formic acid. All samples were diluted 10 times in $H_2O$:MeCN 50:50 (+ 0.1% formic acid) and infused in the LTQ-Orbitrap via the Nanomate**.**




**Figures 3a & 3b: Kinetics of 3-36 NPY degradation by AfuS28 during 15 min measured by MS.**

The histidine signal (m/z 156.0766, present from the elution of $His_6$-tagged AfuS28) was used as reference to normalize peptide fragment intensities (Figure 3), thus allowing us to follow the progression of NPY3-36 degradation. At t = 0 min, intact

NPY3-36 was observed at different m/z ratios corresponding to various charge states (z=3-7). Less than 10 % of full length NPY3-36 was still present after 15 min incubation. Complete digestion of the substrate was obtained after 45 minutes (Suppplementary Figure 4a).Fragments NPY6-36, NPY9-36 and NPY14-36 were detected concomitantly with SKP (residues 3-5), SKPDNP (residues 3-8) and GEDAP (residues 9-13) after 3 min of digestion by AfuS28. NPY6-36 and DNP (residues 6-8) were detected only in low amount as SKPDNP appeared to be highly resistant to AfuS28. At t = 1 min, there was more NPY9-36 than NPY14-36 and following 6 min, NPY14-36 increased at the expense of NPY9-36. The latter disappeared after 60 min reaction (supplementary Figure 4). Importantly, a peak corresponding to fragment SKPDNPGEDAP (NPY3-13) was never detected. Therefore, cleavage between amino acids P8 and G9 seemed necessary for cleavage after the proline residue in position 13. These results are in agreement with AfuS28 having an exoprotease activity.

## Large peptide digestion into short assimilable peptides at acidic pH.

Large peptide digestion at acidic pH was investigated with SedB, the major exoprotease secreted at acidic pH. NPY3-36 was not digested by recombinant SedB (Figure 2a), but this enzyme removed tripeptides (YPS, KPD and NPG) from the N-terminus of NPY1-36 until position 10 (Figure 2b). In conclusion, SedB appeared to be active only when the amino acid in P1 or P'1 position (amino acids in positions 3 and 4 from the N-terminus of any substrate peptide) is not a proline. In contrast, a proline residue in position P'2 or P2 does not constitute an impediment for SedB during sequential digestion.

AfuS28 and SedB combined were able to degrade NPY3-36 and NPY1-36 in Y, di- and tri-peptides (Figure 2). Based on the peptide fragments produced during incubation with AfuS28 and SedB, two different degradation pathways could be proposed for NPY3-36 degradation. In the first one, SedB cleaves NPY9-36 generated by AfuS28 into tri-peptides by "jumping" over proline residue in position 5 (position 13 in NPY1-36) to generate the APA tripeptide. In the second pathway, AfuS28 first acts on P13 before further SedB digestion. Tripeptides such as INL, ITR or QRY which would result from other modes of degradation were not detected. In contrast to NPY3-36, one single pathway has been established for NPY1-36. In our

experimental conditions, cleavage after proline was only detected after P13 attesting for a more rapid degradation of the N-terminus by SedB.

Proline containing peptide digestion into amino acids, di- and tripeptides by synergistic action of SedB and AfuS28 at acidic pH was confirmed with other substrates such as apelin-13, dynorphin A and KDKRSFPFF peptide (supplementary Figures 5, 6 and 7, and supplementary Tables 6, 7 and 8). Of particular note, dynorphin A and KDKRSFPFF were not cleaved by AfuS28 alone. However, cleavage by AfuS28 after Pro was observed in presence of SedB which removed 6 and 3 amino acids from the N-terminus of dynorphin A and KDKRSFPFF, respectively.

# DISCUSSION

A comprehensive analysis of secreted proteins reveals that *A. fumigatus* grows in a protein medium at neutral and acidic pH using two different sets of secreted proteases. At neutral pH, the fungus secretes a set of neutral and alkaline proteases which includes Alp1, Mep1 as endoproteases and Laps, DppIV and AfuS28 as exoproteases. At acidic pH, the fungus secretes another set of proteases which includes the aspartic protease Pep1[32] as an endoprotease together with tripeptidyl-peptidases of the sedolisin family, AfuS28 and carboypeptidases of the S10 family as exoproteases. During protein digestion the main function of endoproteases is to produce a large number of free ends on which exoproteases may act. Both sets of enzymes secreted by *A. fumigatus* at neutral and acidic pH contain proteases able to sequentially cleave large peptides both internally and from their N-terminus. The importance of secreted carboxypeptidases in large peptide digestion at acidic pH was not investigated in the present study.

Among the 20 amino acids found in proteins, proline is exceptional because of its cyclic structure and constitutes a block to sequential protein hydrolysis by Laps and Seds at neutral and acidic pH, respectively.[12,19,20] However, both sets of proteases secreted by *A. fumigatus* contain exoproteases able to cut after proline residues which cannot be bypassed by non specific aminopeptidases. DppIV is active and secreted at neutral pH only, while AfuS28 is active and secreted at neutral and acidic pH. Therefore, DppIV can be substituted by Afu28 at neutral pH. In contrast,

the latter peptidase may play a major function in peptide digestion from the N-terminus with tripeptidylpeptidases of the sedolisin family at acidic pH, since *A. fumigatus* apparently does not possess other secreted prolyl exopeptidases.[34]

Comparison between the sequence of the *A fumigatus* genome and the sequences of reverse transcriptase PCR products used to produce AfuS28 in *P. pastoris* shows that the gene coding for AfuS28 consists of 10 exons. As a secreted protein, AfuS28 is synthesized as a preprotein precursor. The deduced amino acid sequence of the open reading frame encoded by the AfuS28 gene shows a 21-amino acid signal peptide with a hydrophobic core of 13 amino acid residues and a putative signal peptidase cleavage site Ala-Ser-Ala in accordance with Von Heijne's rule.[35,36] The AfuS28 protein generated after signal peptidase cleavage is 504 amino acids long. The polypeptidic chain of the mature protein has a calculated molecular mass of 55 kDa, which is in accordance with that estimated for the deglycosylated protein by SDS-PAGE (supplementary Figure 2). The amino acid sequence of AfuS28 contains six potential N-linked glycosylation (Asn-X-Thr) sites, and the carbohydrate content of the secreted enzyme is about 20% (Supplementary Figure 3). AfuS28 contains a Gly-Gly-Ser-Tyr-Gly sequence (residue 173-177) in accordance with the consensus sequence Gly-X-Ser-X-Gly for the catalytic site of serine proteases. In addition to Ser 175, alignment of AfuS28 with the above cited S28 peptidases reveals Asp and His residues of the catalytic triad in position 453 and 486, respectively. AfuS28 is closely related to the recently described *A. niger* prolyl endopeptidase, with around 75% identity.

In our experiments, recombinant AfuS28 exclusively hydrolyzed Pro-X bonds but some bonds appear to be more resistant than others as evidenced by the accumulation of SKPDNP during NPY3-36 digestion. In contrast to DppIV, AfuS28 is able to cleave peptides between and after two proline residues as revealed by products found from bradykinin digestion. *Aspergillus niger* prolyl endopeptidase has shown a lower specificity than that of AfuS28 and is able to digest after amino acids other than proline.[37,38] However, these experiments were performed with native enzyme from raw digestion extracts and the possibility of minor endopeptidase contaminants such as subtilisin or fungalisin could not be excluded. For this reason, our experiments were performed with recombinant enzymes. Although AfuS28 cleaves substrates which are Suc-blocked (succinyl function added at the N-

terminus), several facts support the conclusion that AfuS28 rather behaves as an Xn-prolyl exopeptidase as follows: (i) AfuS28 does not attack full length protein substrates such as casein, (ii) NPY3-36 digestion was found to occur sequentially from the N-terminus. (iii) Cleavage of dynorphin A and KDKRSFPFF by AfuS28 was observed only in presence of SedB which itself cleaved 6 and 3 N-terminal amino acids, respectively, from these peptides. This substantiates the preference of AfuS28 for cleaving after proline residues in position 3 and 4. AfuS28 and *A. niger* prolyl endopeptidase are homologous to human lysosomal Pro-Xaa carboxypeptidase and DppII which have a substrate specificity similar to that of DppIV. While all proteases of the S28 family are specialized in hydrolyzing prolyl bonds, no crystal structure has yet been reported. This could help to understand the differences in substrate specificity among family members.

In contrast to obligatory pathogenic species, *A. fumigatus* does not possess specific large expanded gene families encoding secreted proteases. The emergence of multigenic families is most frequently due to ancient gene duplication processes allowing organisms to better adapt to different environmental conditions and marked differences occur from one pathogenic species to another. For instance, dermatophytes, which are highly specialized in hard keratin degradation, secrete multiple endoproteases such as subtilisins and fungalysins.[22,23] Another example is the human yeast *C. albicans* which expresses different genes encoding closely related secreted aspartic proteases in reaction to specific environmental conditions during infection.[39] The absence of specific large gene families encoding secreted proteases may well be a feature of non-specialized opportunistic fungi which are saprophytic in the environment and degrade proteins into amino acids and short peptides during the recycling of soil nitrogen.

Alp1, Mep and Pep1 were detected on the surface of fungal hyphae in the setting of infection by indirect immunofluorescence studies using specific antisera. These results indicate that neutral and acidic protease sets are likely produced during invasive infection. While anti-DppV antibodies were detected in patients with aspergilloma which allows the discrimination between aspergilloma patients and healthy controls,[13,24,40] data on in vivo secretion of most acidic and neutral exoproteases are lacking. The function of different *A. fumigatus* secreted individual exoproteases and exoprotease sets during infection as well as the pH of the fungal

microenvironment in the host would be of interest in determining pathogenesis in humans.

## Acknowledgements

We thank Dr. Massimo Lurati for critical review of the manuscript and assistance with the English as well as Marie-jeanne Voirol, Marina Fratti and Olympia Bontems for technical assistance. This work was supported by the Swiss National Foundation for Scientific Research, grant 320030-1179641.

# REFERENCES


1. Latgé, J.P. *Aspergillus fumigatus* and aspergillosis. *Clin. Microbiol. Rev.* **1999**, *12* (2), 310-50.
2. Reichard, U.; Büttner, S.; Eiffert, H.; Staib, F.; Rüchel, R. Purification and characterisation of an extracellular serine proteinase from *Aspergillus fumigatus* and its detection in tissue. *J. Med. Microbiol.* **1990**, *33* (4), 243-51.
3. Monod, M.; Togni,G.; Rahalison, L.; Frenk, E. Isolation and characterisation of an extracellular alkaline protease of *Aspergillus fumigatus*. *J. Med. Microbiol.* **1991**, *35* (1), 23-8.
4. Perry, J. R.; Basrai,M. A.; Steiner, H. Y.; Naider, F.; Becker, J. M. Isolation and characterization of a *Saccharomyces cerevisiae* peptide transport gene. *Mol. Cell Biol.* **1994**, *14* (1), 104-15.
5. Basrai, M. A.; Lubkowitz, M. A.; Perry, J. R.; Miller, D.; Krainer, E.; Naider, F.; Becker, J. M. Cloning of a *Candida albicans* peptide transport gene. *Microbiology* **1995**, *141* (Pt5), 1147-56.
6. Hauser, M.; Narita, V.; Donhardt, A. M.; Naider, F.; Becker, J. M. Multiplicity and regulation of genes encoding peptide transporters in *Saccharomyces cerevisiae. Mol. Membr. Biol.* **2001**, *18* (1), 105-12.
7. Koh, S.; Wiles, A. M.; Sharp, J. S.; Naider, F. R.; Becker, J. M.; Stacey, G. An oligopeptide transporter gene family in *Arabidopsis*. *Plant. Physiol.* **2002**, *128* (1), 21-9.
8. Lubkowitz, M. A.; Hauser, L.; Breslav, M.; Naider, F.; Becker, J. M. An oligopeptide transport gene from *Candida albicans*. *Microbiology* **1997**, *143* (Pt2), 387-96.
9. Reuss, O.; Morschhäuser, J. A family of oligopeptide transporters is required for growth of *Candida albicans* on proteins. *Mol. Microbiol.* **2006**, *60* (3), 795-812. Erratum in: *Mol. Microbiol.* **2006**, *62* (3), 916.
10. Monod, M.; Paris, S.; Sanglard, D.; Jaton-Ogay, K.; Bille, J.; Latgé, J. P. Isolation and characterization of a secreted metalloprotease of *Aspergillus fumigatus*. *Infect. Immun.* **1993**, *61* (10), 4099-104.
11. Jaton-Ogay, K.; Paris, S.; Huerre, M.; Quadroni, M.; Falchetto, R.; Togni, G.; Latgé, J. P.; Monod, M. Cloning and disruption of the gene encoding an extracellular metalloprotease of *Aspergillus fumigatus*. *Mol. Microbiol.* **1994**,

*14* (5), 917-28.

12. Monod, M.; Lechenne, B.; Jousson, O.; Grand, D.; Zaugg, C.; Stocklin, R.; Grouzmann, E. Aminopeptidases and dipeptidyl-peptidases secreted by the dermatophyte *Trichophyton rubrum*. *Microbiology* **2005**, *151* (Pt 1), 145-55.
13. Beauvais, A.; Monod, M. ; Debeaupuis, J. P. ; Diaquin, M.; Kobayashi, H. ; Latgé, J. P. Biochemical and antigenic characterization of a new dipeptidyl-peptidase isolated from *Aspergillus fumigatus*. *J. Biol. Chem.* **1997**, *272* (10), 6238-44.
14. Beauvais, A.; Monod, M.; Wyniger, J.; Debeaupuis, J. P.; Grouzmann, E.; Brakch, N.; Svab, J.; Hovanessian, A. G.; Latge, J. P. Dipeptidyl-peptidase IV secreted by *Aspergillus fumigatus*, a fungus pathogenic to humans. *Infect. Immun.* **1997**, *65* (8), 3042-7.
15. Doumas, A.; van den Broek, P.; Affolter, M.; Monod, M. Characterization of the prolyl dipeptidyl peptidase gene (dppIV) from the koji mold *Aspergillus oryzae*. *Appl. Environ. Microbiol.* **1998**, *64* (12), 4809-15.
16. Doumas, A.; Crameri, R.; Léchenne, B.; Monod. M. Cloning of the gene encoding neutral protease I of the koji mold *Aspergillus oryzae* and its expression in *Pichia pastoris*. *J. Food Mycol.* **1999**, *2* (1), 271-9.
17. Blinkowsky, A. M.; Byun, T.; Brown, K. M.; Golightly, E. J.; Klotz, A. V. A non-specific aminopeptidase from *Aspergillus*. *Biochim. Biophys. Acta* **2000**, *1480* (1-2), 171-81.
18. Chien, H. C.; Lin, S. H.; Chao, S. H.; Chen, C. C.; Wang, W. C.; Shaw, C. Y.; Tsai, Y. C.; Hu, H. Y.; Hsu, W. H. Purification, characterisation, and genetic analysis of a leucine aminopepeptidase from *Aspergillus sojae*. *Biochim Biophys Acta* **2002**, *1576* (1-2), 119-26.
19. Byun, T.; Kofod, L.; Blinkovsky, A. Synergistic action of an X-prolyl dipeptidyl aminopeptidase and a non-specific aminopeptidase in protein hydrolysis. *J. Agric. Food Chem.* **2001**, *49* (4), 2061-3.
20. Reichard U.; Léchenne, B.; Asif, A.R.; Streit, F.; Grouzmann, E.; Jousson, O.; Monod, M. Sedolisins, as new class of secreted proteases from *Aspergillus fumigatus* with endoprotease or tripeptidyl-peptidase activity at acidic pH. *Appl. Environ. Microbiol.* **2006**, *72* (3), 1739-48.
21. Borg-von Zepelin, M.; Beggah, S.; Boggian, K.; Sanglard, D.; Monod, M. The expression of the secreted aspartyl proteinases Sap4 to Sap6 from *Candida*

*albicans* in murine macrophages. *Mol. Microbiol.* **1998**, *28* (3), 543-54.

22. Jousson, O.; Léchenne, B.; Bontems, O.; Capoccia, S.; Mignon, B.; Barblan, J.; Quadroni, M.; Monod, M. Multiplication of an ancestral gene encoding secreted fungalysin preceded species differentiation in the dermatophytes *Trichophyton* and *Microsporum*. *Microbiology* **2004**, *150* (Pt 2), 301-10.
23. Jousson, O.; Léchenne, B.; Bontems, O.; Mignon, B.; Reichard, U.; Barblan, J.; Quadroni, M.; Monod, M. Secreted subtilisin gene family in *Trichophyton rubrum*. *Gene* **2004**, *339*, 79-88.
24. Sarfati, J.; Monod, M.; Recco, P.; Sulahian, A.; Pinel, C.; Candolfi, E.; Fontaine, T.; Debeaupuis, J. P.; Tabouret, M.; Latgé, J. P. Recombinant antigens as diagnostic markers for aspergillosis. *Diagn. Microbiol. Infect. Dis.* **2006**, *55* (4), 279-91.
25. Chevallet, M.; Luche, S.; Rabilloud, T. Silver staining of proteins in polyacrylamide gels. *Nat. Protocols* **2006**, *1* (4), 1852-8.
26. Wilm, M.; Shevchenko, A.; Houthaeve, T.; Breit, S.; Schweigerer, L.; Fotsis, T.; Mann, M. Femtomole sequencing of proteins from polyacrylamide gels by nano-electrospray mass spectrometry. *Nature* **1996**, *379* (6564), 466-9.
27. Shevchenko, A.; Wilm, M.; Vorm, O.; Mann, M. Mass spectrometric sequencing of proteins silver-stained polyacrylamide gels. *Anal Chem.* **1996**, *68* (5), 850-8.
28. Keller, A.; Nesvizhskii, A. I.; Kolker, E.; Aebersold, R. Empirical statistical model to estimate the accuracy of peptide identifications made by MS/MS and database search. *Anal Chem.* **2002**, *74* (20), 5383-92.
29. Nesvizhskii, A. I.; Keller, A.; Kolker, E.; Aebersold, R. A statistical model for identifying proteins by tandem mass spectrometry. *Anal Chem.* **2003**, *75* (17), 4646-58.
30. Liu, H.; Sadygov, R. G.; Yates, J. R. 3rd. A model for random sampling and estimation of relative protein abundance in shotgun proteomics. *Anal Chem.* **2004**, *76* (14), 4193-201.
31. Edens, L.; Dekker, P.; van der Hoeven, R.; Deen, F.; de Roos, A.; Floris, R. Extracellular prolyl endoprotease from Aspergillus niger and its use in the debittering of protein hydrolysates. *J Agric Food Chem.* **2005**, *53* (20), 7950-7.
32. Reichard, U.; Monod, M.; Rüchel, R. Molecular cloning and sequencing of the gene encoding an extracellular aspartic proteinase from *Aspergillus fumigatus*.

*FEMS Microbiol. Lett.* **1995**, *130* (1), 69-74.

33. Zaugg, C.; Jousson, O.; Léchenne, B.; Staib, P.; Monod, M. *Trichophyton rubrum* secreted and membrane-associated carboxypeptidases. *Int. J. of Med.Microbiol.* **2008,** *298* (7-8), 669–82.
34. Monod, M.; Jousson, O.; Reichard, U. *Aspergillus fumigatus* secreted proteases. In *Aspergillus fumigatus* and aspergillosis; Latgé, J.-P.; Steinbach, W. J. Eds. ASM Press: Washington, D.C., **2009**, pp 87-106.
35. von Heijne, G. A new method for predicting signal sequence cleavage sites. *Nucleic Acids Res.* **1986***, 14* (11), 4683-90.
36. Bendtsen J. D.; Nielsen, H.; von Heijne, G.; Brunak, S. Improved prediction of signal peptides: SignalP 3.0. *J. Mol. Biol.* **2004**, *340* (4), 783-95.
37. Kubota, K.; Tanokura, M.; Takahashi, K. Purification and characterization of a novel prolyl endopeptidase from *Aspergillus niger*. *Proc. Japan Acad.* **2005,** *81* (10), 447-53.
38. Sebela, M.; Rehulka, P.; Kábrt, J.; Rehulková, H.; Ozdian, T.; Raus, M.; Franc, V.; Chmelík, J. Identification of N-glycosylation in prolyl endoprotease from Aspergillus niger and evaluation of the enzyme for its possible application in proteomics*. J Mass Spectrom.* **2009** , *44* (11), 1587-95.
39. Naglik, J.R.; Challacombe, S.J.; Hube, B. *Candida albicans* secreted aspartyl proteinases in virulence and pathogenesis. *Microbiol Mol Biol Rev.* **2003**, *67* (3), 400-28.
40. Biguet, J.; Tran, Van Ky P.; Fruit, J.; Andrieu, S. Identification of chymotryptic activity in remarkable fractions of an antigenic extract of *Aspergillus fumigatus*. Significance for the immunologic diagnosis of aspergillosis. *Rev Immunol Ther Antimicrob.* **1967**, *31* (4), 317-28.

# Part 2

## Secreted glutamic protease rescues aspartic protease Pep deficiency in *Aspergillus fumigatus* during growth in acidic protein medium.

**Dev Sriranganadane**, Utz Reichard, Karine Salamin, Marina Fratti, Olivier Jousson, Patrice Waridel, Manfredo Quadroni, Jean-Marc Neuhaus and Michel Monod. *Microbiology. 2011 feb. 24*.
Key words: *Aspergillus fumigatus*, secreted proteases, glutamic protease, aspartic protease, *Pichia pastoris*.

# INTRODUCTION

*Aspergillus fumigatus* is an important opportunistic pathogen which is the main causative agent of invasive aspergillosis in neutropenic patients (Latgé, 1999). In composts, this fungus plays an important role in the decomposition of organic material and in recycling environmental carbon and nitrogen (Beffa *et al.*, 1998). Like many other Ascomycetes from the soil, *A. fumigatus* grows well in a medium containing protein as the sole nitrogen and carbon source and produces secreted proteolytic activity (Reichard *et al*., 1990; Monod *et al*., 1991; Sriranganadane *et al*., 2010). At neutral pH, the fungus secretes two endoproteases, an alkaline protease (Alp) of the subtilisin family (Reichard *et al*., 1990; Monod *et al*., 1991) and a metalloprotease (Mep) of the fungalisin family (Monod *et al.*, 1993a; 1993b; Jaton-Ogay *et al.*, 1994). In addition, *A. fumigatus* secretes leucine aminopeptidases (Laps) which are non-specific monoaminopeptidases, dipeptidyl-peptidases (DppIV and DppV) and a prolyl-peptidase (AfuS28) as exopeptidases (Beauvais *et al*., 1997a; 1997b; Monod *et al*., 2005; Sriranganadane *et al*., 2010). *Alp*Δ *mep*Δ knockout mutants are unable to grow in a medium containing protein as the sole nitrogen source at neutral and alkaline pH while single *alp*Δ and *mep*Δ mutants produced 30 and 70% of the proteolytic activity of the wild type strain, respectively (Monod *et al.*, 1993a; 1993b; Jaton-Ogay *et al.*, 1994).

In a protein medium at acidic pH, *A. fumigatus* was found to secrete another set of proteases which includes the aspartic protease Pep as an endoprotease (Reichard et al., 1995), tripeptidyl-peptidases (Tpp) of the sedolisin family (SedB to SedD) (Reichard et al., 2006), AfuS28 and carboxypeptidases of the S10 family as exoproteases (Sriranganadane *et al*., 2010). In addition, we identified by mass spectrometry analysis a not yet characterized putative glutamic endoprotease AfuGprA (XP_748619, MER107323) (encoding gene at the locus AFUA_3G02970) (Monod *et al*., 2009; Sriranganadane *et al*., 2010) homologous to *Aspergillus niger* proteinase A or aspergillopepsin II (Takahashi, 2004) and to *Scytalidium lignicolum* Scytalidopepsin B (Oda, 2004). *Aspergillus niger* aspergillopepsin II is not inhibited by pepstatin and was previously considered as a non-pepsin-type acid protease (Sasaki *et al*., 1995; Takahashi, 2004). The active site of scytalidopepsin B and aspergillopepsin II was identified with a catalytic dyad formed by residues Glu (E) and

Gln (Q) (Fujinaga *et al.*, 2004; Yabuki *et al*.; 2004; Kataoka *et al*., 2005). This finding led to the constitution of a novel family of proteases called eqolisins (Fujinaga *et al*., 2004) or glutamic peptidases (Yabuki *et al*.; 2004; Kataoka *et al*., 2005) separated from the aspartic proteases (see MEROPS proteolytic enzyme database, hyperlink http://merops.sanger.ac.uk).

*Aspergillus fumigatus pep* mutants were constructed in order to test the role of Pep in virulence (Reichard *et al*., 1997). However, no difference in pathogenicity was observed between the wild type strain and *pep* mutants. Mortality curves were not statistically different, and histopathological studies of lungs from infected guinea pigs showed a similar extent of mycelium growth. In a control analysis, we have found that *A. fumigatus pep* mutants grew well at acidic pH in a protein medium. Because this result suggested that other endoproteases could rescue a lack of Pep activity we have characterized AfuGprA and investigated its function with respect to protein digestion. We have shown that AfuGprA and Pep constitute a pair of endoproteases in which one of them is necessary for fungal growth in a protein medium at acidic pH.

# MATERIALS AND METHODS

## Fungal and bacterial strains.

*Aspergillus fumigatus* D141 (NRRL 6585; U.S. Department of Agriculture, Peoria, IL) and an isogenic *pep*Δ mutant (Reichard *et al*., 1997) were used in this study. All plasmid subcloning experiments were performed in *E. coli* XL1 blue. *Pichia pastoris* GS115 (Invitrogen) was used to produce recombinant AfuGprA.

## *Aspergillus fumigatus* growth on solid media.

*Aspergillus fumigatus* was grown on 2% (w/v) malt agar medium (Oxoid) for production of spores, bovine serum albumin (BSA) agar medium and soy protein agar medium at pH 7.0 or pH 4.0. BSA agar medium contained 1.2% (w/v) yeast carbon base (YCB, Difco), 0.2% (w/v) BSA (Fraction V, Serva) and 1.5 % (w/v) agar. Nine hundred milliliters of a solution containing YCB and agarose were adjusted to pH 7.0 or pH 4.0 with 1 M NaOH or 1 M HCl, respectively, and sterilized by autoclaving at 120°C. A BSA solution (2.0 % [w/v], 100 ml) with a pH adjusted at 7.0 or 4.0 using 1 M NaOH or 1 M HCl, respectively, was sterilized by filtration and added to cooled YCB-agarose and the mix was poured into Petri dishes. Soy protein agar medium contained 0.2% (w/v) soy protein (Supro 1711, Protein Technologies International), and 1.5 % (w/v) agar. To test the growth of *A. fumigatus* at acidic pH, soy protein was dissolved in 68 mM citrate buffer (pH 4.0). Neutral and acidic soy protein media were sterilized by autoclaving at 120°C and poured into Petri dishes.

## *Aspergillus fumigatus* liquid cultures.

In order to promote production of proteolytic activity at neutral pH, *A. fumigatus* was grown in liquid media containing protein as the sole nitrogen source (1.2% [w/v] BSA supplemented with 1.2% [w/v] YCB or 0.2% [w/v] soy protein). The pH was adjusted to 7.0 with 1M NaOH and was observed to increase slightly to 7.5 during growth of the fungus. To test proteolytic activity at acidic pH in BSA liquid medium, 1.2% (w/v) BSA supplemented with 1.2% (w/v) YCB was adjusted to pH 4.0 with 1 M HCl. To test proteolytic activity at acidic pH in soy protein liquid medium, 0.2% (w/v) soy protein was dissolved in 68 mM citrate buffer (pH 4.0). One hundred milliliter flasks containing 50 ml of medium were inoculated with approximately $10^7$

spores each and incubated for 96 h at 30°C on an orbital shaker at 200 rpm. The pH of the medium was measured at 48 h, 72h and at the end of the incubation and was found to remain constant.

## Gene replacement.

*AfugprA* replacement was performed in *A. fumigatus* D141 and in the D141 *pep*Δ mutant. The plasmid p∆*gprA* was designed to function as a one-step gene replacement vector in transformation experiments and was constructed using pSK397 (Krappmann *et al*., 2005; 2006). The p∆*gprA* plasmid contained *AfugprA* replaced by the *E. coli* hygromycin B phosphotransferase gene (*hph*) under the control of a truncated *gpd* promoter and the *trpC* terminator of *Aspergillus nidulans* (Fig. 1). For plasmid construction, two approximately 1.2 kb sized fragments covering the 5'- and 3'-flanking regions of *AfugprA* and small portions (< 100 bp) of this gene (*Nflr* and *Cflr*, respectively) were PCR-amplified using genomic *A. fumigatus* DNA as a template. P1/P2 and P3/P4 primers which contained additional restriction sites at the 5' extremity for subsequent cloning steps (Table 1) were used to amplify *Nflr* and *Cflr*, respectively. *Nflr* and *Cflr* were subsequently digested with *Not*I/*Xma*I, and *Pac*I/*Hin*DIII, repectively and were inserted end-to-end into a modified pBluescript II SK(+) (Stratagene) in which a *Pac*I restriction site has been previously added in the multiple cloning site. The generated pBluescript construction contained between unique *Not*I and *Pac*I sites, *Nflr* and *Cflr* in the same orientation as in the *A. fumigatus* genome, and two different *Sfi*I sites located between *Nflr* and *Cflr* for subsequent directive cloning (Kämper, 2004). Thereafter, the hygromycin-resistance gene containing cassette was excised with *Sfi*I from pSK397 and cloned into the *Sfi*I site of the pBluescript construction to generate p∆*gprA*.

Transformation of wild type *A. fumigatus* D141 and the D141 *pep*Δ mutant was accomplished according to a protocol that has been used for *A. nidulans* and *A. fumigatus* (Tilburn *et al*., 1983). Transformation of $10^7$ protoplasts with 5 µg of *Pac*I and *Not*I double-digested p∆*gpr*A typically yielded 100 to 200 hygromycin-resistant colonies. After overnight *hph* expression the transformants were incubated on agar, based on GYE-medium (1% [w/v] glucose, 0.5% [w/v] yeast extract) containing 200 µg/ml hygromycin (Sigma, St Louis, MO), and selected after 5 days of incubation at 20°C followed by an overnight incubation at 42°C. Transformants initially identified as

hygromycin resistant were picked and subcultured again on agar containing hygromycin.

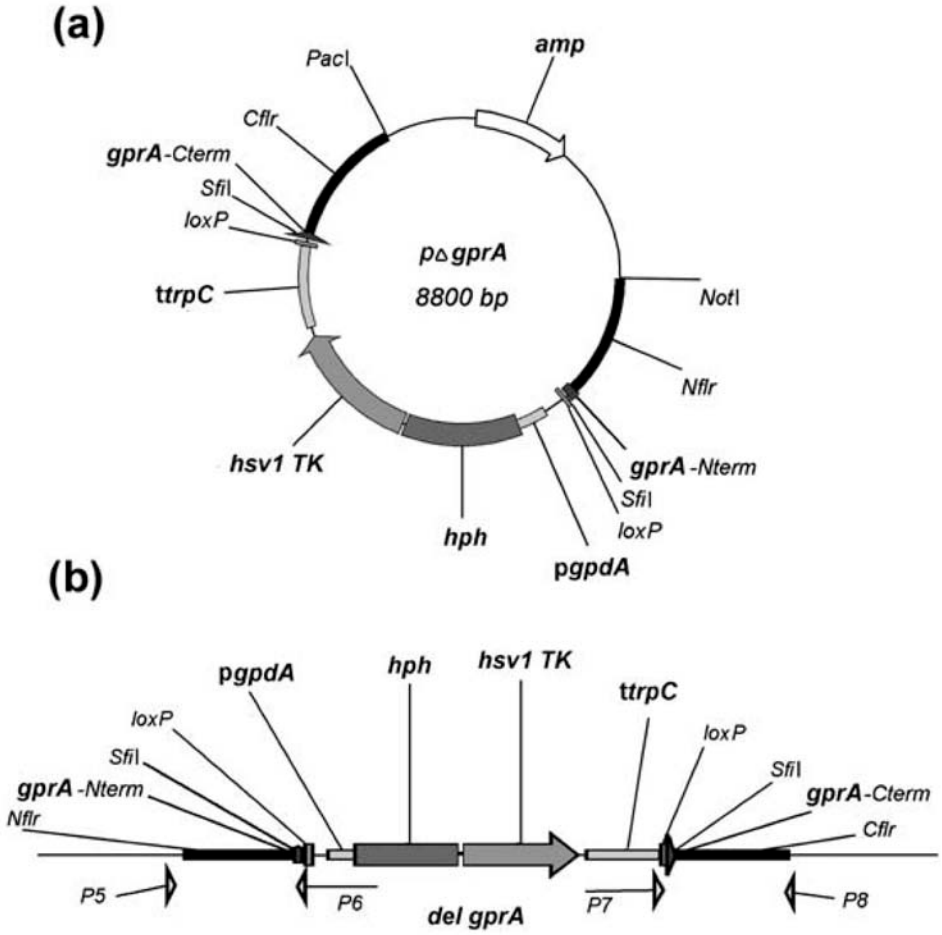



**Fig. 1. A: map of pΔ*gprA* used for *AfugprA* replacement. B: genomic DNA of generated *A. fumigatus gprA*Δ mutants.** The transforming DNA was excised from pΔ*gpr*A via double digestion with *Not*I and *Pac*I. Screening for homologous integration was done by PCR with p5/p6 and p7/8 primer pairs (see Methods). *amp*: ampicillin resistance gene; *gprA*-Nterm: 3'-extremity of *AfugprA*; *gprA*-Cterm: 5'-extremity of *AfugprA*; *hph*: *E. coli* hygromycin B phosphotransferase gene; *Nflr* and *Cflr*: 1.2 kb sized fragments covering the N-terminal and C-terminal flanking regions including small portions of *AfugprA* (< 100 bp); *hsv1 TK*: thymidine kinase-encoding sequence from the herpes simplex virus type 1; *loxP*: specific recombinase binding sites of plasmid pSK397 (18-19); p*gpdA*: truncated *A. nidulans gpd* promoter; t*rpC*: terminator region of the *Aspergillus nidulans trpC* gene.

## Table 1. Primers used in this study*

| Primer | Oligonucleotide sequence§ | Location | PCR product size with cloning sites |
|---|---|---|---|
| P1 | 5'-TATAT***GCGGCCGC***CCATATTCGTCTGGCTCACC-3' | Upstream sequence in *AfugprA* | 1192 bp (*Nflr* fragment) |
| P2 | 5'-ATATTA***CCCGGG<u>GGCCTGAGTGGCC</u>***CTTGACGCTGTTCTGTGAGG-3' | Complement of *AfugprA, N-terminal region* | *Not*I----***<u>Sfi</u>*****<u>I</u>**-*Xma*I |
| P3 | 5'-ATATTA***AAGCTT<u>GGCCATCTAGGCC</u>***AGCGGCAAGGTTTTGACTT-3' | Downstream sequence in *AfugprA* | 1219 bp (*Cflr* fragment) |
| P4 | 5'-TATCG***TTAATTAA***ATGAACCGCCTTCAGAGCTA-3' | Complement of *AfugprA, C-terminal region* | *Hin*DIII-***<u>Sfi</u>*****<u>I</u>**----*Pac*I |
| P5 | 5'-GGGCTGAAGGAAGAATACCC-3' | Upstream sequence in *AfugprA* | 1343 bp. |
| P6 | 5'-AGGTGATATCGGCCTGAGTG-3' | pSK397 | |
| P7 | 5'-CGTTTACCCAGAATGCACAG-3' | Downstram sequence in *AfugprA* | 1350 bp. |
| P8 | 5'-GCACAGTGATATCGGACGAC-3' | pSK397 | |
| P9 | 5'-GTT***CTCGAG***CCATCGCTGCTCCCCTCACA-3' | *AfugprA* | 782 bp. |
| P10 | 5'-GTT***GGATCC***TTAGACATACTTAACAGTGAC-3' | Complement of *AfugprA* | *Xho*I----*Bam*HI |

* Primers P1 to P4 were used to construct *Nflr* and *Cflr* fragments. Primers P5 to P8 were used for screening *A. fumigatus gprA*Δ mutants. For location of those primers in the *A. fumigatus gprA*Δ mutant compare Fig.1. Primers P9 and P10 were used for *AfugprA* gene expression in *P. pastoris*.

§ Italicized and boldfaced nucleotides represent cloning sites.

*Aspergillus fumigatus gprA*Δ mutants were identified by PCR of genomic DNA from various numbers of the hygromycin-resistant colonies as previously performed for selection of other protease-and transporter-deficient mutants (Reichard *et al*., 2006; Léchenne *et al*., 2007).

Two pairs of specific Δ*gprA* primers (P5/P6 and P7/P8, Table 1) were used to verify correct *gprA* replacement. In each primer pair, one primer hybridized with a part of the former transformation plasmid sequence and the other primer hybridized with genomic DNA near the desired homologous integration locus (Fig. 1). The predicted PCR product sizes were 1343 and 1350 bp with P5/P6 and P7/P8 primer pairs, respectively, if the transforming DNA was integrated at the homologous site. Two *A. fumigatus* D141 *gprA*Δ and two D141 *pep*Δ *gprA*Δ mutant strains were identified among 20 and 30 hygromycin-resistant colonies, respectively, which were tested for gene disruption.

## Recombinant protease production.

To construct *P. pastoris* strains producing GprA, DNA encoding N-terminal and C-terminal portions of the protein was amplified by PCR with a standard protocol using homologous sense and antisense primers (P9 and P10, Table 1) and 200 ng of *A. fumigatus* genomic DNA as no intron was predicted in the gene encoding AfuGprA (*AfugprA*). The PCR product was digested with *Xho*I and *Bgl*II for which a site was previously designed at the 5' end of the primers and inserted into pKJ113 (Borg-von Zepelin *et al*., 1998) digested with *Xho*I/*Bam*HI to generate the expression plasmids pAfu*gpr*A. *Pichia pastoris* GS115 transformation with *Eco*RI linearized plasmid DNA, selection of transformants, and production of recombinant AfuGprA in methanol medium, were performed as previously described (Borg-von Zepelin *et al.*, 1998).

## Purification of heterologously produced AfuGprA.

AfuGprA was purified at 4°C as following: secreted proteins from 250 ml of *P. pastoris* culture supernatant were concentrated by ultrafiltration to 6 ml using a Centricon Plus-70 (10 kDa cut-off) (Millipore). Thereafter, the concentrate was desalted with a PD10 column (Amersham Pharmacia) and applied to a DEAE-Sepharose column which had previously been equilibrated with a 100 mM Tris HCl buffer (pH 9.5). After washing the column with the same buffer, the recombinant

protein was eluted with a 100 mM Tris HCl buffer (pH 8). Enzymatic activity was tested with resorufin-labelled casein as a substrate (see below) and active fractions were pooled.

## Protein gel electrophoresis.

Extracts were analysed by SDS-PAGE with a separation gel of 12% polyacrylamide (Laemmli, 1970). The gels were stained either with 0.1% Coomassie Brilliant blue R-250 (Bio-Rad) in ethanol-acetic acid-water (40:10:50) or with silver nitrate according to Chevallet *et al*.(2006).

## Protein identification by liquid chromatography (LC)-MS/MS.

Identification and confirmation of heterologously expressed proteins were obtained by a LC-MS/MS analysis approach. Recombinant enzyme at pH 6.0 or after 60 min incubation at room temperature in formic acid buffer (pH 4.0) was subjected to regular sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (12%). Coomassie blue stained bands were excised from the SDS-PAGE and in-gel digested with sequencing-grade chymotrypsin (Promega) as previously described (Shevchenko et al., 1996; Wilm et al., 1996). Extracted peptides were analyzed on a hybrid linear trap LTQ-Orbitrap mass spectrometer (Thermo Fisher) interfaced *via* a TriVersa Nanomate (Advion Biosciences) to an Agilent 1100 nano HPLC system (Agilent Technologies). Solvents used for the mobile phase were 95:5 H2O:acetonitrile (v/v) with 0.1 % (v/v) formic acid (**A**) and 5:95 H2O:acetonitrile (v/v) with 0.1 % (v/v) formic acid (**B**). Peptides were loaded onto a trapping microcolumn ZORBAX 300SB C18 (5 mm x 300 µm ID, 5 µm, Agilent) in H2O: acetonitrile 97:3 (v/v) + 0.1 % (v/v) formic acid at a flow rate of 10 µl $min^{-1}$. After 5 min, they were back-flush eluted and separated on a reversed-phase nanocolumn ZORBAX 300SB C18 column (75 µm ID x 15 cm, 3.5 µm, Agilent) at a flow rate of 300 nl/min with a gradient from 5 to 85 % acetonitrile in 0.1% formic acid: 5 min at 0% of solvent **B**, from 0 to 25 % of **B** in 35 min, 25 to 50 % **B** in 15 min, 50 to 90 % in 5 min, 90 % **B** during 10 min, 90 to 0 % in 5 min and 15 min at 0 % (total time: 90 min).

For spraying, a 400 nozzle ESI Chip (Advion Biosciences) was used with a voltage of 1.65 kV, and the mass spectrometer capillary transfer temperature was set at 200°C. In data dependent acquisition controlled by Xcalibur 2.0.7 software

(ThermoFisher), the six most intense precursor ions detected in the full MS survey performed in the Orbitrap (range 350-1500 *m/z*, resolution 60000 at *m/z* 400) were selected and fragmented. MS/MS was triggered by a minimum signal threshold of 10000 counts, carried out at relative collision energy of 35% and with isolation width of 4.0 amu. Only precursors with a charge higher than one were selected for CID fragmentation and fragment ions were analyzed in the LTQ linear trap. The *m/z* of fragmented precursors was then dynamically excluded, with a tolerance of 0.01 amu, from any selection during 120 s.

From *raw* files, MS/MS spectra were exported as *mgf* (Mascot Generic File, text format) files using the extract_msn.exe script (Thermo Fisher) with the following settings: peptide mass range: 500-5000, minimum total ion intensity threshold: 500, minimum number of fragment ions: 15, minimum signal-to- noise ratio needed for a peak to be written: 3.

Samples were analyzed using Mascot 2.2 (Matrix Science, London, UK). Mascot was set up to search a custom-built database containing the sequences of the glutamic protease AfuGprA and of contaminants (enzymes, keratins). Semi-specific cleavage at F, L, W and Y (not before P) were used as the enzyme definition with a maximum of two missed cleavages allowed. Mascot was searched with a fragment ion mass tolerance of 0.50 Da and a parent ion tolerance of 10 ppm. The iodoacetamide derivative of cysteine was specified in Mascot as a fixed modification. Deamidation of asparagine and glutamine, oxidation of methionine and protein N-acetylation were specified as variable modifications.

## Proteolytic activities.

Endoproteolytic activity was measured with 0.02% (w/v) resorufin-labeled casein as a substrate (Roche Diagnostics) at different pHs in sodium citrate buffer (50 mM final concentration; pH 2.0 to 7.0) in a total volume of 0.5 ml (Borg-von Zepelin *et al*., 1998; Reichard *et al*., 2006). After incubation at 37°C, the undigested substrate of the enzyme-substrate mix was precipitated by trichloroacetic acid (4% [v/v] final concentration) and separated from the supernatant by centrifugation. Five hundred µl Tris-HCl buffer (500 mM; pH 9.4) were added to the collected supernatant (neutralization step) and the $A_{574}$ of the mixture (1 ml) was measured. For practical purposes, one mU of activity was defined as producing an increase in absorbance of

0.001 per min in a proteolytic assay (1 ml) at optimal pH for activity. Activities were measured in culture supernatant without and with the presence of the aspartic protease inhibitor pepstatin at a 10 µg $ml^{-1}$ concentration.

Tripeptidyl-peptidase and AfuS28 activities were measured as previously described using Phe-Pro-Ala-pNA and Ala-Ala-Ala-pNA as substrates (Bachem), respectively (Reichard *et al*., 1997; Sriranganadane *et al*., 2010). The reaction mixture contained 80 µl culture supernatant, a concentration of 5 mM substrate, 50 mM citrate buffer (pH 4) in total volume of 100 µl. After incubation at 37°C for 0.5 to 4 h, the reaction was terminated by adding 5 µl of glacial acetic acid. The released pNA was measured by spectrometry as a change in $A_{405}$. A control with a blank substrate and blank culture broth was carried out in parallel. Enzyme activities were expressed in mU (nmoles of released pNA $min^{-1}$).

## Phylogenetic analyses.

Amino acid sequences of a selection of putative glutamic proteases from *Aspergillus* spp., and previously characterized proteases from the Ascomycetes *Talaromyces emersonii*, *Botryotinia fuckeliana*, *Sclerotinia sclerotium*, *Scytalidium lignicolum*, and *Cryphonectria parasitica* (MEROPS G01 family) were aligned using ClustalW as implemented in BioEdit Sequence Alignment Editor software (Hall, 1999). Phylogenetic analyses were performed using the following reconstruction methods and parameters: PhyML (Guindon & Gascuel, 2003) with SH-like approximate likelihood ratio test, 4 substitution rate categories and estimation of gamma distribution parameter and proportion of invariable sites; BIONJ (Gascuel, 1997), using a Dayhoff PAM substitution matrix; TNT (Goloboff *et al*., 2008) with sectorial search and tree fusing options; MrBayes (Ronquist & Huelsenbeck, 2003) with GTR likelihood model and 100.000 Markov Chain Monte Carlo generations. Phylogenetic trees were edited using Dendroscope (Huson *et al*., 2007).

# RESULTS

## Importance of AfuGprA in extracellular protein digestion.

In order to evaluate the importance of AfuGprA in protein digestion at acidic pH, *AfugprA* was replaced in *A. fumigatus* wild type D141 and *pep*Δ mutant as described in Materials and Methods. *Aspergillus fumigatus* wild type D141, *pep*Δ, *gprA*Δ and *pep*Δ *gprA*Δ mutants were first tested for their ability to grow using protein solid and liquid media at neutral and acidic pH (Fig. 2). Two different protein sources, BSA and soy protein which is a heterogeneous peptide mixture (Fig. 3, panel C), were used.

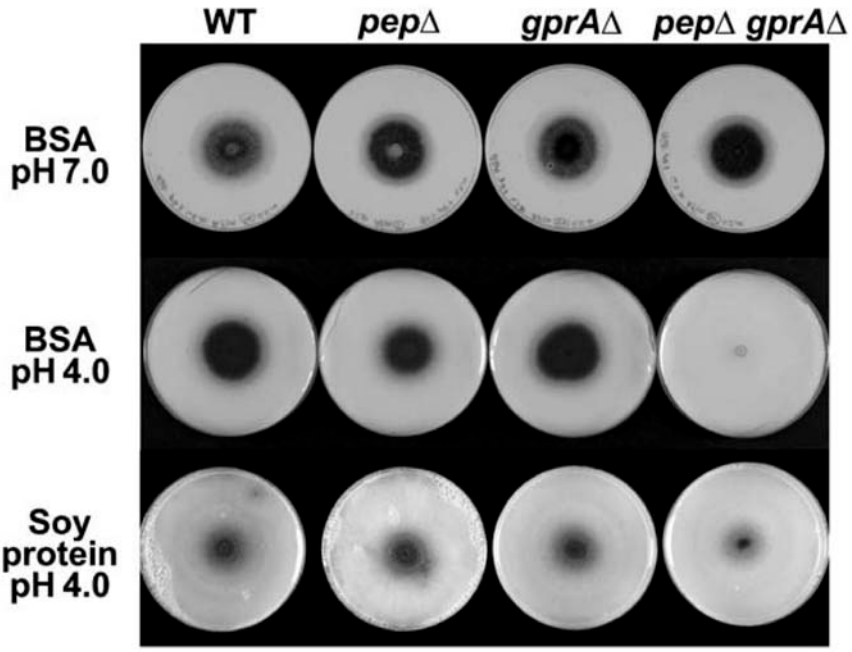



**Fig. 2. *Aspergillus fumigatus* wild type D141 (WT), and *pep*Δ, *gprA*Δ and *pep*Δ *gprA*Δ mutants on BSA and soy protein agar medium.**

No phenotypic difference was observed between wild type and mutant strains at neutral pH. At acidic pH, *A. fumigatus gprA*Δ could not be differentiated from wild type D141 strain using both protein sources. In contrast, *A. fumigatus pep*Δ *gprA*Δ was unable to grow on BSA agar medium at acidic pH whereas *A. fumigatus pep*Δ showed only a slightly reduced size of the colony (Fig. 2). The four *A. fumigatus* strains showed comparable growth rate on soy protein agar medium at acidic pH (Fig. 2).

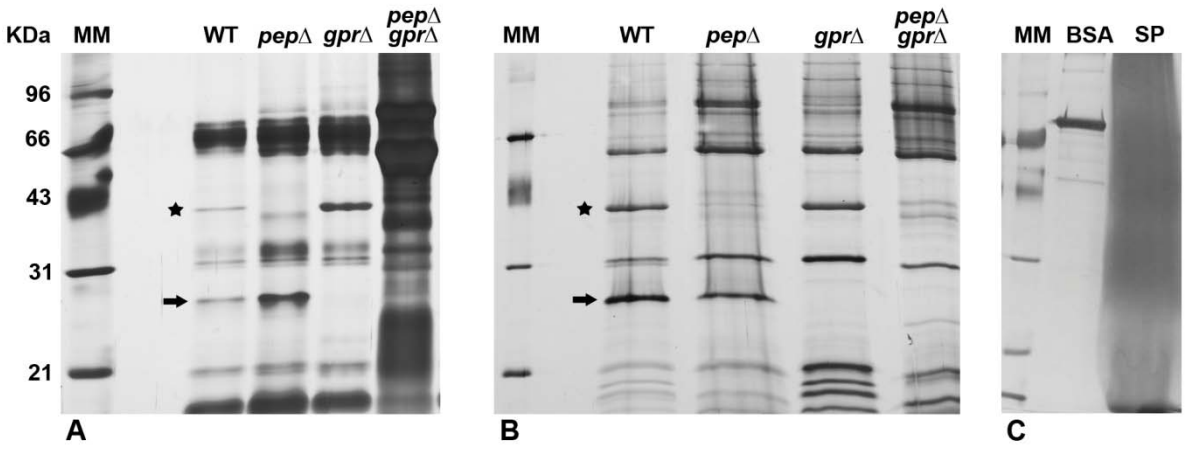



**Fig. 3. A and B: Pep and GprA identification in culture supernatant of *A. fumigatus* wild type D141(WT) and *pep*Δ, *gprA*Δ and *pep*Δ *gprA*Δ mutant strains growing in BSA (A) and soy protein (B) acidic liquid medium (pH 4.0).** Two hundred ml flasks containing 50 ml of medium were inoculated with approximately $10^7$ spores and incubated for 96 h at 30°C on an orbital shaker at 200 rpm. Supernatant extracts were concentrated by ultrafiltration and acidic precipitation as previously described (Sriranganadane, 2010). The precipitated molecules of 2.0 ml culture supernatant was resuspended in 10µl SDS-PAGE buffer, and subsequently loaded on SDS-12% polyacrylamide gels. C: SDS-12% polyacrylamide gels with 1 µl of non digested BSA and soy protein liquid medium (1 µg protein). The gels were stained with silver nitrate according to Chevallet *et al*. (2006).

Similar results were obtained with liquid cultures at acidic pH. (i) *Aspergillus fumigatus* wild type D141, *pep*Δ and *gprA*Δ mutants grew well in BSA and soy protein liquid medium. (ii) Only a residual growth of *A. fumigatus pep*Δ *gprA*Δ without clarification of the medium was observed with BSA liquid medium, while the fungus grew well with soy protein (data not shown). After 96 h of growth in the latter medium, the secreted activity of the *gprA*Δ and *pep*Δ mutants was approximately 70% and 20% of that of *A. fumigatus* wild type D141, respectively (Fig. 4). In presence of the aspartic protease inhibitor pepstatin the activity of *gprA*Δ mutant was totally inhibited and the activity of wild type strain was reduced to the rate of that of the *pep*Δ mutant. *Aspergillus fumigatus pep*Δ *gprA*Δ soy protein culture supernatant was completely devoid of extracellular endoproteolytic activity. Comparable tripeptidyl-peptidase and AfuS28 activities were found to be secreted at acidic pH by *A. fumigatus* wild type D141, *pep*Δ, *gprA*Δ and *pep*Δ *gprA*Δ mutants in soy protein medium, as well as by *A. fumigatus* D141, *pep*Δ, and *gprA*Δ in BSA medium (7.6 ± 1.0 and 0.03 ± 0.01 mU,

respectively). The production of these exoproteases was apparently not changed by deleting *pep* and *gprA*.

## Characterization of AfuGprA.

SDS-PAGE of culture supernatants of *A. fumigatus* wild type D141, *pep*Δ, *gprA*Δ and *pep*Δ *gprA*Δ are shown in Figure 3. The absence of a 36 kDa band in *A. fumigatus pep*Δ and a 26 kDa band in *A. fumigatus gprA*Δ mutants identified Pep and GprA, respectively. AfuGprA was characterized as a recombinant enzyme using *P. pastoris* as an expression system. A yield of 25 µg/ml of recombinant protein in culture supernatant was obtained. Recombinant AfuGprA in *P. pastoris* culture supernatant showed an apparent molecular mass of 30 kDa, which is higher than that of AfuGprA secreted by *A. fumigatus* at pH 4 (Fig. 5, line 1). However, after pre-incubation at pH 4.0, recombinant AfuGprA showed the same molecular mass as native AfuGprA (compare Fig. 5, line 2, and Fig. 3). These results suggest that AfuGprA, like other G1 proteases, is made as a preproprotein and that the propeptide was cleaved and removed to generate the active enzyme through an autoproteolytic reaction.

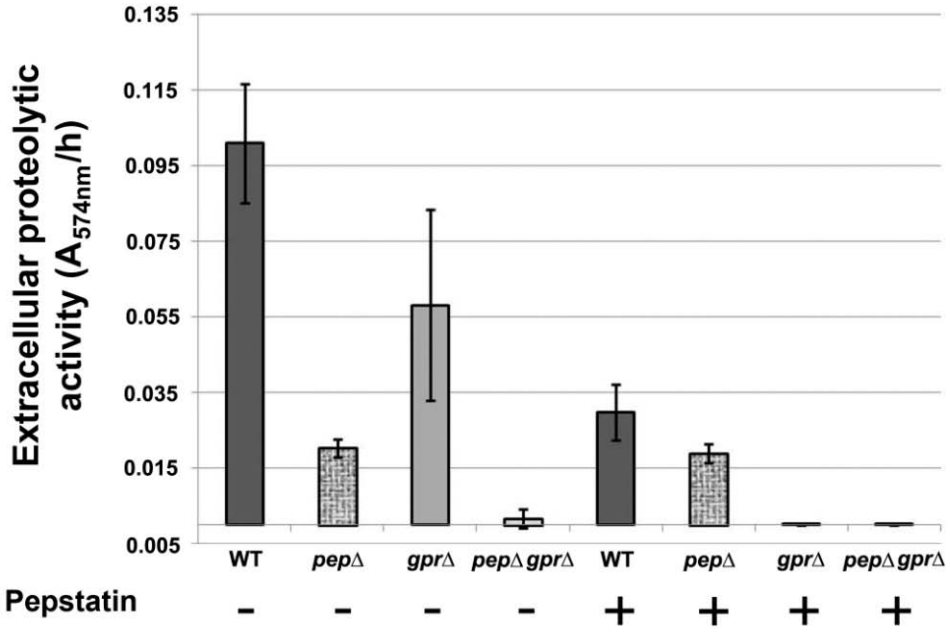



**Fig. 4. Secreted endoproteolytic activity of *A. fumigatus* wild type D141, *pep*Δ, *gprA*Δ and *pep*Δ *gprA*Δ mutants in soy protein medium (pH 4.0) after 96 h of growth at 30°C.** Activities from 50 µl culture supernatant were measured as described in Methods using resorufin labeled casein as a substrate in the absence and in the presence of pepstatin (10 µg

$ml^{-1}$). Means and standard deviations of values recorded in three independent experiments are indicated.

The deduced amino acid sequence of the *AfugprA* open reading frame shows a 16-18-amino acid hydrophobic signal peptide with a putative signal peptidase cleavage site Ala-Ile-Ala in accordance with Von Heijne's rule (Von Heijne, 1986) (Fig. S1 in the supplemental material). The AfuGprA proprotein generated after signal peptidase cleavage is made of 252 amino acids with a theoretical mass of 26.34 kDa. Therefore, the molecular mass of reduced proAfuGprA was overestimated in SDS-PAGE gels as it migrates as a 30 kDa protein. Of particular note, a similar observation was made with *Botrytis cinerea* secreted G1 protease (Rolland *et al*., 2009). Non-reduced proAfuGprA and AfuGprA showed a higher electrophoretic mobility than reduced proAfuGprA and AfuGprA, respectively, after a 2 min heating treatment in SDS-PAGE buffer (Fig. 5, lanes 3 and 4).

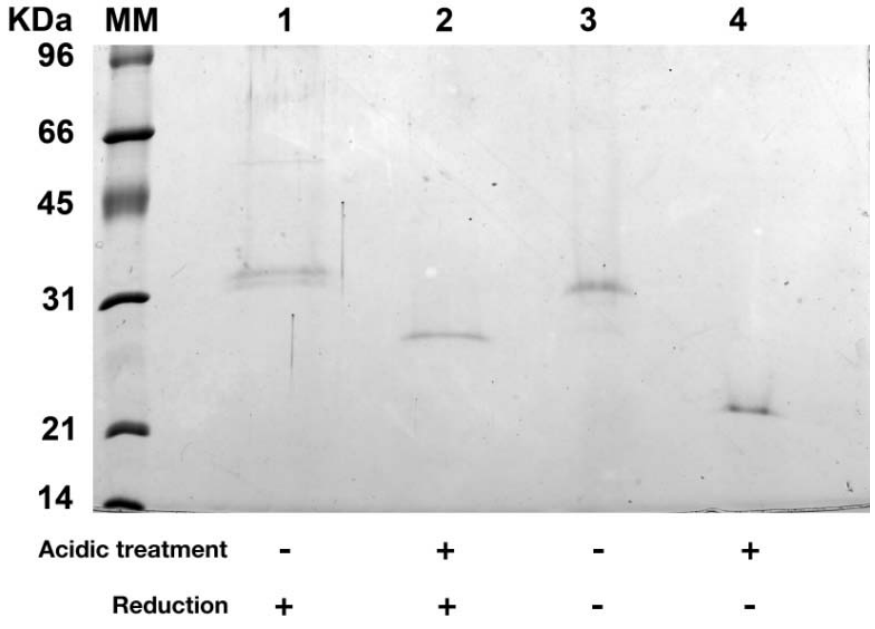



**Fig. 5. SDS-PAGE (9% gel) of recombinant AfuGprA secreted by *P. pastoris*. The gel was stained with Coomassie blue.** Approximately 0.3 µg of recombinant reduced (lanes 1 and 2) and non-reduced (lanes 3 and 4) AfuGprA was loaded on the gel. AfuGprA reduction was performed after heating treatment in SDS-PAGE buffer containing 2% (v/v) β-mercaptoethanol. In lanes 2 and 4, recombinant AfuGprA was incubated at pH 4.0 for 30 min at 30° C. The double band observed for native proGprA attests for two different signal peptidase cleavage sites (see Fig 6).

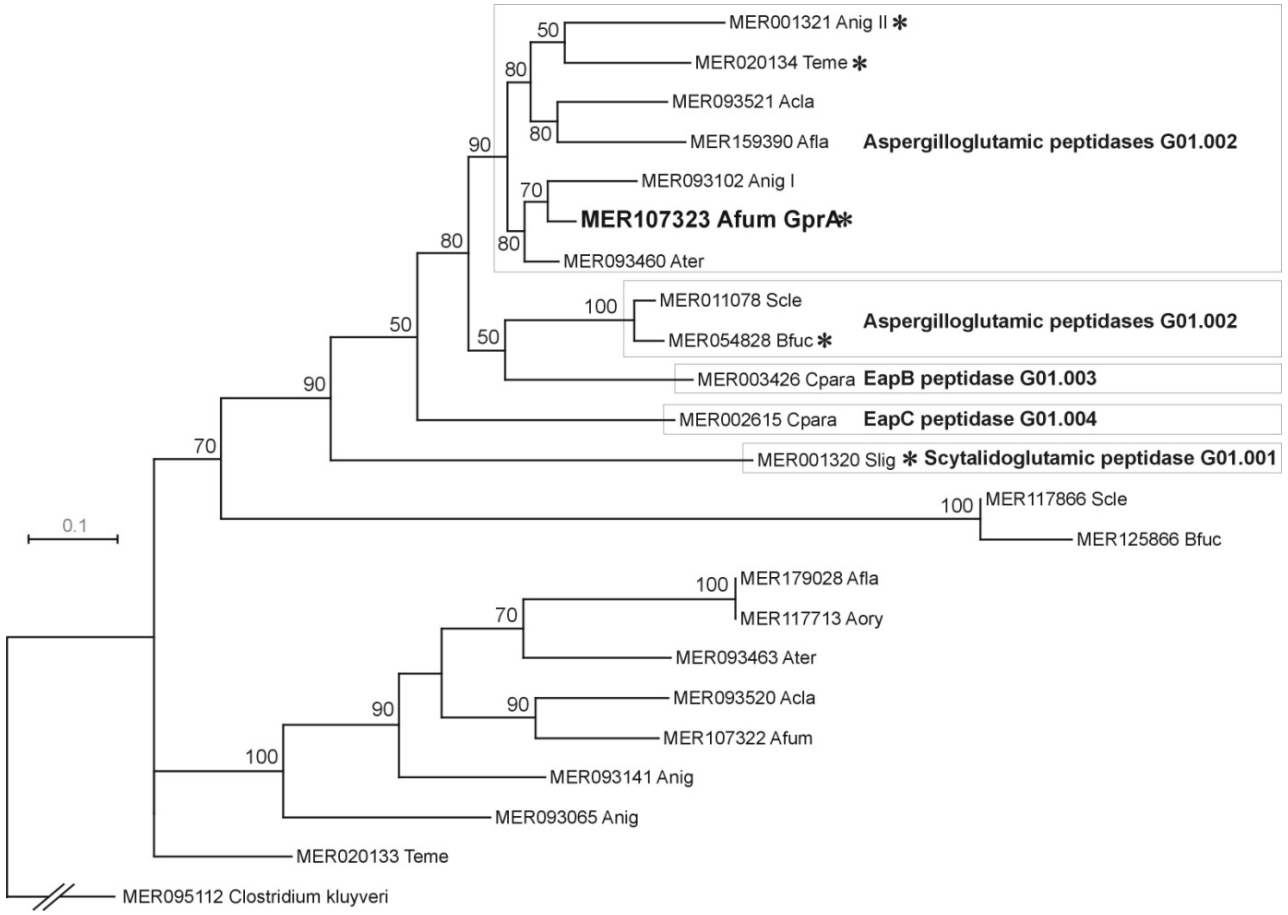
Identification of the heterologously produced AfuGprA was also confirmed using ESI-LC-MS/MS. Chymotryptic digestion was performed in gel slices containing

the 30 kDa protein secreted by *P. pastoris* at neutral pH, and the 26 kDa protein obtained after acidic treatment at pH 4.0 (Fig 5). MS analysis allowed the detection of different AfuGprA peptides matching with residues 42 to 269 in the 30 kDa secreted protein. Peptides matching with residues 61 to 269 but not with residues 1 to 61 were detected in the 26 kDa protein (Fig. 6). These results confirmed that active AfuGprA was generated after cleavage of a prosequence at acidic pH at residue 61 or few residues before as suggested from alignment with other glutamic proteases. Gln and Glu residues of the catalytic dyad of AfuGprA were identified in position 120 and 206, respectively (Fig. S1, in the supplemental material).


**Fig. 6. PreproAfuGprA amino acid sequence.** Detected MS peptides generated by chymotryptic digestion of proAfuGprA secreted by *P. pastoris* and mature AfuGprA after incubation at acidic pH are underlined and dotted, respectively. Vertical arrows indicate two potential signal peptidase cleavage sites in amino acid positions 16 and 18, and the cleavage site in the proprotein generating the mature enzyme. Active site residues Gln and Glu forming the catalytic diad are indicated by solid triangles.

Characterization assays of the recombinant AfuGprA were accomplished after purification by ion exchange chromatography. The enzyme was found to be active between pH 2.0 and 6.0 with an optimum activity at pH 5.0 on resorufin-labeled casein. The specific activity of AfuGprA was measured as 2.2 mU µg protein$^{-1}$ at pH 5.0. The enzyme was not inhibited by pepstatin (aspartic protease inhibitor), E64 and iodoacetamide (cystein protease inhibitor), EDTA and phosphoramidon (metalloprotease inhibitors), PMSF, antipain, chymostatin, leupeptin and aprotinin (serine protease inhibitors). Being resistant to class specific inhibitors of aspartic, cystein, metallo and serine proteases, AfuGprA behaves like other previously described glutamic proteases (Oda, 2004; Takahashi, 2004; O'Donoghue *et al*., 2008).

**Fig. 7. Phylogenetic position of *Aspergillus fumigatus* GprA with respect to other fungal glutamic proteases obtained by a maximum likelihood analysis using PhyML.** Asterisks indicate proteases that have been characterized biochemically. A glutamic protease from *Clostridium kluyveri* (Firmicutes, Bacteria) was used a potential outgroup. MEROPS identifiers and names of type proteases are indicated. Numbers at nodes are approximate Likelihood-Ratio Test (aLRT) values of branch support. Scale bar indicates the number of expected changes per site. Abbreviations are: Afum, *Aspergillus fumigatus*; Anig, *A. niger*; Acla, *A. clavatus*; Ater, *A. terreus*; Afla, *A. flavus*; Teme, *Talaromyces emersonii*; Scle, *Sclerotinia sclerotium*; Bfuc, *Botryotinia fuckelania*; Cpara, *Cryphonectria parasitica*; Slig, *Scytalidium lignicolum*.



# DISCUSSION

Two endoproteases, Pep and GprA, are secreted by *A. fumigatus* in an acidic protein medium. We have shown that either *A. fumigatus* Pep or GprA was necessary for fungal growth in BSA medium at acidic pH. AfuGprA deficiency alone does not affect *A. fumigatus* growth while a slight growth rate reduction of the single *pep*Δ mutant was detected. These results apparently contrast with those obtained with *T. emersonii* whose growth is strongly inhibited with glutamic peptidase antagonists (O'Donoghue *et al*., 2008). However, the tested G1 inhibitors could affect functions in the fungus other than G1 protease activity.

During the process of protein digestion the main function of the endoproteases is to produce large numbers of free end peptides on which exopeptidases may act. Synergism of non-specific amino peptidases (Lap at neutral pH, Seds at acidic pH) and prolyl peptidases (DppIV or AfuS28 at neutral pH, AfuS28 at acidic pH) allows sequential degradation of large peptides previously generated by endoproteolysis into amino acids, di- and tripeptides (Byun *et al*., 2001; Sriranganadane *et al*., 2010). Large peptide cleavage by further exoproteolytic activity is also essential for fungi using protein as sole nitrogen and carbon source since only amino acids and short peptides can be assimilated by transporters. When using soy protein source which is a heterogeneous peptide mixture, exoproteolytic activity alone was sufficient for fungal growth.

Two major proteases, aspergillopepsin A (Pep) and aspergillopepsin B (aspergillopepsin II), were found in the culture filtrate of *A. niger* (Koaze *et al*., 1964). An *A. niger* mutant deficient in the secretion of both proteases was generated by UV mutagenesis (Mattern *et al*., 1992). This strain in which a mutation is located in a gene encoding a regulator of extracellular protease genes called PrtT (Punt *et al*., 2008) has only 1-2% extracellular protease activity at pH 5.3. In contrast, *A. fumigatus pep*Δ *gprA*Δ produces substantial proteolytic activity and grows well in a protein medium at this pH value because of the secretion of Alp and Mep (data not shown). Both enzymes are still active at pH 5.3 (Monod et al., 1991; 1993b). The *A. fumigatus* genome contains an orthologue of *A. niger PRTT*, which was shown to control the expression of multiple secreted proteases such as Alp, Mep, Pep, SedB and DppIV (Sharon *et al*., 2009; Bergmann *et al*., 2009). However, functional PrtT

dependence of *A. fumigatus* secreted proteases was investigated at neutral pH only, and the role of PrtT in protein gene expression was not addressed at acidic pH. Virulence studies were not in the scope of the present investigations, but *A. fumigatus prtT* deletion mutants were shown to be not attenuated in virulence in immunosuppressed mice (Sharon *et al.*, 2009; Bergmann *et al.*, 2009).

Being a single chain protein, mature AfuGprA resembles other previously characterized fungal glutamic proteases from *T. emersonii*, *S. lignicolum* and *B. fuckeliana*. However, it differs from *A. niger* apergillopepsin II which is a two-chain protein composed of a 39-residue light chain and a 173-residue heavy chain. Both chains of apergillopepsin II are noncovalently bound and originate from a single precursor of 282 amino acids which contains a 14 amino acid intervening peptide (Fig. S1 in the supplemental material) that is absent in the AfuGprA preproprotein. The heterodimer results from autoproteolytic cleavage at the level of the intervening peptide as for heterodimer yapsins where an autocleavage occurs within an internal loop of the polypeptidic chain (Gagnon-Arsenault *et al*., 2006). In fact, none of the currently available sequences of fungal glutamic proteases possesses the intervening peptide found in *A. niger* aspergillopepsin II. This two-chain glutamic protease seems therefore to be an exception.

Sequence searches in GenBank and in the MEROPS database, sequence alignments, and phylogenetic analyses indicated that putative orthologues of AfuGprA occur in all *Aspergillus* species (Fig. 7). Two G1 proteases are found in the *A. fumigatus* genome, GprA (MER107323), and a second G1 protease (MER107322) which was not detected in the present study. AfuGprA belongs to a robust clade including all aspergilloglutamic peptidases (MEROPS identifier G01.002) from *Aspergillus* spp. and *T. emersonii*. AfuGprA is most closely related to MER093102 of *A. niger* (76% identity, 89% similarity), a putative protease. Characterized aspergilloglutamic peptidases from *S. sclerotinium* and *B. fuckeliana*, as well as glutamic proteases from *C. parasitica* and *S. lignicolum* (G01.001, G01.003 and G01.004) branch basally with respect to *Aspergillus* G01.002 peptidases. It can be noted that most fungal species possess at least two paralogous genes encoding glutamic proteases. Each species typically harbours an aspergilloglutamic-like protease belonging to the G01.001-G01.004 clades and a glutamic protease designated as “unassigned” in the MEROPS database and branching at the base of

the tree. None of these unassigned glutamic proteases have been characterized experimentally to date. The relatively high amino acid sequence divergence between the glutamic proteases found in a given genome (only 37% identity between *A. fumigatus* GprA and *A. fumigatus* unassigned protease MER107322) together with the scattered distribution of sequences in the phylogenetic tree suggest that these paralogues arose by ancient gene duplication.

In conclusion, we have shown that secreted glutamic protease rescues aspartic protease Pep deficiency in *A. fumigatus* and that exoproteolytic activity is not sufficient for complete protein hydrolysis and fungal growth in an acidic protein medium. Pep and GprA constitute a pair of acidic endoproteases analogous to the *A. fumigatus* Alp/Mep pair (Jaton-Ogay *et al*., 1994) at neutral pH, in which expression of either one of them was shown to be sufficient for fungal growth in a protein medium.

**ACKNOWLEDGEMENTS**

We thank Sven Krappmann for providing pSK397 plasmid, Massimo Lurati for critical review of the manuscript and assistance with the English, Olympia Bontems for helpful discussion and technical assistance. This work was supported by the Swiss National Foundation for Scientific Research, grant 320030-1179641.

# REFERENCES

**Beauvais, A., Monod M., Debeaupuis, J. P., Diaquin, M., Kobayashi, H. & Latgé, J. P. (1997a).** Biochemical and antigenic characterization of a new dipeptidyl-peptidase isolated from *Aspergillus fumigatus*. *J Biol Chem* **272,** 6238-6244.

**Beauvais, A., Monod, M., Wyniger, J., Debeaupuis, J. P., Grouzmann, E., Brakch, N., Svab, J., Hovanessian, A. G. & Latgé, J. P. (1997b).** Dipeptidyl-peptidase IV secreted by *Aspergillus fumigatus*, a fungus pathogenic to humans. *Infect Immun* **65,** 3042-3047.

**Beffa, T., Staib, F., Lott Fischer, J., Lyon, P. F., Gumowski, P., Marfenina, O. E., Dunoyer-Geindre, S., Georgen, F., Roch-Susuki, R., Gallaz, L. & Latgé, J. P. (1998).** Mycological control and surveillance of biological waste and compost. *Med Mycol* **36 Suppl 1,** 137-145.

**Bergmann, A., Hartmann, T., Cairns, T., Bignell, E. M. & Krappmann, S. (2009).** A regulator of Aspergillus fumigatus extracellular proteolytic activity is dispensable for virulence. *Infect Immun* **77,** 4041-4050.

**Borg-von Zepelin, M., Beggah, S., Boggian, K., Sanglard, D. & Monod, M. (1998).** The expression of the secreted aspartyl proteinases Sap4 to Sap6 from *Candida albicans* in murine macrophages. *Mol Microbiol* **28,** 543-554.

**Byun, T., Kofod, L. & Blinkovsky, A. (2001).** Synergistic action of an X-prolyl dipeptidyl aminopeptidase and a non-specific aminopeptidase in protein hydrolysis. *J Agric Food Chem* **49,** 2061-2063.

**Chevallet, M., Luche, S. & Rabilloud, T. (2006).** Silver staining of proteins in polyacrylamide gels. *Nat Protoc* **1,** 1852-1858.

**Fujinaga, M., Cherney, M. M., Oyama, H., Oda, K. & James, M. N. (2004).** The molecular structure and catalytic mechanism of a novel carboxyl peptidase from *Scytalidium lignicolum*. *Proc Natl Acad Sci USA* **101,** 3364-3369.

**Gagnon-Arsenault, I., Tremblay, J. & Bourbonnais, Y. (2006).** Fungal yapsins and cell wall: a unique family of aspartic peptidases for a distinctive cellular function. *FEMS Yeast Res* **6,** 966-978.

**Gascuel, O. (1997).** BIONJ: an improved version of the NJ algorithm based on a simple model of sequence data. *Mol Biol Evol* **14,** 685-695.

**Goloboff, P., Farris, J. & Nixon, K. (2008).** TNT: a free program for phylogenetic analysis. *Cladistics* **24,** 774-786.

**Guindon, S. & Gascuel, O. (2003).** A simple, fast and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst Biol* **52,** 696-704.

**Hall, T. A. (1999).** BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* **41,** 95-98.

**Huson, D. H., Richter, D. C., Rausch, C., Dezulian, T., Franz, M. & Rupp, R. (2007).** Dendroscope: An interactive viewer for large phylogenetic trees. *BMC Bioinformatics* **8,** 460.

**Jaton-Ogay, K., Paris, S., Huerre, M., Quadroni, M., Falchetto, R., Togni, G., P. Latgé, J. & Monod, M. (1994).** Cloning and disruption of the gene encoding an extracellular metalloprotease of *Aspergillus fumigatus*. *Mol Microbiol* **14,** 917-928.

**Kämper, J. (2004).** A PCR-based system for highly efficient generation of gene replacement mutants in *Ustilago maydis*. *Mol Genet Genomics* **271,** 103-110.

**Kataoka,Y., Takada, K., Oyama, H., Tsunemi, M., James, M. N. & Oda, K. (2005).** Catalytic residues and substrate specificity of scytalidoglutamic peptidase, the first member of the eqolisin in family (G1) of peptidases. *FEBS Lett* **579,** 2991-2994.

**Koaze, Y., Goi, H., Ezawa, K., Yamada, Y. & Hara, T. (1964).** Fungal proteolytic enzymes. PartI. Isolation of two kinds of acid proteases excreted by *Aspergillus niger* var *macrosporus*. *Agric Biol Chem* **28,** 216-223.

**Krappmann, S., Bayram, O. & Braus, G. H. (2005).** Deletion and allelic exchange of the *Aspergillus fumigatus* veA locus via a novel recyclable marker module. *Eukaryot Cell* **4,** 1298-1307.

**Krappmann, S., Sasse, C. & Braus, G. H. (2006).** Gene targeting in *Aspergillus fumigatus* by homologous recombination is facilitated in a nonhomologous end-joining-deficient genetic background. *Eukaryot Cell* **5,** 212-215.

**Laemmli, U. K. (1970).** Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227,** 680-685.

**Latgé, J. P. (1999).** *Aspergillus fumigatus* and aspergillosis. *Clin Microbiol Rev* **12**, 310-350.

**Léchenne, B., U. Reichard, C. Zaugg, M. Fratti, J. Kunert, O. Boulat, and Monod, M. (2007).** Sulphite efflux pumps in *Aspergillus fumigatus* and dermatophytes. *Microbiology* 153:905-913.

**Mattern, I. E., van Noort, J. M., van den Berg, P., Archer, D. B., Roberts, I. N. & van den Hondel, C. A. (1992).** Isolation and characterization of mutants of *Aspergillus niger* deficient in extracellular proteases. *Mol Gen Genet* **234,** 332-336.

**Monod, M., Togni, G., Rahalison, L. & Frenk, E. (1991)**. Isolation and characterisation of an extracellular alkaline protease of *Aspergillus fumigatus*. *J Med Microbiol* **35,** 23-28.

**Monod, M., Paris, S., Sarfati, J., Jaton-Ogay, K., Ave, P. & Latgé, J. P. (1993a).** Virulence of alkaline protease-deficient mutants of *Aspergillus fumigatus*. *FEMS Microbiol Lett* **106,** 39-46.

**Monod, M., Paris, S., Sanglard, D., Jaton-Ogay, K., Bille, J. & Latgé, J. P. (1993b).** Isolation and characterization of a secreted metalloprotease of *Aspergillus fumigatus*. *Infect Immun* **61,** 4099-4104.

**Monod, M., Léchenne, B., Jousson, O., Grand, D., Zaugg, C., Stocklin, R. & Grouzmann, E. (2005).** Aminopeptidases and dipeptidyl-peptidases secreted by the dermatophyte *Trichophyton rubrum*. *Microbiology* **151,** 145-155.

**Monod, M., Jousson, O. & Reichard, U. (2009).** *Aspergillus fumigatus* secreted proteases. In *Aspergillus fumigatus and Aspergillosis*, pp 87-106. Edited by J.-P. Latgé & W. J. Steinbach. Washington, D.C: ASM Press.

**Oda, K. (2004).** Scytalidopepsin B. In *Handbook of Proteolytic Enzymes*, 2nd ed., pp. 219-221. Edited by A. J. Barrett, N. D. Rawlings & J. F. Woessner. London, United Kingdom: Elsevier.

**O'Donoghue, A. J., Mahon, C. S., Goetz, D. H., O'Malley, J. M., Gallagher, D. M., Zhou, M., Murray, P. G., Craik, C. S. & Tuohy, M. G. (2008).** Inhibition of a secreted glutamic peptidase prevents growth of the fungus *Talaromyces emersonii*. *J Biol Chem* **283,** 2986-2995.

**Punt, P. J., Schuren, F. H., Lehmbeck, J., Christensen, T., Hjort, C. & van den Hondel, C. A. (2008).** Characterization of the *Aspergillus niger* prtT, a unique regulator of extracellular protease encoding genes. *Fungal Genet Biol* **45,** 1591-1599.

**Reichard, U., Büttner, S., Eiffert, H., Staib, F. & Rüchel, R. (1990).** Purification and characterisation of an extracellular serine proteinase from *Aspergillus fumigatus* and its detection in tissue. *J Med Microbiol* **33,** 243-251.

**Reichard, U., Monod, M. & Rüchel, R. (1995).** Molecular cloning and sequencing of the gene encoding an extracellular aspartic proteinase from *Aspergillus fumigatus*. *FEMS Microbiol Lett* **130,** 69-74.

**Reichard, U., Monod, M., Odds, F. & Rüchel, R. (1997).** Virulence of an aspergillopepsin-deficient mutant of *Aspergillus fumigatus* and evidence for another aspartic proteinase linked to the fungal cell wall. *J Med Vet Mycol* **35,** 189-195.

**Reichard, U., Léchenne, B., Asif, A. R., Streit, F., Grouzmann, E., Jousson, O. & Monod, M. (2006).** Sedolisins, as new class of secreted proteases from *Aspergillus fumigatus* with endoprotease or tripeptidyl-peptidase activity at acidic pH. *Appl Environ Microbiol* **72,** 1739-1748.

**Rolland S., Bruel, C., Rascle, C., Girard, V., Billon-Grand, G. & Poussereau, N. (2009).** pH controls both transcription and post-translational processing of the protease BcACP1 in the phytopathogenic fungus *Botrytis cinerea*. *Microbiology*. **155,** 2097-2105.

**Ronquist, F. & Huelsenbeck, J. P. (2003).** MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19,** 1572-1574.

**Sasaki, H., Tanokura, M., Muramatsu, T., Nakagawa, A., Iwata, S., Hamaya, T., Takizawa, T., Kono, T. & Takahashi, K. (1995).** X-ray crystallographic study of a non-pepsin-type acid proteinase, *Aspergillus niger* proteinase A. *Adv Exp Med Biol* **362**, 605-609.

**Sharon, H., Hagag, S. & Osherov, N. (2009).** Transcription factor PrtT controls expression of multiple secreted proteases in the human pathogenic mold Aspergillus fumigatus. *Infect Immun* **77,** 4051-4060.

**Shevchenko, A., Wilm, M., Vorm, O. & Mann, M. (1996).** Mass spectrometric sequencing of proteins silver-stained polyacrylamide gels. *Anal Chem* **68,** 850-858.

**Sriranganadane, D., Waridel, P., Salamin, K., Reichard, U., Grouzmann, E., Neuhaus, J. M., Quadroni, M. & Monod, M. (2010).** *Aspergillus* protein degradation pathways with different secreted protease sets at neutral and acidic pH. *J Proteome Res* **9,** 3511-3519.

**Takahashi, K. (2004).** Apergillopepsin II**,** In *Handbook of Proteolytic Enzymes*, 2nd ed., pp. 221-224. Edited by A. J. Barrett, N. D. Rawlings & J. F. Woessner. London, United Kingdom: Elsevier.

**Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994).** CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22,** 4673-4680.

**Tilburn, J., Scazzocchio, C., Taylor, G. G., Zabicky-Zissman, J. H., Lockington,**

**R. A. & Davies, R. W. (1983).** Transformation by integration in *Aspergillus nidulans*. *Gene* **26,** 205-221.

**Von Heijne, G. (1986).** A new method for predicting signal sequence cleavage sites. *Nucleic Acids Res* **14**, 4683-4690.

**Wilm, M., Shevchenko, A., Houthaeve, T., Breit, S., Schweigerer, L., Fotsis, T. & Mann, M. (1996).** Femtomole sequencing of proteins from polyacrylamide gels by nano-electrospray mass spectrometry. *Nature* **379,** 466-469.

**Yabuki, Y., Kubota, K., Kojima, M., Inoue, H. & Takahashi, K. (2004).** Identification of a glutamine residue essential for catalytic activity of aspergilloglutamic peptidase by site-directed mutagenesis. *FEBS Lett* **569,** 161-164.

# Project 3

## Identification of novel secreted proteases during extracellular proteolysis by dermatophytes at acidic pH

Dev Sriranganadane, Patrice Waridel, Karine Salamin, Marc Feuermann, Bernard Mignon, Peter Staib, Jean-Marc Neuhaus, Manfredo Quadroni and Michel Monod. Submitted on May 2011 on Proteomics.

Key words: *Dermatophytes,* Microsporum canis*,* Arthroderma benhamiae*, secreted proteases, shotgun mass spectrometry.*

# INTRODUCTION

Dermatophytes are the most common agents of human and animal cutaneous mycoses [1]. Since these highly specialized pathogenic fungi are almost exclusively localized in keratinized tissues, research on their virulence factors primarily focused on secreted proteolytic activity. A particularity of dermatophytes is their ability to increase the pH of a growth medium in vitro through the secretion of alkaline metabolites [2-3]. It is thus not surprising that the vast majority of the secreted proteases isolated from these fungi are neutral or alkaline enzymes. In a medium containing protein as the sole nitrogen source dermatophytes were found to secrete multiple subtilisins (Sub, serine proteases of the S8 family) and fungalysins (Mep, metalloproteases of the M36 family) as endopeptidases [4-7]. So far characterized dermatophyte exopeptidases are leucine aminopeptidases (Lap1 and Lap2), dipeptidyl-peptidases (DppIV and DppV) and a major metallocarboxypeptidase homologous to the human pancreatic carboxypeptidase A [8-10]. The protein sequence of each of these proteases is highly conserved across dermatophyte species, but its level of secretion varies from one species to another [6].

The genomes of seven dermatophytes (*Arthroderma benhamiae*, *Trichophyton verrucosum*, *Trichophyton rubrum*, *Microsporum canis*, *Microsporum gypseum*, *Trichophyton tonsurans* and *Trichophyton equinum*) have now been fully sequenced (http://www.broadinstitute.org/annotation/genome/dermatophyte_comparative/MultiHome.html) [11]. More than 200 proteases were recorded in these species among which 72 were found to possess a signal sequence. Several of these proteases which remain so far hypothetical are homologous to acidic proteases (aspartic proteases and sedolisins) that were characterized in *Aspergillus fumigatus* [12-13]. In addition, five secreted proteases belonging to the deuterolysin family (M35) as well as new members of the S28 family were hypothetical. To date only one acidic serine endoprotease was isolated and characterized from *Trichophyton mentagrophytes* grown in a keratin medium at neutral pH [14].

The validation of genome predictions inspired the present study. We tested the ability of two dermatophyte species, *M. canis* and *A. benhamiae*, to grow at acidic pH in a medium promoting secretion of proteases. We show that both species in these growth conditions secrete proteases different from those secreted at neutral pH. Our

investigation reveals new dermatophyte secreted proteases and suggests several pathways of protein degradation into amino acids and short assimilable peptides.

# MATERIALS AND METHODS

## *M. canis and A. benhamiae* liquid cultures

*M. canis* IHEM21239 [15] and *A. benhamiae* LAU2354-2 [11,16] were used in this study. In order to promote production of proteolytic activity at acidic and neutral pH, both dermatophyte species were grown in soy protein (SP) liquid medium [4-5,8] prepared by dissolving 2g soy protein (Supro 1711, Protein Technologies International) in 1 L distilled water. In acidic conditions, citrate buffer (pH 4.0) was added to the medium at a 68 mM final concentration. For neutral pH cultures, the pH was adjusted to 7.0 with few drops of NaOH. All media were sterilized by autoclaving at 120°C for 15 min.

One hundred mL of each medium were poured into 500 mL tissue culture flasks and inoculated with four 4 mm diameter mycelium fragments taken from a 10 to 15-day-old culture on Sabouraud chloramphenicol agar medium (Bio-Rad, Hercules, CA). Liquid cultures were incubated for 14 days at 30°C without shaking. The pH of the medium was measured after one week and at the end of the incubation and was found to remain constant at acidic pH. Using neutral pH medium, the pH of the culture supernatant increased slightly from 7.0 to 7.5 after 14 days of fungal growth.

## Proteolytic activities in culture supernatant

All substrates used to measure proteolytic activities in *M. canis* and *A. benhamiae* culture supernatants are given in Table 1. Endoproteolytic activity was measured with 50 µL dermatophyte culture supernatants and 50 µL of 0.2% resorufin-labeled casein in 50 mM sodium citrate buffer (pH 4.0) or in 25 mM Tris-HCl buffer (pH 7.0) in a total volume of 0.5 mL. After incubation at 37°C, the undigested substrate of the enzyme-substrate mix was precipitated with trichloroacetic acid (TCA, 4% final concentration) and separated from the supernatant by centrifugation. Subsequently, 500 µL of Tris-HCl buffer (500 mM; pH 9.4) were added to the collected supernatant (neutralization step) and the $A_{574}$ of the mixture (1 mL) was

measured. A blank was performed with substrate without culture supernatant. For practical purposes, one milliunit (mU) of endoproteolytic activity was arbitrarily defined as producing an increase in absorbance of 0.001 per min. Activities were measured in culture supernatant without and with the presence of the aspartic protease inhibitor pepstatin (Sigma-Aldrich, Buchs, Switzerland) at a 10 µg/ mL concentration.

Aminopepeptidase activities were tested with synthetic substrates supplied by Genecust (Dunedange, Luxembourg). Stock solutions were prepared at 100 mM concentration and stored at –20°C. Leu-p-nitroanilide (pNA), Ala-Pro-pNA, Ala-Ala-pNA, Phe-Pro-Ala-pNA and Ala-Ala-Pro-pNA were dissolved in DMSO. The reaction mixture contained 80 µL culture supernatant, 5 mM substrate, 50 mM sodium citrate buffer (pH 4.0) or 25 mM Tris-HCl buffer (pH 7.0) in a total volume of 0.1 mL. After incubation at 37°C for 10 min, the reaction was stopped by adding 5 µL of glacial acetic acid followed by adding 0.9 mL of water to the mixture. The released pNA was measured by spectrometry as a change in $A_{405}$. A control with substrate and culture broth was carried out in parallel. Activities were expressed in mU (nmoles of released pNA/min) using the different tested substrates.

Carboxypeptidase activity was tested with hippuryl-L-phenylalanine (Sigma, St.Louis, MO). The substrate was dissolved at 20 mM concentration in DMSO. The reaction mixture of 0.2 mL total volume contained substrate at 1 mM concentration, 10 µL of culture supernatant 5 fold concentrated, 500 mM NaCl as well as 0.05 M Tris at pH 7.0 or 50 mM Na acetate at pH 4.0. After incubation at 37°C for 60 min, released hippuric acid was measured by spectrometry as a change in $A_{254}$. A control with substrate and culture broth was carried out in parallel. Activities were expressed in mU (nmoles of released hippuric acid/min).

## Precipitation and separation of proteins from dermatophyte culture supernatants by 1D-SDS-PAGE

The mycelium was separated from culture medium by paper filtration (Miracloth Calbiochem). Thereafter, 50 mL of supernatant were centrifuged for 10 min at 5000× g to remove debris, followed by concentration down to 1 mL using a Centricon Plus-70 with a 10000 Da cutoff. Concentrated media were subsequently

precipitated as follows: 0.9 mL of 0.2% (w/v) sodium deoxycholate was mixed with 100 µL of concentrated medium and incubated for 10 min at room temperature. 100 µL of 6.1 N TCA was added to this mixture and gently shaken. The sample was incubated for 10 min at 4 °C, and then centrifuged at 12,000 g for 10 min to obtain a pellet. After removal of the supernatant, the pellet was washed twice with 100% acetone and dried. For 1D-SDS-PAGE analysis, the pellet was dissolved in 20 µL of 20 mM Tris-HCl, pH 7.4 and mixed with SDS sample buffer. Proteins were separated on a 12% SDS polyacrylamide gel followed by staining with Coomassie brilliant blue R-250 (Bio-Rad). The total optical density in every lane was determined by densitometry and used to calibrate sample loadings onto a preparative gel. Equal amounts of protein for every sample were subjected to limited electrophoretic separation on a 10% minigel, that is, the migration was stopped after the front had moved by about 2.5 cm into the separating gel. At this time all bands up to 250 kDa of a prestained molecular weight marker had moved into the gel and were distinguishable. Gels were fixed for 10 min, partially stained with Coomassie Brilliant blue G (15 min) and then destained for 30 min. Every lane was cut into 4-5 sections beginning with high molecular weights.

## Shotgun MS Experiments

Proteins in 1D gel slices were digested with trypsin according to a described protocol [17,18]. Tryptic peptides were recovered in the supernatant of the digestion, dried by evaporation, reconstituted in 20 µL of $H_2O$/acetonitrile (ACN) 97:3 (v/v) with 0.1% formic acid and analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Samples were loaded onto a trapping microcolumn ZORBAX 300SB C18 (5 mm × 300 µm ID, 5 µm, Agilent) in $H_2O$/ACN 97:3 (v/v) + 0.1% formic acid at a flow rate of 10 µL/min. After 5 min they were back-flush eluted and separated on a reversed-phase nanocolumn ZORBAX 300SB C18 column (75 µm ID × 15 cm, 3.5 µm, Agilent Technologies) with an Agilent 1100 nano HPLC system (Agilent Technologies). Solvents used for the mobile phase were 95:5 $H_2O$/ACN (v/v) with 0.1% formic acid (A) and 5:95 $H_2O$/ACN (v/v) with 0.1% formic acid (B). The flow rate was 300 nL/min and the gradient used lasted 90 min (5 min at 0% of solvent B, from 0 to 25% of B in 35 min, 25 to 50% B in 15 min, 50 to 90% in 5 min, 90% B during 10 min, 90 to 0% in 5 and 15 min at 0%). The chromatographic system was interfaced to

a LTQOrbitrap mass spectrometer (Thermo Fisher Scientific) through a TriVersa Nanomate (Advion Biosciences). In data-dependent acquisition controlled by Xcalibur 2.0 software (Thermo Fisher), the four most intense precursor ions detected in the full MS survey performed in the Orbitrap (range 350-1500 m/z, resolution 60 000 at m/z 400) were selected and fragmented. MS/MS was triggered by a minimum signal threshold of 10 000 counts, carried out at relative collision energy of 35% and with isolation width of 4.0 amu. Only precursors with a charge higher than one were selected for CID fragmentation and fragment ions were analyzed in the linear trap. Spectra were acquired under automated gain control, with maximal ion injection time of 100 ms. The m/z of fragmented precursors was then dynamically excluded, with a tolerance of 0.01 amu, from any selection during 120 s.

## Database Searching with MS Data

From *raw* files, MS/MS spectra were deisotoped and exported as mgf files (Mascot Generic File, text format) using MascotDistiller 2.1.1 (Matrix Science, London, UK). MS/MS spectra from *M. canis* samples were searched with Mascot (Matrix Science, London, UK; version 2.2.0) against UniProt database (www.uniprot.org) restricted to *M. canis* (suffix _MICCA and _NANOT for *Nannizzia otae,* 8'805 sequences). The UniProt database release used was version 15.14 of February, 9th 2010. MS/MS spectra from *A. benhamiae* were searched against sequences from the same organism obtained from the Dermatophyte Comparative Sequencing Project, Broad Institute of Harvard and MIT, (http://www.broadinstitute.org/) (arthroderma_benhamiae_cbs_112371_proteins database).

The digestion enzyme trypsin was specified with one missed cleavage. Mascot searches were performed with a fragment ion mass tolerance of 0.50 Da and a parent ion tolerance of 10.0 ppm. Iodoacetamide derivative of cysteine was specified in Mascot as a fixed modification. N-terminal acetylation of protein, deamidation of asparagine and glutamine, and oxidation of methionine were specified in Mascot as variable modifications.

## Criteria for Protein Identification

Scaffold (version Scaffold_ 3_00_08, Proteome Software Inc., Portland, OR) was used to validate MS/MS based peptide and protein identifications. Peptide identifications were accepted if they could be established at greater than 90.0% probability as specified by the Peptide Prophet algorithm.28. Protein identifications were accepted if they could be established at greater than 95.0% probability and contained at least 1 identified peptide. Protein probabilities were assigned by the Protein Prophet algorithm.29. Proteins that contained similar peptides and could not be differentiated based on MS/MS analysis alone were grouped to satisfy the principles of parsimony.

# RESULTS AND DISCUSSION

## Dermatophyte secreted proteolytic activity at neutral and acidic pH

Soy protein was previously found to be an adequate protein source to promote secreted proteolytic activity by dermatophytes, thereby governing protease gene expression patterns similar to those observed during growth of these fungi in keratin medium [4-8, 19-20]. In addition, fungal growth is faster with soy protein than with other protein sources such as keratin, and results are reproducible from one experiment to another. In acidic as in neutral pH conditions, *M. canis* and *A. benhamiae* grew well in SP medium. After 14 days of culture at neutral and acidic pH, the amount of protein was 200-300 µg/ mL in culture supernatants. At the indicated time point, growth in protein media was accompanied by clarification of the medium along with maximal endoproteolytic activity as determined using resorufin-labeled casein as a substrate. Various aminopeptidase and carboxypeptidase activities were concomitantly measured using different substrates (Fig. 1, Table 1).

Growth of *M. canis* and *A. benhamiae* was possible in keratin medium at neutral pH after adding soy protein to initiate protease secretion [8,19-20]. Following several unsuccessful trials, experimental conditions were not yet found for these fungi to digest keratin at acidic pH in vitro.

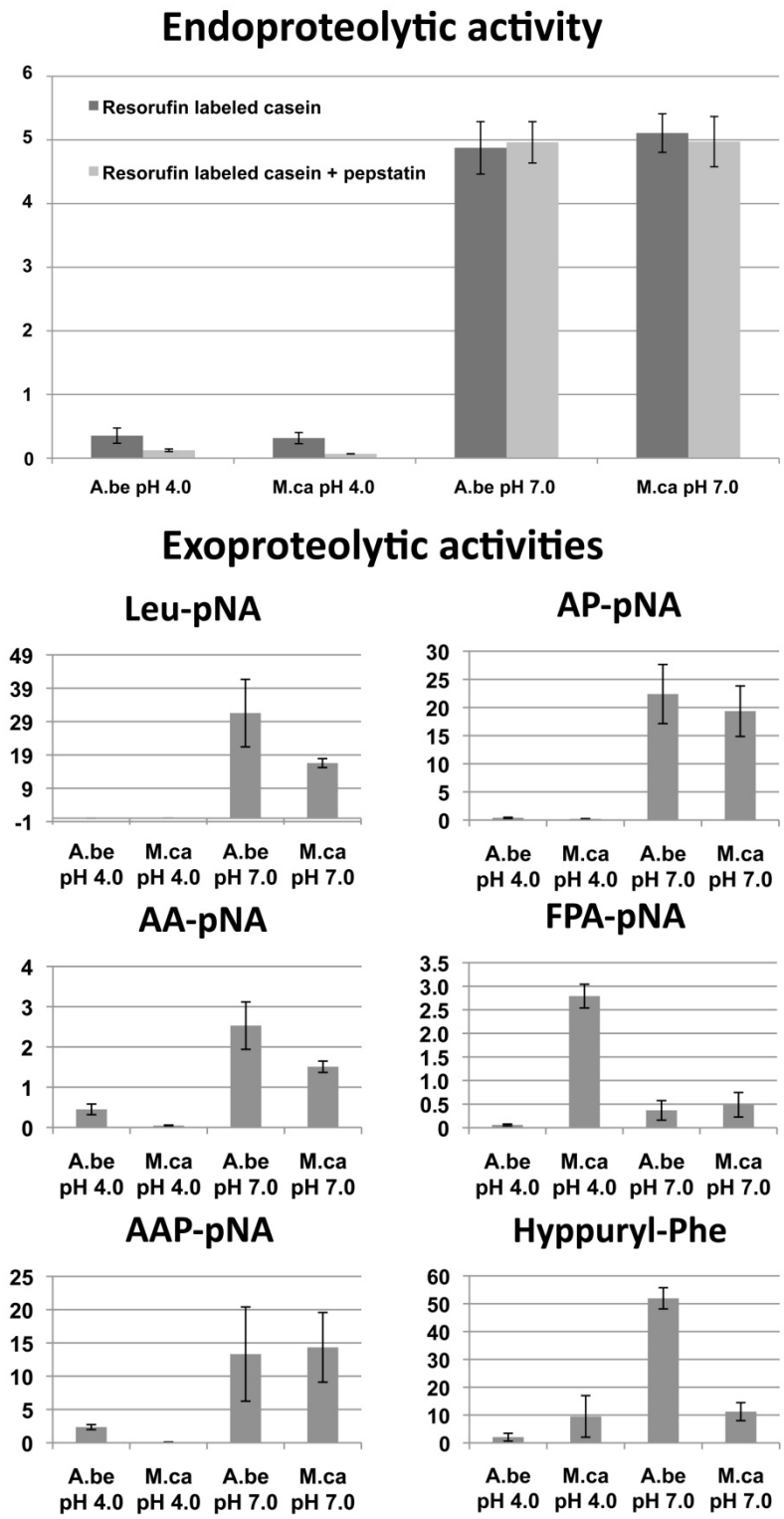



**Figure 1: Endo- and exoproteolytic activities (mU/ mL) in *M. canis* (Mca) and *A. benhamiae* (Abe) culture supernatants after 14 days of growth in soy protein medium.** Activities in three culture supernatants were measured as described in material and methods at the pH values of the culture medium. Endoproteolytic activity was measured in the absence and in the presence of pepstatin (10 µg/ mL).

**Table 1: Substrates used to measure proteolytic activities in *M. canis* and *A. benhamiae* culture supernatants.**

| Substrates | Usage | Enzyme activity detected in *A. fumigatus* culture supernatants | Enzyme activity detected in dermatophyte culture supernatants |
|---|---|---|---|
| Resorufin-labeled casein | Endoproteolytic activity detection | Pep1, GprA at acidic pH [32]<br>Alp, Mep at neutral pH [unpublished results] | Pep[a] at acidic pH [This work][b]<br>Subs, Meps at neutral pH [4,5] |
| Leu-pNA | Detection of Lap (non specific amino peptidase) activity | Lap1, Lap2 (neutral pH) [27] | Lap1, Lap2 (neutral pH) [8, This work][c] |
| Ala-Pro-pNA | Detection of X-prolyl peptidase activity | DppIV (neutral pH) [25, 27] | DppIV (neutral pH) [8, This work][c] |
| Ala-Ala-pNA | Detection of X-alalyl peptidase activity | DppIV, DppV and Lap (neutral pH) [27] | DppIV, DppV and Lap (neutral pH) [This work][c] |
| Phe-Pro-Ala-pNA | Detection of tripeptidyl peptidase activity | Sed1, Sed2 at acidic pH [13]<br>Mix of DppIV and Lap1 or Lap2 at neutral pH [27] | Seds[a] at acidic pH [This work][b]<br>Mix of DppIV and Lap1 or Lap2 at neutral pH [This work][c] |
| Ala-Ala-Pro-pNA | Detection of prolyl peptidase activity | AfuS28 at acidic pH [28]<br>AfuS28 or mix of DppIV and Lap1 or Lap2 at neutral pH [27] | S28 proteases[a] at acidic pH [This work][b]<br>AfuS28 or mix of DppIV and Lap1 or Lap2 at neutral pH [This work][c] |
| Hippuryl-L-Phe | Carboxypeptidase activity detection | Serine carboxypeptidases of the S10 family at acidic pH [9][c] | Serine carboxypeptidases of the S10 family at acidic pH [This work][c]<br>McpA at neutral pH [9, This work][c] |

[a] Putative orthologues of *A. fumigatus* secreted proteases.
[b] Not formally proved using purified native or recombinant proteases. Deduced from knowledge on A. *Fumigatus* secreted proteolytic activity.
[c] Substrate tested using recombinant dermatophyte aminopeptidases [8,9]

## 1D-SDS-PAGE analysis of secreted proteins in soy protein medium

Culture supernatants from acidic and neutral pH cultures were collected after 14 days, concentrated, size-fractionated on SDS-PAGE and stained with Coomassie Brilliant Blue (see Materials and Methods). SDS-PAGEs of proteins precipitated from culture supernatants of the same fungus at pH 7.0 and pH 4.0 showed distinct complex band patterns (Fig. 2). Important differences were also observed between *A. benhamiae* and *M. canis* at the same pH. In contrast, comparable band patterns were observed at the same pH with other strains of either *A. benhamiae* or *M. canis* (data not shown). Band patterns were previously found to be species specific [6].

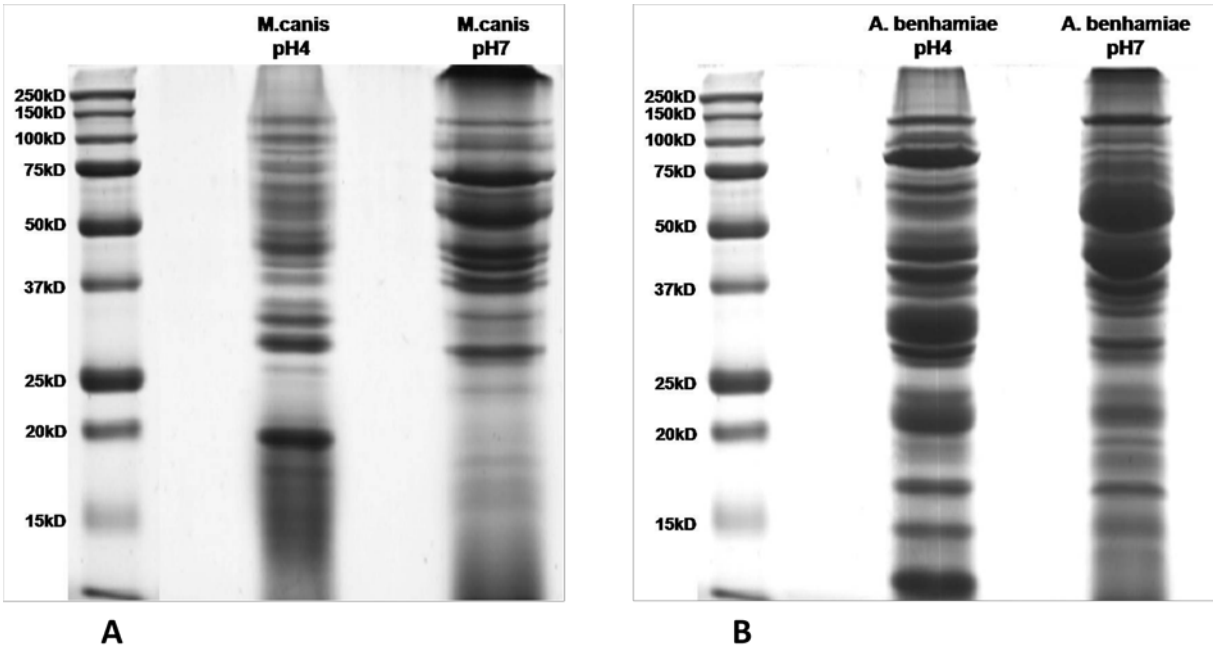



**Figure 2: 12% SDS-PAGE stained with Coomassie blue of *M. canis* (A) and *A. benhamiae* (B) secreted proteins at pH 4.0 and pH 7.0.**

In an attempt to map a maximum number of proteins secreted by these two dermatophytes at different pH values, a systematic shotgun protein identification experiment was implemented. After sample fractionation by limited 1D-SDS-PAGE, five identical gel bands corresponding to different molecular weights were excised from every lane and proteins were in-gel digested. After peptide gel extraction, every fraction was analyzed by LC-MS/MS on a LTQ-orbitrap mass spectrometer.

Lists of spectra for each lane were merged to obtain a data set that was used to search sequence databases as described in the methods section (Supporting

Information Tables 1 and 2). From three independent shotgun analyses, the total number of MS/MS spectra assigned to peptides after database searches were 14076 (5172+5241+3663) and 10531 (3019+4480+2632) for *A. benhamiae* samples at pH 7.0 and 4.0, respectively. Analogously they were 9516 (2643+1808+5065) and 6450 (2427+1155+2868) for *M. canis* samples at those same two pHs. Spectral counting coupled with redundant sampling of a mixture has been established as a reliable method to quantify protein abundances in shotgun experiments [21]. All proteins listed have at least one validated unique peptide. Here we have assumed that percentages of matched spectra represent semi-quantitative estimates of the relative protein abundances between samples. Extensive details on the results of identifications are reported in Supporting Information Tables 1 and 2. Overall, 175 and 196 different sequences were blasted against the complete dermatophyte database (Broad institute of MIT and Harvard, Boston, United States) and a predictive function was assigned to them. Among all the proteins detected, 56 hydrolases (4 lipases, 9 phosphatases, 27 glycosidases and 16 other hydrolases) for *M. canis* and 54 hydrolases (6 lipases, 5 phosphatases, 29 glucosidases and 14 other hydrolases) for *A. benhamiae* were identified (Supporting Information Table 3). Other identified protein classes included isomerases, oxidoreductases, and transferases. No function could be assigned based on sequence similarity for 39 and 27 sequences on both acidic and neutral secretomes for *M. canis* and *A. benhamiae*, respectively.

## Proteases identified by shotgun analysis in *M. canis* and *A. benhamiae* culture supernatants in soy protein medium

A total of 72 proteases with a signal sequence distributed over 16 families of the MEROPS proteolytic enzyme database, (http://merops.sanger.ac.uk) were recorded in dermatophyte genomes [11]. As expected, *M. canis* and *A. benhamiae* proteases constituted a significant fraction of all identified proteins, with 38 and 39 predicted proteases respectively (Fig. 3, Table 2 and Supporting Information Table 4). In *M. canis*, proteases accounted for 32% and 50% of total matched spectra in the shotgun analysis at neutral and acidic pH, respectively. In *A. benhamiae*, proteases accounted for 37% and 48% of total matched spectra in the shotgun analysis at neutral and acidic pH. These results highlight the dominance of proteases in the

dermatophyte secretomes when fungi are grown in a protein medium. Several *M. canis* and *A. benhamiae* secreted proteases were detected at both neutral and acidic pH (Figs. 2 and 3). However, most of these proteases were substantially secreted in only one pH condition and at a lower level in the other pH condition, or at a trace level in both pH conditions. Common major secreted proteases at neutral and at acidic pH in *M. canis*, as well as in *A. benhamiae* were the endoproteases Sub3 and Mep3.

## Comparison between proteases secreted by *M. canis* and *A. benhamiae* in soy protein medium

Seventeen proteases produced by *M. canis* and *A. benhamiae* are highly similar and are putative orthologues (Table 2). An aspartic protease of the pepsin family (Pep1), Sub3, SedB, DppV, and various carboxypeptidases of the S10 family were secreted in substantial amounts at acidic pH by both species. It is the first time that aspartic proteases and sedolisins were found to be secreted by dermatophytes. Most endoproteolytic activity at acidic pH was inhibited by pepstatin (Fig. 1) leading to the conclusion that Pep1 was the major secreted endoprotease at this pH condition. Sub3 Mep3, Mep4, and DppIV were major proteases secreted by *M. canis* and *A. benhamiae* at neutral pH. These findings attest for substantial endoproteolytic activities, as well as different amino peptidase and carboxypeptidase activities measured in the culture supernatants (Fig. 1). Our results are comparable to those obtained for *T. rubrum*, *T. violaceum* and *A. benhamiae* in previous studies where Sub3, Mep3, Mep4, and DppIV were shown to be major proteases secreted at neutral pH in different protein media [6,11].

Other proteases detected in culture supernatants of both species were produced at different levels. Strikingly, Lap1 was predominant in *M. canis*, while Lap2 was detected in larger quantities in *A. benhamiae* at neutral pH. Correlating with this finding, substantial Lap activities were measured in both dermatophyte culture supernatants (Fig. 1). Lap2 was found to be the major exoprotease secreted by *Trichophyton rubrum*, *Trichophyton violaceum* and *A. benhamiae* at neutral pH in SP and keratin media [7,8]. A deuterolysin (NPIIA) and a S41 protease were exclusively detected in supernatants of *M. canis* at acidic pH. As a general conclusion, although

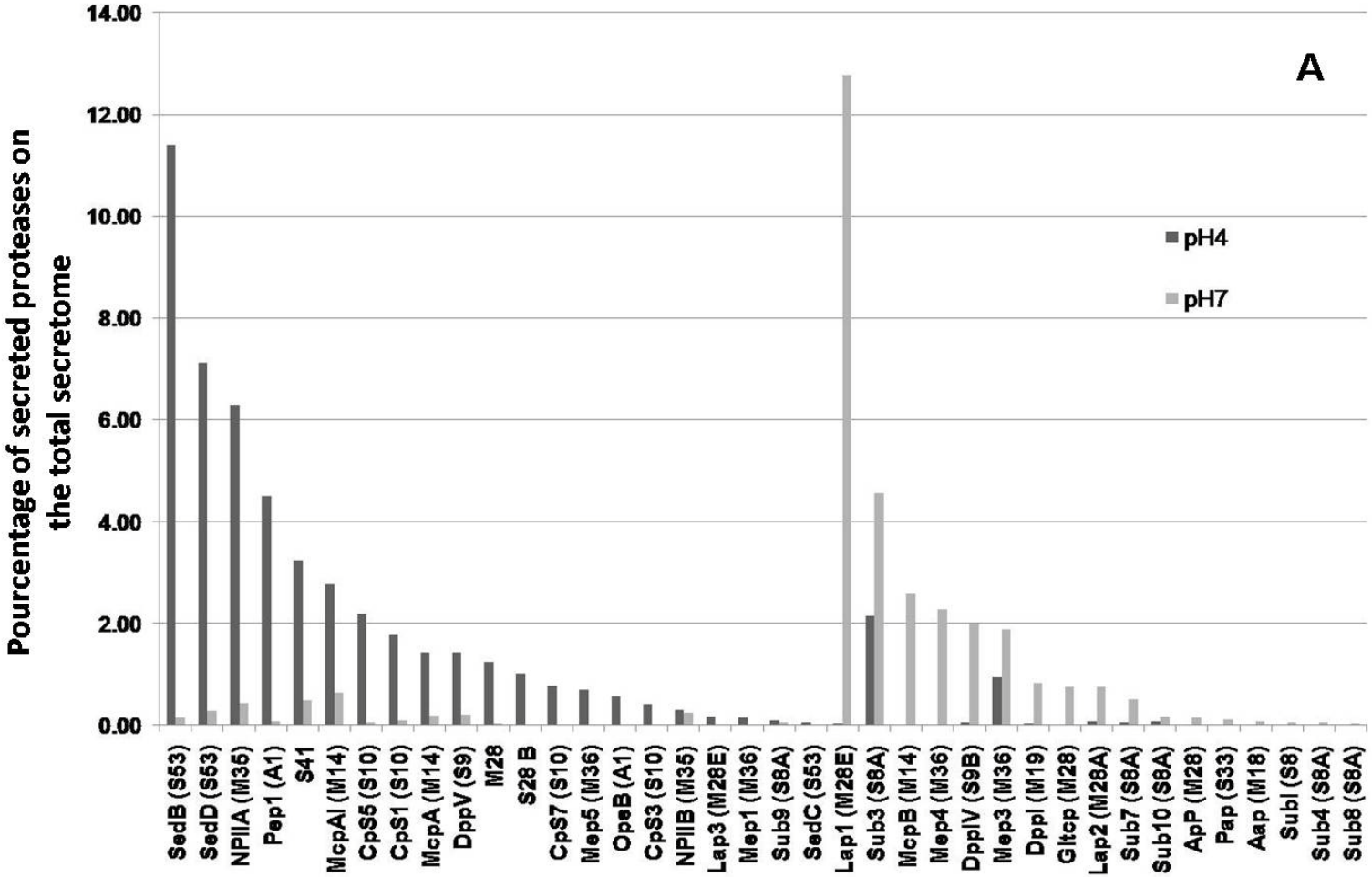
the protein sequence of each protease remains highly conserved across dermatophyte species, their level of secretion is highly variable.

## Comparison between proteases secreted by dermatophytes and *A. fumigatus*

Proteases secreted in a protein medium by *A. benhamiae* and *M. canis* were compared to those belonging to *A. fumigatus,* of which many members have been previously characterized [8-9,12-13,22-26] (see [27] for a review). Comparison of the complete secreted protease repertoire encoded in the genomes of *A. fumigatus*, *A. benhamiae* and *M. canis* (Supporting Information Table 5) highlights the expansion in dermatophytes of the subtilisin (S8), deuterolysin (M35) and fungalysin (M36) endoprotease families as well as of the exopeptidases of the M14 and M28 families. Genes encoding glutamic protease (G1) were not detected in either dermatophytes (Table 2 and Supporting Information Table 5).

In *A. fumigatus*, an acidic pH environment promotes the secretion of an aspartic protease of the pepsin family (Pep1), and a glutamic protease (GprA) as endoproteases as well as tripeptidyl peptidases of the sedolisin family (SedB and SedD), an X-prolyl peptidase (AfuS28) and carboxypeptidases of the S10 family as exoproteases [28]. Similar enzymes are secreted by dermatophytes with the exception of glutamic proteases, as genes encoding glutamic proteases are lacking in dermatophyte genomes [11]. During protein digestion, the main function of endoproteases is to produce a large number of free ends on which exoproteases may act. Large peptides generated by *Aspergillus* endoproteolytic activity can be further digested by sedolisins and AfuS28 [28]. During this process, *A. fumigatus* sedolisins were shown to be nonspecific tripeptidyl peptidases blocked by proline residues in P1 and P'1 position. However, X-X-Pro and X-X-X-Pro sequences can be removed by AfuS28 thus allowing sedolisins further sequential proteolysis. We hypothesize that protein degradation by dermatophytes at acidic pH occurs in a way similar to that in *Aspergillus* spp with aspartic protease activity and sedolisins which are abundantly secreted. Moreover, a S28 protease and concomitant prolyl peptidase activity similar to that of the previously characterized AfuS28 [28] were detected in *A. benhamiae* culture supernatants at acidic pH (Figs 1 and 3). Prolyl peptidase activity was not detected in *M. canis.*

At neutral pH *A. fumigatus* secretes one subtilisin (Alp for alkaline protease) and one metalloprotease (Mep) belonging to the fungalisin family as endoproteases, and leucine aminopeptidases (Laps), and an X-prolyl dipeptidase (DppIV) as major exoproteases [28]. *Aspergillus* spp. genomes do not encode McpA proteases [9,27]. Dermatophytes and *Aspergillus* spp. subtilisins, fungalisins, Lap1, Lap2, DppIV and DppV secreted at neutral pH revealed comparable substrate specificity [8]. Thus, protein degradation by dermatophytes at neutral pH can be performed in a way similar to that in *Aspergillus* spp. Subtilisins and fungalisins digest proteins into large peptides, which are subsequently digested into amino acids and short peptides by the synergistic action of Laps and DppIV. During this process, *Aspergillus* Laps were shown to degrade large peptides from their N-terminus until they reach an X-Pro sequence acting as a stop. In a complementary manner, X-Pro sequences can be removed by DppIV, thus allowing Laps to access to the next residue [29]. Dermatophytes prefer alkaline conditions [2,3] and it is likely that in their natural habitat proteolysis by subtilisins, fungalisins, Laps and DppIV play a pivotal role in keratin degradation and nitrogen assimilation.


A
Pourcentage of secreted proteases on the total secretome
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
pH4
pH7
SedB (S53)
SedD (S53)
NPIIA (M35)
Pep1 (A1)
S41
McpAl (M14)
CpS5 (S10)
CpS1 (S10)
McpA (M14)
DppV (S9)
M28
S28 B
CpS7 (S10)
Mep5 (M36)
OpeB (A1)
CpS3 (S10)
NPIIB (M35)
Lap3 (M28E)
Mep1 (M36)
Sub9 (S8A)
SedC (S53)
Lap1 (M28E)
Sub3 (S8A)
McpB (M14)
Mep4 (M36)
DpplV (S9B)
Mep3 (M36)
Dppl (M19)
Gltcp (M28)
Lap2 (M28A)
Sub7 (S8A)
Sub10 (S8A)
ApP (M28)
Pap (S33)
Aap (M18)
Subl (S8)
Sub4 (S8A)
Sub8 (S8A)

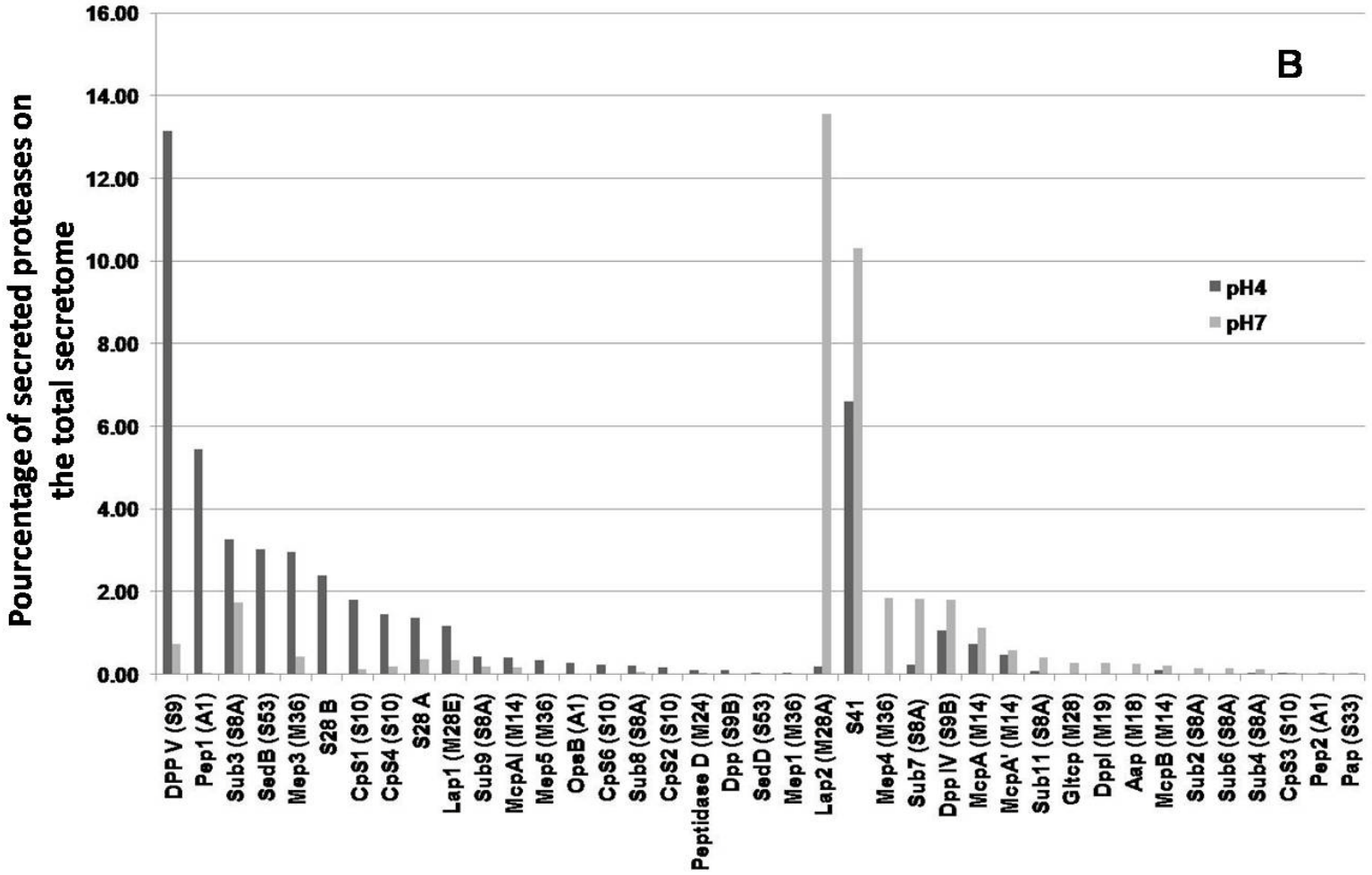



**Figure 3: Percentage of matched spectra for proteases secreted by *M. canis* (A) and *A. benhamiae* (B) on a soy protein medium at 4.0 and pH 7.0.** Data are averages of percentages of matched spectra assigned by Scaffold 3.0 in three independent shotgun experiments at both pH values. Therefore, numbers represent the percentage of matched spectra over the total secretome. The major proteases secreted in an acidic environment are listed in the left part of the graph and the major proteases secreted in a neutral condition, in the right part. Plots are normalized to the total number of matched spectra for each independent shotgun experiment. Database accession numbers for each protease are listed in Table 1. All the average values for each protease are listed in the supplementary Tables 3a and 3b.

**Table 2: *M. canis* and *A. benhamiae* proteases secreted at pH 4.0 and pH 7.0 in SP medium and homologous proteases in *A. fumigatus* [8].** *M. canis* and *A. benhamiae* specific proteases are shaded in light and dark grey, respectively.

| Family/ Sub-family | Protein description | *M.Canis*[a] | pH4 (50.91%)[d] | pH7 (32.40%)[d] | *A.Benhamiae*[b] | pH4 (46.79%)[d] | pH7 (37.45%)[d] | *A.fumigatus*[c] | pH4[e] | pH7[e] |
|---|---|---|---|---|---|---|---|---|---|---|
| A1 | Aspartic protease **Pep2** (Vacuolar protease A) | MCYG_05527 C5FS55 | | | ARB_02919 D4B385 | 0 | 0.01 | AFUA_3G11400 O42630 | | |
| A1 | Aspartic protease **Pep1** (Aspergillopepsin I) | MCYG_00144 C5FBS2 | 4.51 | 0.08 | ARB_05728 D4ANC3 | 5.25 | 0.03 | AFUA_5G13300P41748 | ++++ | - |
| A1 | Aspartic protease **OpsB** | MCYG_05372 C5FRQ0 | 0.55 | 0 | ARB_04170 D4AIS3 | 0.26 | 0 | AFUA_6G05350 Q4WDN4 | | |
| G1 | Glutamic protease **GpA** (Aspergillopepsin II) | - | | | - | | | AFUA_3G02970Q4WF96 | + | - |
| M14 | Metallocarboxypeptidase A **McpA** | MCYG_06789 C5FVN6 | 1.43 | 0.19 | ARB_07026 / ARB_07027 D4AS12 | 0.70/0.48 | 1.10/0.57 | - | | |
| M14 | Metallocarboxypeptidase A-like protein **McpAl** | MCYG_01475 C5FH26 | 2.76 | 0.63 | ARB_03789 D4B5N0 | 0.40 | 0.17 | - | | |
| M14 | Metallocarboxypeptidase B **McpB** | MCYG_07414 C5FYJ7 | 0 | 2.58 | ARB_02407 D4B1S6 | 0.08 | 0.22 | - | | |
| M18* | Aspartyl aminopeptidase **Aap** | MCYG_06746 C5FVJ3 | 0 | 0.06 | ARB_06983 D4ARW8 | 0 | 0.24 | - | | |
| M19 | Dipeptidase 1 **Dppl** | MCYG_02918 C5FK77 | 0.03 | 0.83 | ARB_02715 D4B2N2 | 0 | 0.27 | - | | |
| M24 | **Peptidase D** | MCYG_06503 C5FUV0 | | | ARB_01886 D4B0B2 | 0.10 | 0.01 | AFUA_1G14920 Q4WRV9 | | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| M28 | Putative glutamate carboxypeptidase **Gltcp** | MCYG_07431 C5FYL4 | 0.01 | 0.76 | ARB_02390 D4B1R0 | 0 | 0.28 | - | | |
| M28 | Peptidase family M28 protein **M28** | MCYG_04217 C5FP82 | 1.23 | 0.03 | ARB_04732 D4AM42 | | | - | | |
| M28* | Aminopeptidase P **ApP** | MCYG_01718 | 0 | 0.15 | - | | | - | | |
| M28A | Leucine aminopeptidase 2 **Lap2** | MCYG_06199 C5FTZ6 | 0.07 | 0.74 | ARB_00494 D4AWC9 | 0.14 | 13.65 | AFUA_3G00650 Q4WFX9 | - | +++ |
| M28E | Leucine aminopeptidase 1 **Lap1** | MCYG_02286C5 FFM0 | 0.02 | 12.77 | ARB_03568 D4B528 | 1.23 | 0.35 | AFUA_4G04210 Q4W9P4 | | |
| M28E | Probable leucine aminopeptidase **Lap3** | MCYG_04170 C5FNB5 | 0.17 | 0 | ARB_00576 D4AWL0 | | | - | | |
| M35 | Deuterolisin **NpIIA** (*Aspergillus* neutral protease 2 homolog) | MCYG_07155 C5FWQ2 | 6.29 | 0.43 | ARB_03949 D4B639 | | | - | | |
| M35 | Deuterolisin **NpIIB** (*Aspergillus* neutral protease 2 homolog) | MCYG_05201 C5FR79 | 0.30 | 0.24 | ARB_04336 D4AJ87 | | | - | | |
| M36 | Fungalysin **Mep1** (Extracellular metalloproteinase 1) | MCYG_07415 C5FYJ8 | 0.15 | 0.02 | ARB_02406 D4B1S5 | 0.03 | 0 | - | | |
| M36 | Fungalysin **Mep3** (Extracellular metalloproteinase 3) | MCYG_04620 C5FNU8 | 0.94 | 1.88 | ARB_05085 D4AL88 | 2.89 | 0.43 | - | | |
| M36 | Fungalysin **Mep4** (Extracellular metalloproteinase 3) | MCYG_02088 C5FIK0 | 0 | 2.27 | ARB_00762 D4AX35 | 0 | 1.84 | - | | |
| M36 | Fungalysin **Mep5** (Extracellular metalloproteinase 3) | MCYG_07688 C5FX29 | 0.68 | 0.01 | ARB_06472 D4AQG5 | 0.34 | 0 | - | | |
| M36 | Aspergillus Metalloprotease **Mep** | - | | | - | | | AFUA_8G07080 P46075 | - | + |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| S8* | Subtilisin-like protease **SubI** | MCYG_01699 C5FHQ0 | 0 | 0.06 | ARB_06723 D4AR10 | | | - | | |
| S8A | Subtilisin 2 **Sub2** | MCYG_08690 C5G168 | | | ARB_01495 D4AZ75 | 0 | 0.15 | AFUA_4G11800 P28296 | - | ++++ |
| S8A | Subtilisin 3 **Sub3** | MCYG_04040 C5FMY5 | 2.15 | 4.55 | ARB_00701 D4AWY5 | 3.06 | 1.72 | - | | |
| S8A | Subtilisin 4 **Sub4** | MCYG_07213 C5FXZ6 | 0 | 0.04 | ARB_01032 D4AXW3 | 0.04 | 0.13 | - | | |
| S8A | Subtilisin 6 **Sub6** | MCYG_04955 C5FQI3 | | | ARB_05307 D4ALV9 | 0 | 0.13 | - | | |
| S8A | Subtilisin 7 **Sub7** | MCYG_02345 C5FJA5 | 0.05 | 0.5 | ARB_06076 D4APA9 | 0.22 | 1.82 | - | | |
| S8A | Subtilisin 8 **Sub8** | MCYG_02070 C5FII2 | 0 | 0.04 | ARB_00777 D4AX50 | 0.22 | 0.04 | AFUA_5G09210 P87184 | + | - |
| S8A | Subtilisin 9 **Sub9** | MCYG_01476 C5FH27 | 0.08 | 0.05 | ARB_03790 D4B5N1 | 0.46 | 0.18 | - | | |
| S8A | Probable alkaline serine protease **Sub10** | MCYG_02748 C5FGP4 | 0.06 | 0.16 | - | | | - | | |
| S8A | Subtilisin 11 **Sub11** | MCYG_07961 C5FZ39 | | | ARB_06111 D4APE3 | 0.07 | 0.42 | - | | |
| S9B | Dipeptidyl peptidase 4 **DppIV** | MCYG_02383 C5FJE3 | 0.06 | 1.99 | ARB_06110 D4APE2 | 1.08 | 1.82 | AFUA_4G09320 Q4WPH9 | - | ++ |
| S9B | Dipeptidyl aminopeptidase **Dpp** | MCYG_07560 C5FYZ3 | | | ARB_06590 D4AQT0 | 0.09 | 0 | AFUA_3G07850 Q4WX13 | | |
| S9; not assigned to subfamily | Dipeptidyl-peptidase 5 **DppV** | MCYG_01626 C5FH88 | 1.42 | 0.21 | ARB_06651 D4ARB1 | 13.05 | 0.69 | AFUA_2G09030 **P0C959** | + | ++++ |
| S10 | Carboxypeptidase S1 homolog A | MCYG_06933 | 1.78 | 0.08 | ARB_04046 | 1.90 | 0.11 | AFUA_5G07330 | | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **CpS1** | C5FW30 | | | D4AIF1 | | | Q5VJK9 | | |
| S10 | Carboxypeptidase S1 homolog B **CpS2** | MCYG_02825 C5FGX1 | 0.40 | 0 | ARB_06019 D4AP52 | 0.17 | 0 | AFUA_8G04120 Q4WCK3 | + | - |
| S10 | Carboxypeptidase S1 homolog **CpS3** | MCYG_07817 C5FXF8 | | | ARB_06361 D4AQ54 | 0.03 | 0.03 | - | | |
| S10 | Carboxypeptidase CpdS homolog **CpS4** | MCYG_00613 C5FD41 | | | ARB_02032 D4B0Q6 | | | AFUA_6G00310 Q4W8Y5 | ++ | - |
| S10 | Carboxypeptidase CpdS homolog **CpS5** | MCYG_07753 C5FX94 | 2.18 | 0.04 | ARB_06414 D4AQA7 | 1.42 | 0.18 | AFUA_2G03510 Q5VJG7 | ++ | - |
| S10 | Carboxypeptidase Y homolog **CpS6** | MCYG_00135 C5FBR3 | | | ARB_05721 D4ANB6 | 0.22 | 0 | AFUA_5G14610 Q4WW68 | | |
| S10 | Carboxypeptidase Y homolog **CpS7** | MCYG_05486 C5FS14 | 0.77 | 0.01 | ARB_07161 D4ASE6 | | | - | | |
| S10 | Carboxypeptidase Y homolog | | | | - | | | AFUA_5G01200 Q4WDZ3 | +++ | - |
| S28 | Prolyl peptidase | - | | | - | | | AFUA_2G01250 Q4WIN2 | ++ | ++ |
| S28 | Putative prolyl peptidase **S28A** | MCYG_00366 C5FC55 | | | ARB_00083 D4AV74 | 1.34 | 0.38 | AFUA_4G03790 Q4W9T5 | | |
| S28 | Putative prolyl peptidase **S28B** | MCYG_02207 C5FJ58 | 1.02 | 0 | ARB_01345 D4AYS6 | 2.34 | 0 | AFUA_2G17330 Q4WZD5 | | |
| S33 | Prolyl aminopeptidase **Pap** | MCYG_04539 C5FNL7 | 0 | 0.11 | ARB_04981 D4AKY5 | 0 | 0.01 | | | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| S41 | **S41** peptidase | MCYG_03610<br>C5FM69 | 3.23 | 0.47 | ARB_02220<br>D4B191 | 6.18 | 10.44 | - | | |
| S53 | Sedolisin-B (Tripeptidyl-peptidase in *A. fumigatus*)<br>**SedB** | MCYG_00184<br>C5FBW2 | 11.41 | 0.15 | ARB_05765<br>D4ANG0 | 2.97 | 0.03 | AFUA_4G03490<br>Q70J59 | ++++ | - |
| S53 | Sedolisin-C (Tripeptidyl-peptidase in *A. fumigatus*)<br>**SedC** | MCYG_06077<br>C5FTQ5 | 0.06 | 0 | - | | | AFUA_3G08930<br>Q70GH4 | | |
| S53 | Sedolisin-D (Tripeptidyl-peptidase in *A. fumigatus*)<br>**SedD** | MCYG_05446<br>C5FRX4 | 7.11 | 0.28 | ARB_04101<br>D4AIK6 | 0.03 | 0 | AFUA_4G14000<br>Q4WQU0 | + | - |

[a] *M. canis* complete genome of strain CBS 113480 (Cuomo *et al*., Submitted to GenBank/EMBL in 2008)
[b] *A. benhamiae* complete genome of strain CBS 113480 (Burmester *et al*., Submitted to GenBank/EMBL in 2009) [11]
[c] *A. fumigatus* complete genome of strain Af293 / CBS 101355 / FGSC A1100 (Nierman *et al*., 2005) [33]
[a, b, c] Proteins are from UniProtKB database (http://www.uniprot.org/). UniProtKB accession numbers are indicated below the gene name. Closest homologs were identified by Blast search using the *A. fumigatus* and/or *M. canis* proteins as query.
[d] Ratio between matched spectra of secreted proteases and the total matched spectra in the shotgun analysis at pH 4.0 and pH 7.0 (Supporting information Table 4).
[e] *A. fumigatus* secretome data [28]

# CONCLUDING REMARKS

Analysis of the repertoire of secreted proteases suggests common basic mechanisms for extracellular protein digestion in dermatophytes and in *Aspergillus* spp. at acidic and neutral pH. However, in comparison to *A. fumigatus* more proteases were found to be secreted by dermatophytes at neutral as well as acidic pH. Families of genes encoding endoproteases of the subtilisin (S8), deuterolisin (M35) and fungalisin (M36) families have expanded in dermatophytes, and several members were shown to be secreted [4-7,11]. Therefore, the pathway of protein degradation at acidic pH and that at neutral pH appear to be less strict than those in *A. fumigatus*.

Dermatophytes originate in soil where conditions may vary from acidic to alkaline. The capacity to secrete multiple subtilisins and metalloproteases as alkaline proteases, as well as various acidic proteases, may reflect the initial ability of these fungi to multiply in different environmental conditions during saprophytic growth in soil. These properties could have been a decisive evolutionary advantage favoring invasion of keratinized tissues and could explain the very high frequency of dermatophytes in both animals and humans in comparison to other filamentous fungi. Though the pH of skin surface is rather acidic in humans, this is not the case in all animal species [30]. Moreover, skin is a complex environment where pH varies according to many both endogenous and exogenous factors [31]. Therefore, the large panel of acidic, neutral and alkaline proteases secreted by dermatophytes, including the newly recognized acidic ones, should instigate further investigations in order to define their role during skin infection.

**Acknowledgments**

We thank Dr. Massimo Lurati for critical review of the manuscript and assistance with the English, Marina Fratti and Olympia Bontems for technical assistance. This work was supported by the Swiss National Foundation for Scientific Research, grant 320030-117964. The Swiss-Prot group is part of the Swiss Institute of Bioinformatics (SIB) and of the UniProt Consortium. Swiss-Prot group activities are supported by the Swiss Federal Government through the Federal Office of Education and Science and by the National Institutes of Health (NIH) grant 2 U01 HG02712-04. Additional support comes from the European Commission contract SLING (226073).

# REFERENCES


1. Weitzman, I., Summerbell, R. C., The dermatophytes. *Clin. Microbiol. Rev.* 1995, *8*, 240-259.
2. Taplin, D., Zaias, N., Rebell, G., Blank, H., Isolation and recognition of dermatophytes on a new medium (DTM). *Arch. Derm.* 1969, *99*, 203-209.
3. Li, X. F., Shen, Y. N., Chen, W., Chen, H. *et al.*, A new medium for diagnosis of dermatophyte infection. *Eur. J. Dermatol.* 2009, *19*, 34-37.
4. Jousson, O., Léchenne, B., Bontems, O., Capoccia, S. *et al.*, Multiplication of an ancestral gene encoding secreted fungalysin preceded species differentiation in the dermatophytes *Trichophyton* and *Microsporum*. *Microbiology* 2004, *150*, 301-310.
5. Jousson, O., Léchenne, B., Bontems, O., Mignon, B. *et al.*, Secreted subtilisin gene family in *Trichophyton rubrum. Gene* 2004, *339*, 79-88.
6. Giddey, K., Favre, B., Quadroni, M., Monod, M., Closely related dermatophyte species produce different patterns of secreted proteins. *FEMS Microbiol. Lett.* 2007, 267, 95-101.
7. Giddey, K., Monod, M., Barblan, J., Potts, A. *et al.*, Comprehensive analysis of proteins secreted by *Trichophyton rubrum* and *Trichophyton violaceum* under in vitro conditions. *J. Proteome Res.* 2007, *6*, 3081-3092.
8. Monod, M., Léchenne, B., Jousson, O., Grand, D. *et al.*, Aminopeptidases and dipeptidyl-peptidases secreted by the dermatophyte *Trichophyton rubrum*. *Microbiology* 2005; *151*, 145-155.
9. Zaugg, C., Jousson, O., Léchenne, B., Staib, P., Monod, M., *Trichophyton rubrum* secreted and membrane-associated carboxypeptidases. *Int. J. Med. Microbiol.* 2008, *298*, 669-682.
10. Vermout, S., Baldo, A., Tabart, J., Losson, B, Mignon, B., Secreted dipeptidyl peptidases as potential virulence factors for *Microsporum canis*. *FEMS Immunol. Med. Microbiol.* 2008, *54*, 299-308.
11. Burmester, A., Shelest, E., Glöckner, G., Heddergott, C. *et al.*, Comparative and functional genomics provide insights into the pathogenicity of dermatophytic fungi. *Genome Biol.* 2011, *12*, R7.
12. Reichard, U., Monod, M., Rüchel, R., Molecular cloning and sequencing of the gene encoding an extracellular aspartic proteinase from *Aspergillus fumigatus*. *FEMS Microbiol Lett* 1995, *130*, 69-74.

13. Reichard, U., Léchenne, B., Asif, A. R., Streit, F. *et al.*, Sedolisins, as new class of secreted proteases from *Aspergillus fumigatus* with endoprotease or tripeptidyl-peptidase activity at acidic pH. *Appl. Environ. Microbiol.* 2006, *72*, 1739-1748.
14. Tsuboi, R., Ko, I. J., Takamori, K., Ogawa, H., Isolation of a keratinolytic proteinase from *Trichophyton mentagrophytes* with enzymatic activity at acidic pH. *Infect. Immun*. 1989, *57*, 3479-3483.
15. Mathy, A., Baldo, A., Schoofs, L., Cambier, L. *et al.*, Fungalysin and dipeptidyl-peptidase gene transcription in *Microsporum canis* strains isolated from symptomatic and asymptomatic cats. *Vet. Microbiol.* 2010, *146*, 179-182.
16. Fumeaux, J., Mock, M., Ninet, B., Jan, I. *et al.*, First report of *Arthroderma benhamiae* in Switzerland. *Dermatology* 2004, *208*, 244-250.
17. Wilm, M., Shevchenko, A., Houthaeve, T., Breit, S. *et al.*, Femtomole sequencing of proteins from polyacrylamide gels by nano-electrospray mass spectrometry. *Nature* 1996, *379*, 466-469.
18. Shevchenko, A., Wilm, M., Vorm, O., Jensen, O. N. *et al.*, A strategy for identifying gel-separated proteins in sequence databases by MS alone. *Biochem. Soc. Trans*. 1996, *24*, 893-896.
19. Zaugg, C., Monod, M., Weber, J., Harshman, K. *et al.*, Gene expression profiling in the human pathogenic dermatophyte *Trichophyton rubrum* during growth on proteins. *Eukaryotic Cell* 2009, *8*, 241-250.
20. Staib, P., Zaugg, C., Mignon, B., Weber, J. *et al.*, Differential gene expression in the pathogenic dermatophyte *Arthroderma benhamiae* in vitro versus during infection. *Microbiology* 2010, *156*, 884-895.
21. Liu H, Sadygov R G, Yates J R 3rd. A model for random sampling and estimation of relative protein abundance in shotgun proteomics. *Anal. Chem.* 2004, *76*, 4193-4201.
22. Reichard, U., Büttner, S., Eiffert, H., Staib, F., Rüchel, R., Purification and characterisation of an extracellular serine proteinase from *Aspergillus fumigatus* and its detection in tissue. *J. Med. Microbiol.* 1990, *33*, 243-251.
23. Monod, M., Paris, S., Sanglard, D., Jaton-Ogay, K. *et al.*, Isolation and characterization of a secreted metalloprotease of *Aspergillus fumigatus*. *Infect. Immun.* 1993, *61*, 4099-4104.
24. Reichard, U., Eiffert, H., Rüchel, R., Purification and characterization of an extracellular aspartic proteinase from *Aspergillus fumigatus*. *J. Med. Vet. Mycol*. 1994, *32*, 427-436.
25. Beauvais, A., Monod, M., Debeaupuis, J. P., Diaquin, M. *et al.*, Biochemical and antigenic characterization of a new dipeptidyl-peptidase isolated from *Aspergillus fumigatus*. *J. Biol. Chem.* 1997, *272*, 6238-6244.

26. Beauvais, A., Monod, M., Wyniger, J., Debeaupuis, J. P. *et al.*, Dipeptidyl-peptidase IV secreted by *Aspergillus fumigatus*, a fungus pathogenic to humans. *Infect. Immun.* 1997, *65*, 3042-3047.
27. Monod, M., Jousson, O., Reichard, U., *Aspergillus fumigatus* secreted proteases, in: Latgé, J. P., Steinbach, W. J. (Eds), *Aspergillus fumigatus and Aspergillosis*, ASM Press, Washington, D.C 2009, pp 87-106.
28. Sriranganadane, D., Waridel, P., Salamin, K., Reichard, U. *et al.*, *Aspergillus* protein degradation pathways with different secreted protease sets at neutral and acidic pH. *J. Proteome Res.* 2010, *9*, 3511-3519.
29. Byun, T., Kofod, L., Blinkovsky, A. Synergistic action of an X-prolyl dipeptidyl aminopeptidase and a non-specific aminopeptidase in protein hydrolysis. *J. Agric. Food Chem.* 2001, *49*, 2061-2063.
30. Scott, D. W., Miller, W. H., Griffin, C. E., Fungal skin diseases, in: Muller G. H. (Ed.), Small animal dermatology, 5th ed., W. B. Saunders, Philadelphia, Pa. 1995, pp. 329–391.
31. Schmid-Wendtner, M. H., Korting, H. C. The pH of the skin surface and its impact on the barrier function. *Skin Pharmacol. Physiol.* 2006, *19*, 296-302.
32. Sriranganadane, D., Reichard, U., Salamin, K., Fratti, M. *et al*., Secreted glutamic protease rescues aspartic protease Pep deficiency in *Aspergillus fumigatus* during growth in acidic protein medium. *Microbiology* 2011, *157*, 1541-1550.
33. Nierman, W. C., Pain, A., Anderson, M. J., Wortman, J. R., *et al.*, Genomic sequence of the pathogenic and allergenic filamentous fungus Aspergillus fumigatus. *Nature* 2005, *438*, 1151-1156.

# General conclusion

The two first projects unraveled the mechanisms by which endo- and exoproteases are secreted by *Aspergillus fumigatus* to produce pentapeptides to amino acids from a protein substrate. Transporters can absorb these molecules to support their growth. The projects also highlight the faculty of this fungus to grow in an acidic environment, and have allowed the "discovery" of new proteases, such as AfuS28, sedolisin and glutamic proteases. *Aspergillus fumigatus* uses several endoproteases such as pepsin and glutamic proteases (in a rescue mechanism) at acidic pH, and subtilisins and metalloproteases (Meps) at neutral pH. In a second step, to produce small peptides and amino acids, *Aspergillus fumigatus* secretes exopeptidases such as sedolisins and carboxypeptidases at acidic pH, as well as leucine amino peptidases (Laps), dipeptidylpeptidase (DppV) and other carboxypeptidases at neutral pH. For proline rich proteins, the fungus uses at acidic pH AfuS28 which cuts from the N-terminus after proline residues. At neutral pH, this task is accomplished by the DppIV peptidase which cuts after an X-Pro from the N-terminus. This information permitted to build a proteolytic model from which all of our hypothesis on dermatophytes secretomes will be based on. In this regard, the aim of our latest project was the prediction of proteolytic mechanisms used by these fungi.

The analysis of secreted proteases suggests a common basic mechanism for extracellular protein digestion in dermatophytes as well as in *Aspergillus* spp at acidic and neutral pH. However, families of genes encoding endoproteases of the subtilisin (S8), deuterolisin (M35) and fungalisin (M36) families have expanded in dermatophytes, several members of which are secreted by these fungi in a protein medium. In addition, various proteases belonging to other families are secreted by dermatophytes both at neutral and acidic pH. Therefore, alternative pathways of protein degradation are possible in dermatophytes (pathways of protein degradation appear not to be so strict as they were observed in Aspergillus). To summarize, both A. *benhamiae* and *M. canis* use the Subtilisin and Mep endoproteases system, and the combination of DppIV and Lap exopeptidases at neutral pH to completely degrade the substrate. We can notice the preference towards Lap2 instead of Lap1 in *A. benhaniae*. In acidic condition both dermatophytes use pepsin as an endoprotease and SedB and SedD as exopeptidases with a preference for SedB for *A. benhamiae*. A deuterolysin protease (NPII) is present only in M. canis in the acidic secretome; we believe that it could help Pep1 in his enzymatic activity. The presence of the S41 protease family in a high amounts in acidic and neutral conditions demonstrate a possible large range of endoproteolytic activity. We can make the hypothesis that NPII and S41 proteases provide Pep1 the same rescue mechanism that the glutamic protease provides to the *A. fumigatus* pepsin. While DppIV could

remove proline in a neutral condition, its function is carried out by a S28 protease in *A. benhamiae* and *M.canis*, similarly to AfuS28, in an acidic pH.

While dermatophytes grow well in hard keratin at neutral pH, they were however not able to grow well in a same substrate at acidic pH. Dermatophytes are alkaline fungi and it is likely that in natural conditions subtilisins, fungalisins, Laps and DppIV play a pivotal role in keratin degradation, recycling nitrogen from keratin. Dermatophytes originated in the soil where conditions are often acidic (acidic proteases can help the fungus to adapt to extreme conditions). The capacity to secrete different subtilisins, metalloproteases as well as acid and alkaline proteases likely reflects a selection process during evolution conferring these fungi the ability to adapt to different environmental conditions during infection and saprophytic growth.

This fundamental work was carried out to understand which proteases were necessary during the first step of dermatophyte skin infection which is the contact between dermatophytes and the epidermis keratin that fosters fungus growth. Understanding of how the proteolytic machinery is implemented in this fungus could give us valuable information on how the development of protease inhibitors could stop fungal expansion.

These proteases could be used for other applications, such as in the Coeliac disease which is an autoimmune disorder of the small intestine that occurs in genetically predisposed people of all ages (Cerf.Bensussan *et al.*, 2007). The majority of the proteins found in food that are responsible for the immune reaction in the Coeliac disease are Prolamins. The characterization of AfuS28 in the first project allowed another scientific group to use it to cut Prolamins, proline rich proteins. In the Coeliac disease a poor degradation of this protein leads to severe intestinal inflammation. The aim of the project was to utilize this enzyme to cut prolamin to avoid generation of allergenic peptides.

Another possible application for these proteases is in biochemistry, to analyze proline rich proteins that cannot be completely cut by trypsin. A mix of AfuS28 and trypsin could facilitate the cut and allow for a better MS analysis. In the same way, the characterization and the production of several recombinant enzymes secreted by the fungi could provide new tools in the proteomics fields.

# References

**Barret A.**, Rawlings N.D. and Woessner J.F., Handbook of proteolytic enzymes, 2edn. London: Elsevier, 2004

**Basrai M.A.**, Lubkowitz M.A., Perry J.R., Miller D., Krainer E., Naider F. and Becker J.M. Cloning of a Candida albicans peptide transport gene. *Microbiology.* 1995 May;141 ( Pt 5):1147-56.

**Beauvais A.**, Monod M., Debeaupuis J.P., Diaquin M., Kobayashi H., Latgé J.P. Biochemical and antigenic characterization of a new dipeptidyl-peptidase isolated from *Aspergillus fumigatus*. *J Biol Chem.* 1997a; 272(10):6238-44.

**Beauvais A.**, Monod M., Wyniger J., Debeaupuis J.P., Grouzmann E., Brakch N., Svab J., Hovanessian A.G., Latgé J.P. Dipeptidyl-peptidase IV secreted by *Aspergillus fumigatus*, a fungus pathogenic to humans. *Infect Immun.* 1997b; 65(8):3042-7.

**Beggah S.**, Léchenne B., Reichard U., Foundling S. and Monod M. Intra- and intermolecular events direct the propeptide-mediated maturation of the Candida albicans secreted aspartic proteinase Sap1p. Microbiology. 2000 Nov;146 ( Pt 11):2765-73.

**Biguet J.**, Tran Van Ky P., Fruit J., Andrieu S. Identification of chymotryptic activity in remarkable fractions of an antigenic extract of *Aspergillus fumigatus*. Significance for the immunologic diagnosis of aspergillosis. *Rev Immunol Ther Antimicrob.* 1967, *31* (4), 317-28.

**Blobel G and Dobberstein B.** Transfer of proteins across membranes. I. Presence of proteolytically processed and unprocessed nascent immunoglobulin light chains on membrane-bound ribosomes of murine myeloma. J Cell Biol. 1975 Dec;67(3):835-51.

**Burmester A.**, Shelest E., Glöckner G., Heddergott C., Schindler S., Staib P., Heidel A., Felder M., Petzold A., Szafranski K., Feuermann M., Pedruzzi I., Priebe S., Groth M., Winkler R., Li W., Kniemeyer O., Schroeckh V., Hertweck C., Hube B., White T. C., Platzer M., Guthke R., Heitman J., Wöstemeyer J., Zipfel P. F., Monod M., Brakhage A. A. Comparative and functional genomics provide insights into the pathogenicity of dermatophytic fungi. *Genome Biology* 2011, 12:R7

**Cerf-Bensussan N.**, Matysiak-Budnik T., Cellier C., Heyman M. Oral proteases: a new approach to managing coeliac disease. *Gut.* 2007 Feb;56(2):157-60.

**Doi Y.**, Lee B.R., Ikeguchi M., Ohoba Y., Ikoma T., Tero-Kubota S., Yamauchi S., Takahashi K. and Ichishima E. Substrate specificities of deuterolysin from Aspergillus oryzae and electron

paramagnetic resonance measurement of cobalt-substituted deuterolysin. Biosci Biotechnol Biochem. 2003 Feb;67(2):264-70.

**Doumas A.**, Van den Broek P., Affolter M., Monod M. Characterization of the prolyl dipeptidyl peptidase gene (dppIV) from the koji mold *Aspergillus oryzae*. *Appl Environ Microbiol.* 1998 Dec;64(12):4809-15.

**Edens L.**, Dekker P., van der Hoeven R., Deen F., de Roos A. and Floris R. Extracellular prolyl endoprotease from Aspergillus niger and its use in the debittering of protein hydrolysates. *J Agric Food Chem.* 2005, *53* (20), 7950-7.

**Eder J. and Fersht A.R.** Pro-sequence-assisted protein folding. Mol Microbiol. 1995 May;16(4):609-14.

**Fabre E.**, Nicaud J.M., Lopez M.C., Gaillardin C. Role of the proregion in the production and secretion of the *Yarrowia lipolytica* alkaline extracellular protease. *J Biol Chem.* 1991 Feb 25;266(6):3782-90.

**Frederick G.D.**, Rombouts P. and Buxton F.P. Cloning and characterisation of pepC, a gene encoding a serine protease from Aspergillus niger. Gene. 1993 Mar 15;125(1):57-64.

**Fresenius G.** Breitrage zur Mykologie. (1863)

**Frosco M.**, Chase T. Jr and Macmillan J.D. Purification and properties of the elastase from Aspergillus fumigatus. Infect Immun. 1992 Mar;60(3):728-34.

**Fukuda R.**, Umebayashi K., Horiuchi H., Ohta A., Takagi M. Degradation of *Rhizopus niveus* aspartic proteinase-I with mutated prosequences occurs in the endoplasmic reticulum of *Saccharomyces cerevisiae*. *J Biol Chem.* 1996 Jun 14;271(24):14252-5.

**Galagan J. E.**, Calvo S. E., Cuomo C., Ma L. J., Wortman J. R., Batzoglou S., Lee S. I., Baştürkmen M., Spevak C. C., Clutterbuck J., Kapitonov V., Jurka J., Scazzocchio C., Farman M., Butler J., Purcell S., Harris S., Braus G. H., Draht O., Busch S., D'Enfert C., Bouchier C., Goldman G. H., Bell-Pedersen D., Griffiths-Jones S., Doonan J. H., Yu J., Vienken K., Pain A., Freitag M., Selker E. U., Archer D. B., Peñalva M. A., Oakley B. R., Momany M., Tanaka T., Kumagai T., Asai K., Machida M., Nierman W. C., Denning D. W., Caddick M., Hynes M., Paoletti M., Fischer R., Miller B., Dyer P., Sachs M. S., Osmani S. A., Birren B. W. Sequencing of Aspergillus nidulans and comparative analysis with A. fumigatus and A. oryzae. Nature. 2005 Dec 22;438(7071):1105-15.

**Geiser D.M.**, Klich M.A., Frisvad J.C., Peterson S.W., Varga J., Samson R.A. The current status of species recognition and identification in *Aspergillus. Stud Mycol.* 2007;59:1-10.

**Giddey K.**, Favre B., Quadroni M. & Monod, M. Closely related dermatophyte species produce different patterns of secreted proteins. *FEMS microbial lett* 2007, 267, 95-101.

**Gräser Y.**, Scott J. and Summerbell R.C. The new species concept in dermatophytes, a polyphasic approach. Mycopathologia, 2008, 166, 239-256.

**Hauser M.**, Narita V., Donhardt A.M., Naider F., Becker J.M. Multiplicity and regulation of genes encoding peptide transporters in *Saccharomyces cerevisiae*. *Mol Membr Biol.* 2001 Jan-Mar;18(1):105-12.

**Hibbett D.S.**, Binder M., Bischoff J.F., Blackwell M., Cannon P.F., Eriksson O.E., Huhndorf S., James T., Kirk P.M., Lücking R., Thorsten Lumbsch H., Lutzoni F., Matheny P.B., McLaughlin D.J., Powell M.J., Redhead S., Schoch C.L., Spatafora J.W., Stalpers J.A., Vilgalys R., Aime M.C., Aptroot A., Bauer R., Begerow D., Benny G.L., Castlebury L.A., Crous P.W., Dai Y.C., Gams W., Geiser D.M., Griffith G.W., Gueidan C., Hawksworth D.L., Hestmark G., Hosaka K., Humber R.A., Hyde K.D., Ironside J.E., Kõljalg U., Kurtzman C.P., Larsson K.H., Lichtwardt R., Longcore J., Miadlikowska J., Miller A., Moncalvo J.M., Mozley-Standridge S., Oberwinkler F., Parmasto E., Reeb V., Rogers J.D., Roux C., Ryvarden L., Sampaio J.P., Schüssler A., Sugiyama J., Thorn R.G., Tibell L., Untereiner W.A., Walker C., Wang Z., Weir A., Weiss M., White M.M., Winka K., Yao Y.J., Zhang N. A higher-level phylogenetic classification of the Fungi. *Mycol Res.* 2007 May;111(Pt 5):509-47.

**Idnurm A.**, Bahn Y.S., Nielsen K., Lin X., Fraser J.A. and Heitman J. Deciphering the model pathogenic fungus Cryptococcus neoformans. Nat Rev Microbiol. 2005 Oct, 3(10), 753-64.

**Jaton-Ogay K.**, Suter M., Crameri R., Falchetto R., Fatih A. and Monod M. Nucleotide sequence of a genomic and a cDNA clone encoding an extracellular alkaline protease of Aspergillus fumigatus. FEMS Microbiol Lett. 1992 Apr 15;71(2):163-8.

**Jongeneel C.V.**, Bouvier J. and Bairoch A. A unique signature identifies a family of zinc-dependent metallopeptidases. FEBS Lett. 1989 Jan 2;242(2):211-4.

**Jousson O.**, Léchenne B., Bontems O., Mignon B., Reichard U., Barblan J., Quadroni M. & Monod M. Secreted subtilisin gene family in *Trichophyton rubrum. Gene* 2004a, 339, 79-88.

**Jousson O.**, Léchenne B., Bontems O., Capoccia S., Mignon B., Barblan J., Quadroni M. & Monod M. Multiplication of an ancestral gene encoding secreted fungalysin preceded species differentiation in the dermatophytes *Trichophyton* and *Microsporum. Microbiology* 2004b, 150, 301-310.

**Koh S.**, Wiles A.M., Sharp J.S., Naider F.R., Becker J.M., Stacey G. An oligopeptide transporter

gene family in *Arabidopsis*. *Plant Physiol.* 2002 Jan;128(1):21-9.

**Kolattukudy P.E.**, Lee J.D., Rogers L.M., Zimmerman P., Ceselski S., Fox B., Stein B. and Copelan E.A. Evidence for possible involvement of an elastolytic serine protease in aspergillosis. Infect Immun. 1993 Jun;61(6):2357-68.

**Kwon-Chung K. J. & Bennett J. E.**. Medical Mycology (1992). Philadelphia and London: Lea & Febiger.

**Larcher G.**, Bouchara J.P., Annaix V., Symoens F., Chabasse D. and Tronchin G. Purification and characterization of a fibrinogenolytic serine proteinase from Aspergillus fumigatus culture filtrate. FEBS Lett. 1992 Aug 10;308(1):65-9.

**Lee B.R.**, Furukawa M., Yamashita K., Kanasugi Y., Kawabata C., Hirano K., Ando K. and Ichishima E. Aorsin, a novel serine proteinase with trypsin-like specificity at acidic pH. Biochem J. 2003 Apr 15;371(Pt 2):541-8.

**Lubkowitz M.A.**, Hauser L., Breslav M., Naider F., Becker J.M. An oligopeptide transport gene from *Candida albicans*. *Microbiology.* 1997 Feb;143 ( Pt 2):387-96.

**Machida M.**, Asai K., Sano M., Tanaka T., Kumagai T., Terai G., Kusumoto K., Arima T., Akita O., Kashiwagi Y., Abe K., Gomi K., Horiuchi H., Kitamoto K., Kobayashi T., Takeuchi M., Denning D. W., Galagan J. E., Nierman W. C., Yu J., Archer D. B., Bennett J. W., Bhatnagar D., Cleveland T. E., Fedorova N. D., Gotoh O., Horikawa H., Hosoyama A., Ichinomiya M., Igarashi R., Iwashita K., Juvvadi P. R., Kato M., Kato Y., Kin T., Kokubun A., Maeda H., Maeyama N., Maruyama J., Nagasaki H., Nakajima T., Oda K., Okada K., Paulsen I., Sakamoto K., Sawano T., Takahashi M., Takase K., Terabayashi Y., Wortman J. R., Yamada O., Yamagata Y., Anazawa H., Hata Y., Koide Y., Komori T., Koyama Y., Minetoki T., Suharnan S., Tanaka A., Isono K., Kuhara S., Ogasawara N., Kikuchi H.. Genome sequencing and analysis of Aspergillus oryzae. Nature. 2005 Dec 22;438(7071):1157-61.

**Marie-Claire C.**, Roques B.P. and Beaumont A. Intramolecular processing of prothermolysin. J Biol Chem. 1998 Mar 6;273(10):5697-701.

**Markaryan A.**, Morozova I., Yu H. and Kolattukudy P.E. Purification and characterization of an elastinolytic metalloprotease from Aspergillus fumigatus and immunoelectron microscopic evidence of secretion of this enzyme by the fungus invading the murine lung. Infect Immun. 1994 Jun;62(6):2149-57.

**Mathy A.**, Baldo A., Schoofs L., Cambier L., Defaweux V., Tabart J., Maréchal F., Symoens F., Mignon B. Fungalysin and dipeptidyl-peptidase gene transcription in Microsporum canis strains isolated from symptomatic and asymptomatic cats. Vet Microbiol. 2010 Nov 20;146(1-2):179-82.

**Milstein C.**, Brownlee G.G., Harrison T.M., Mathews M.B. A possible precursor of immunoglobulin light chains. *Nat New Biol.* 1972 Sep 27;239(91):117-20.

**Moehle C.M.**, Tizard R., Lemmon S.K., Smart J., Jones E.W. Protease B of the lysosomelike vacuole of the yeast *Saccharomyces cerevisiae* is homologous to the subtilisin family of serine proteases. *Mol Cell Biol.* 1987 Dec;7(12):4390-9.

**Monod M.**, Baudraz-Rosselet F., Ramelet A.A., Frenk E. Direct mycological examination in dermatology: a comparison of different methods. *Dermatologica*. 1989;179(4):183-6.

**Monod M.**, Togni G., Rahalison L., and Frenk E. Isolation and characterisation of an extracellular alkaline protease of *Aspergillus fumigatus*. *J. Med. Microbiol.* 1991, *35* (1), 23-8.

**Monod M.**, Paris S., Sanglard D., Jaton-Ogay K., Bille J. and Latgé J.P. Isolation and characterization of a secreted metalloprotease of *Aspergillus fumigatus*. *Infect. Immun.* 1993, *61* (10), 4099-104.

**Monod M.**, Jaccoud S., Zaugg C., Léchenne B., Baudraz F. & Panizzon R. Survey of dermatophyte infections in Lausanne area (Switzerland). *Dermatology* 2002, 205, 201-203.

**Monod M.**, Lechenne B., Jousson O., Grand D., Zaugg C., Stocklin R. and Grouzmann E. Aminopeptidases and dipeptidyl-peptidases secreted by the dermatophyte *Trichophyton rubrum*. *Microbiology* 2005, *151* (Pt 1), 145-55.

**Monod M.**, Jousson O., & Reichard U.. *Aspergillus fumigatus* Secreted Proteases edited in *Aspergillus fumigatus* and Aspergillosis by J-P Latgé and W. J. Steinbach, ASM Press (2008).

**Monod M.**, Reichard U., Staib P., Jousson O., Fungal aspartic proteases as possible therapeutic targets. pp. 573-606. In A. K. Ghosh (ed.), Aspartic proteases as therapeutic targets. Methods and principles in medicinal chemistry (Volume 45). Wiley-VCH Verlag, Weinheim (Germany), 2010.

**Newport G.**, Agabian N. KEX2 influences Candida albicans proteinase secretion and hyphal formation. J Biol Chem. 1997 Nov 14;272(46):28954-61.

**Nierman W. C.**, Pain A., Anderson M. J., Wortman J. R., Kim H. S., Arroyo J., Berriman M., Abe K., Archer D. B., Bermejo C., Bennett J., Bowyer P., Chen D., Collins M., Coulsen R., Davies R., Dyer P. S., Farman M., Fedorova N., Fedorova N., Feldblyum T. V., Fischer R., Fosker N.,

Fraser A., García J. L., García M. J., Goble A., Goldman G. H., Gomi K., Griffith-Jones S., Gwilliam R., Haas B., Haas H., Harris D., Horiuchi H., Huang J., Humphray S., Jiménez J., Keller N., Khouri H., Kitamoto K., Kobayashi T., Konzack S., Kulkarni R., Kumagai T., Lafon A., Latgé J. P., Li W., Lord A., Lu C., Majoros W. H., May G. S., Miller B. L., Mohamoud Y., Molina M., Monod M., Mouyna I., Mulligan S., Murphy L., O'Neil S., Paulsen I., Peñalva M. A., Pertea M., Price C., Pritchard B. L., Quail M. A., Rabbinowitsch E., Rawlins N., Rajandream M. A., Reichard U., Renauld H., Robson G. D., Rodriguez de Córdoba S., Rodríguez-Peña J. M., Ronning C. M., Rutter S., Salzberg S. L., Sanchez M., Sánchez-Ferrero J. C., Saunders D., Seeger K., Squares R., Squares S., Takeuchi M., Tekaia F., Turner G., Vazquez de Aldana C. R., Weidman J., White O., Woodward J., Yu J. H., Fraser C., Galagan J. E., Asai K., Machida M., Hall N., Barrell B., Denning D. W. Genomic sequence of the pathogenic and allergenic filamentous fungus Aspergillus fumigatus. Nature. 2005 Dec 22;438(7071):1151-6.

**Perry J.R.**, Basrai M.A., Steiner H.Y., Naider F., and Becker J.M. Isolation and characterization of a Saccharomyces cerevisiae peptide transport gene. *Mol Cell Biol.* 1994 January; 14(1): 104–115.

**Pfeffer S.R. and Rothman J.E.** Biosynthetic protein transport and sorting by the endoplasmic reticulum and Golgi. Annu Rev Biochem. 1987, 56, 829-52

**Rapper K.B. & Fennel D.I.** The genus Aspergillus. (1965)

**Reichard U.**, Büttner S., Eiffert H., Staib F. and Rüchel R. Purification and characterisation of an extracellular serine proteinase from *Aspergillus fumigatus* and its detection in tissue. *J. Med. Microbiol.* 1990, *33* (4), 243-51.

**Reichard U.**, Cole G.T., Rüchel R. and Monod M. Molecular cloning and targeted deletion of PEP2 which encodes a novel aspartic proteinase from Aspergillus fumigatus. Int J Med Microbiol. 2000 Mar;290(1):85-96.

**Reichard U.**, Léchenne B., Asif A. R., Streit F., Grouzmann E., Jousson O. and Monod M. Sedolisins, as new class of secreted proteases from *Aspergillus fumigatus* with endoprotease or tripeptidyl-peptidase activity at acidic pH. Appl. Environ. Microbiol. 2006, 72:1739-1748.

**Reuss O.**, Morschhäuser J. A family of oligopeptide transporters is required for growth of *Candida albicans* on proteins. *Mol Microbiol.* 2006 May;60(3):795-812.

**Samson R.A.** The genus Aspergillus with special regard to the Aspergillus fumigatus group. Contrib Microbiol 1999, 2, 5-20.

**Samson R.A**., Varga J., Witiak S.M. and Geiser D.M. The species concept in *Aspergillus*: recommendations of an international panel. *Stud Mycol.* 2007, 59: 71–73.

**Stepniak D.**, Spaenij-Dekking L., Mitea C., Moester M., de Ru A., Baak-Pablo R., van Veelen P., Edens L. and Koning F. Highly efficient gluten degradation with a newly identified prolyl endoprotease: implications for celiac disease. Am J Physiol Gastrointest Liver Physiol. 2006 Oct;291(4):G621-9.

**Szecsi P.B.**The aspartic proteases. Scand J Clin Lab Invest Suppl. 1992; 210:5-22.

**Szewczyk E., Krappmann S.** Conserved regulators of mating are essential for Aspergillus fumigatus cleistothecium formation. Eukaryotic Cell 2010 May; 9(5):774-83.

**Togni G.**, Sanglard D., Quadroni M., Foundling S.I. and Monod M. Acid proteinase secreted by Candida tropicalis: functional analysis of preproregion cleavages in C. tropicalis and Saccharomyces cerevisiae. Microbiology. 1996 Mar;142 (Pt 3):493-503.

**Von Heijne G.** A new method for predicting signal sequence cleavage sites. *Nucleic Acids Res.* 1986*, 14* (11), 4683-90.

**Weitzman I.**, McGinnis M. R., Padhye A. A. and Ajello L. The genus Arthroderma and its later synonym Nannizzia. Mycotaxon 1986, *25*: 505-18.

**Weitzman I. & Summerbell R. C.**. The dermatophytes. *Clin Microbiol* 1995 *Rev* 8, 240-249.

**Wu Y.**, Yang J., Yang F., Liu T., Leng W., Chu Y. and Jin Q., Recent dermatophy divergence revealed by comparative and phylogenetic analysis of mitochondrial genomes. BMC Genomics, 2009, 10, 238.

**Zaugg C.**, Jousson O., Léchenne B., Staib P. and Monod M. Trichophyton rubrum secreted and membrane-associated carboxypeptidases. Int J Med Microbiol. 2008 Oct;298(7-8):669-82. Epub 2008 Jan 28.

# Annexes

# Gel silver staining

After a SDS PAGE migration of your samples, the gel is successively under different buffer:

- Fixation: 2x30 min in 10% acetic acid (v/v) and 30% ethanol (v/v)
- Sensibilization: Over night on a buffer containing 0.3% sodium thiosulfate (w/v), 0.5M Potassium acetate, 25% ethanol (v/v) and 0.5% Glutaraldehyde(v/v).
- Washing step: 4x10 min with distilled water.
- Staining: one hour on dark room with a solution composed of 0.4% (w/v) of nitrate silver and 0.02% of formaldehyde (v/v).
- Washing step: 2x1 min with distilled water.
- Developing: on 3% potassium carbonate buffer (w/v) on which you add at the last moment 0.04% of formaldehyde (v/v).
- Before the staining become too dark, stop the development with the “Stop” buffer composed of 4% Tris (w/v) and 2% acetic acid (v/v).

All those steps are done under shaking on a rocking chair at room temperature.

# Protein precipitation by Sodium deoxycholate (DOC)/Trichloroacetic acid(TCA)/Acetone

- For 0.1ml of concentrated sample add 0.9mol of DOC 0.2% (already at 4°C), vortex it and incubate it at 4°C during 10min.
- Vortex and add 0.1ml of TCA 100%. The mix must incubate at 4°C during 10min.
- Centrifuge the mix at 13.3 rpm during 12min at 4°C and remove the supernatant.
- Resuspend the pellet with 1ml of Acetone (100%) and vortex it until the complete disaggregation of the pellet.
- Centrifuge one more time, at 13.3 rpm during 12min at 4°C and remove the supernatant.
- Dry the pellet at room temperature.

## Supplementary Tables and Figures Legends for the Part 1.

0.015nmol of SedB (or both of them) and 0.05 mmol of histidine in acidic buffer pH 4 (Formic acid ~0.0125 %) and was incubated at 37°C during 1h. Reaction was stopped by adding 0.5% formic acid. All samples were diluted 10 times in $H_2O$:MeCN 50:50 (+ 0.1% formic acid) and infused in the LTQ-Orbitrap via the Nanomate**. (p. 130)**

**Supplementary Figures 6: Apelin-13 degradation by AfuS28 and SedB**
The reactional medium contains 15 nmol of Apelin-13, 0.02nmol of AfuS28 and/or 0.015nmol of SedB (or both of them) and 0.05 mmol of histidine in acidic buffer pH 4 (Formic acid ~0.0125 %) and was incubated at 37°C during 1h. Reaction was stopped by adding 0.5% formic acid. All samples were diluted 10 times in $H_2O$:MeCN 50:50 (+ 0.1% formic acid) and infused in the LTQ-Orbitrap via the Nanomate**. (p. 130)**

**Supplementary Figures 7: Dynorphin-A degradation by AfuS28 and SedB**
The reactional medium contains 15 nmol of Dynorphin-A, 0.02nmol of AfuS28 and/or 0.015nmol of SedB (or both of them) and 0.05 mmol of histidine in acidic buffer pH 4 (Formic acid ~0.0125 %) and was incubated at 37°C during 1h. Reaction was stopped by adding 0.5% formic acid. All samples were diluted 10 times in $H_2O$:MeCN 50:50 (+ 0.1% formic acid) and infused in the LTQ-Orbitrap via the Nanomate**. (p. 130)**

## Supplementary Table 1

P1: 5'-GTTT*CTCGAG*CACTCATGCCCAGGGCGCCTT-3'
P2: 5'-TGA*GAGCTC*CCAACCCGAACATCTC-3'
P3: 5'-TGG*GAGCTC*TCAAGCATTTTGACT-3'
P4: 5'-GTTT*AGATCT*CATGGCTTCCTATATTTGGG-3'
P5: 5'-GTTT*AGATCT*CAGTGATGGTGATGGTGATGTGGCTTCCTATATTTGGG-3'
P6: 5'-GTT*CCATGG*GTGGCTTTGGCAGGATATGAAT-3'
P7: 5'-CTT*GGATCC*TCATGGCTTCCTATATTTGGG-3'

## Supplementary Table 2

| Identified Proteins (171) | Accession Number | Molecular Weight | Secretome A. fumigatus (number of spectra detected by MS) | |
|---|---|---|---|---|
| | | | pH-3.5 | pH-7 |
| Secreted dipeptidyl peptidase - Aspergillus fumigatus A1163 | B0XRV0 | 80 kDa | 13 | 466 |
| Alkaline serine protease Alp1 - Aspergillus fumigatus A1163 | B0Y708 | 42 kDa | 0 | 433 |
| Tripeptidyl-peptidase - Aspergillus fumigatus A1163 | B0YEL1 | 66 kDa | 315 | 0 |
| Mannosidase MsdS - Aspergillus fumigatus A1163 | B0XMT4 | 54 kDa | 93 | 170 |
| Aspartic endopeptidase Pep1 - Aspergillus fumigatus A1163 | B0Y1V8 | 42 kDa | 240 | 0 |
| Beta-D-glucoside glucohydrolase - Aspergillus fumigatus A1163 | B0YB65 | 78 kDa | 61 | 131 |
| Glutaminase GtaA - Aspergillus fumigatus A1163 | B0XYT5 | 76 kDa | 20 | 161 |
| FG-GAP repeat protein, putative - Aspergillus fumigatus (Sartorya fumigata) | Q4WK08 | 34 kDa | 4 | 171 |
| Aminopeptidase Y, putative - Aspergillus fumigatus A1163 | B0XX53 | 54 kDa | 0 | 133 |
| Mycelial catalase Cat1 - Aspergillus fumigatus A1163 | B0Y0G0 | 80 kDa | 5 | 129 |
| Carboxypeptidase S1, putative - Aspergillus fumigatus A1163 | B0Y1L0 | 54 kDa | 123 | 0 |
| FAD-dependent oxygenase, putative - Aspergillus fumigatus A1163 | B0XX33 | 55 kDa | 67 | 42 |
| Extracellular lipase, putative - Aspergillus fumigatus A1163 | B0Y214 | 31 kDa | 9 | 101 |
| Glucan 1,4-alpha-glucosidase, putative - Aspergillus fumigatus A1163 | B0XSV7 | 67 kDa | 83 | 35 |
| Serine peptidase, putative - Aspergillus fumigatus A1163 | B0XT80 | 59 kDa | 57 | 45 |
| Chitosanase precursor - Aspergillus fumigatus (Sartorya fumigata) | Q709P2 | 25 kDa | 104 | 0 |
| Beta-fructofuranosidase, putative - Aspergillus fumigatus A1163 | B0XT79 | 57 kDa | 50 | 32 |
| Major allergen Asp F1 - Aspergillus fumigatus A1163 | B0Y2B4 | 20 kDa | 70 | 0 |
| GPI-anchored cell wall beta-1,3-endoglucanase EglC - Aspergillus fumigatus A1163 | B0XXF8 | 45 kDa | 33 | 44 |
| Carboxypeptidase 5 - Aspergillus fumigatus (Sartorya fumigata) | Q5VJG7 | 60 kDa | 45 | 0 |
| FAD/FMN-containing isoamyl alcohol oxidase MreA - Aspergillus fumigatus A1163 | B0YD87 | 61 kDa | 36 | 21 |
| Isoamyl alcohol oxidase, putative - Aspergillus fumigatus (Sartorya fumigata) | A4D9R5 | 61 kDa | 32 | 33 |
| Beta-N-acetylhexosaminidase NagA, putative - Aspergillus fumigatus A1163 | B0Y9W3 | 67 kDa | 37 | 24 |
| Cell wall serine-threonine-rich galactomannoprotein Mp1 - Aspergillus fumigatus A1163 | B0YEP2 | 27 kDa | 12 | 47 |
| Extracellular dipeptidyl-peptidase Dpp4 - Aspergillus fumigatus A1163 | B0Y6C5 | 86 kDa | 0 | 54 |
| Beta glucosidase, putative - Aspergillus fumigatus A1163 | B0Y7Q8 | 95 kDa | 0 | 48 |
| Mannosidase I - Aspergillus fumigatus (Sartorya fumigata) | Q6PWQ1 | 55 kDa | 17 | 30 |
| Phytase, putative - Aspergillus fumigatus A1163 | B0YBU1 | 57 kDa | 48 | 0 |
| Cell wall protein, putative - Aspergillus fumigatus (Sartorya fumigata) | Q4WFT1 | 19 kDa | 0 | 46 |
| Exo-beta-1,3-glucanase, putative - Aspergillus fumigatus A1163 | B0XLY8 | 84 kDa | 6 | 36 |
| Beta-galactosidase, putative - Aspergillus fumigatus (Sartorya fumigata) | Q4WS33 | 112 kDa | 38 | 0 |
| Carboxypeptidase 4 - Aspergillus fumigatus (Sartorya fumigata) | Q5VJG8 | 58 kDa | 43 | 0 |
| Putative uncharacterized protein - Aspergillus fumigatus A1163 | B0Y501 | 23 kDa | 37 | 7 |
| Acid phosphatase, putative - Aspergillus fumigatus A1163 | B0YEJ1 | 46 kDa | 44 | 0 |
| Thioredoxin reductase, putative - Aspergillus fumigatus A1163 | B0Y2V0 | 43 kDa | 12 | 25 |
| Putative uncharacterized protein - Aspergillus fumigatus A1163 | B0XVH5 | 42 kDa | 19 | 20 |
| 1,3-beta-glucanosyltransferase Bgt1 - Aspergillus fumigatus A1163 | B0XQR5 | 33 kDa | 31 | 0 |
| Oxidoreductase, FAD-binding, putative - Aspergillus fumigatus A1163 | B0XU27 | 50 kDa | 14 | 12 |
| Bifunctional catalase-peroxidase Cat2 - Aspergillus fumigatus A1163 | B0YAK0 | 84 kDa | 0 | 37 |
| Endonuclease/exonuclease/phosphatase family protein - Aspergillus fumigatus A1163 | B0XY76 | 64 kDa | 3 | 29 |
| Beta galactosidase, putative - Aspergillus fumigatus A1163 | B0XNY2 | 112 kDa | 14 | 18 |

| Identified Proteins (171) | Accession Number | Molecular Weight | Secretome A. fumigatus (number of spectra detected by MS) | |
|---|---|---|---|---|
| | | | pH-3.5 | pH-7 |
| Class V chitinase ChiB1 - Aspergillus fumigatus A1163 | B0YAM7 | 48 kDa | 0 | 30 |
| 1,3-beta-glucanosyltransferase Gel1 - Aspergillus fumigatus A1163 | B0XT72 | 48 kDa | 22 | 4 |
| Tripeptidyl peptidase A - Aspergillus fumigatus A1163 | B0Y502 | 64 kDa | 26 | 0 |
| IgE-binding protein - Aspergillus fumigatus A1163 | B0YDX1 | 20 kDa | 0 | 28 |
| Alpha-galactosidase, putative - Aspergillus fumigatus A1163 | B0YDJ1 | 56 kDa | 15 | 9 |
| Alpha-1,2-mannosidase family protein, putative - Aspergillus fumigatus A1163 | B0XQJ8 | 93 kDa | 19 | 10 |
| Extracellular cell wall glucanase Crf1/allergen Asp F9 - Aspergillus fumigatus A1163 | B0XNL0 | 40 kDa | 5 | 24 |
| Alpha-1,3-glucanase/mutanase, putative - Aspergillus fumigatus A1163 | B0XUS0 | 54 kDa | 0 | 22 |
| Extracellular serine-rich protein, putative - Aspergillus fumigatus A1163 | B0XYK6 | 84 kDa | 0 | 23 |
| Putative uncharacterized protein - Aspergillus fumigatus A1163 | B0YEP7 | 49 kDa | 0 | 27 |
| Extracellular arabinanase, putative - Aspergillus fumigatus A1163 | B0YDT3 | 45 kDa | 0 | 21 |
| 1,3-beta-glucanosyltransferase, putative - Aspergillus fumigatus A1163 | B0XVI5 | 59 kDa | 14 | 0 |
| Alpha-glucosidase, putative - Aspergillus fumigatus A1163 | B0XNL6 | 99 kDa | 0 | 19 |
| Acid sphingomyelinase, putative - Aspergillus fumigatus A1163 | B0Y5K6 | 68 kDa | 0 | 23 |
| Cell wall protein PhiA - Aspergillus fumigatus (Sartorya fumigata) | A4FSH5 | 19 kDa | 4 | 19 |
| Carboxypeptidase S1, putative - Aspergillus fumigatus A1163 | B0YA52 | 61 kDa | 22 | 0 |
| Aspergillopepsin, putative - Aspergillus fumigatus A1163 | B0Y015 | 28 kDa | 23 | 0 |
| Alpha-L-arabinofuranosidase A - Aspergillus fumigatus A1163 | B0XUG6 | 72 kDa | 13 | 10 |
| Alpha,alpha-trehalose glucohydrolase TreA/Ath1 - Aspergillus fumigatus A1163 | B0Y0F9 | 117 kDa | 14 | 4 |
| Alpha-galactosidase - Aspergillus fumigatus A1163 | B0Y224 | 47 kDa | 15 | 0 |
| Extracellular endo-polygalacturonase, putative - Aspergillus fumigatus (Sartorya fumigata) | Q4WBE1 | 38 kDa | 21 | 0 |
| Endo-1,4-beta-xylanase - Aspergillus fumigatus A1163 | B0XXF3 | 24 kDa | 4 | 12 |
| Beta-1,6-glucanase, putative - Aspergillus fumigatus A1163 | B0Y9D8 | 51 kDa | 20 | 0 |
| Alpha-glucosidase AgdA, putative - Aspergillus fumigatus A1163 | B0Y6K4 | 108 kDa | 19 | 0 |
| Extracelular serine carboxypeptidase, putative - Aspergillus fumigatus A1163 | B0XVM7 | 55 kDa | 7 | 6 |
| Endo-1,3(4)-beta-glucanase, putative - Aspergillus fumigatus A1163 | B0Y002 | 31 kDa | 0 | 15 |
| Endo-arabinase, putative - Aspergillus fumigatus A1163 | B0XU55 | 36 kDa | 0 | 16 |
| Putative uncharacterized protein - Aspergillus fumigatus A1163 | B0XP19 | 33 kDa | 9 | 4 |
| Allergen Asp f 15 precursor - Aspergillus fumigatus (Sartorya fumigata) | AL15 | 16 kDa | 2 | 8 |
| Neutral/alkaline nonlysosomal ceramidase, putative - Aspergillus fumigatus A1163 | B0XPL9 | 83 kDa | 0 | 13 |
| Fucose-specific lectin - Aspergillus fumigatus (Sartorya fumigata) | Q8NJT4 | 35 kDa | 4 | 11 |
| Aldose 1-epimerase, putative - Aspergillus fumigatus A1163 | B0XWH7 | 51 kDa | 5 | 0 |
| Mutanase - Aspergillus fumigatus A1163 | B0Y904 | 138 kDa | 0 | 15 |
| Amidase, putative - Aspergillus fumigatus A1163 | B0Y0P8 | 65 kDa | 13 | 0 |
| Alpha-N-acetylglucosaminidase, putative - Aspergillus fumigatus A1163 | B0XRY5 | 86 kDa | 12 | 0 |
| Beta-lactamase, putative - Aspergillus fumigatus A1163 | B0Y2K9 | 48 kDa | 12 | 0 |
| Class V chitinase, putative - Aspergillus fumigatus A1163 | B0XXM2 | 44 kDa | 0 | 16 |
| Thioredoxin reductase GliT - Aspergillus fumigatus A1163 | B0Y818 | 36 kDa | 0 | 15 |
| Endo-1,3-beta-glucanase Engl1 - Neosartorya fischeri | A1D4K0_NEOFI | 78 kDa | 0 | 14 |
| Extracellular phytase, putative - Neosartorya fischeri | A1DJ51_NEOFI | 58 kDa | 14 | 0 |

| Identified Proteins (171) | Accession Number | Molecular Weight | Secretome A. fumigatus (number of spectra detected by MS) | |
|---|---|---|---|---|
| | | | pH-3.5 | pH-7 |
| Cell wall glucanase - Aspergillus fumigatus A1163 | B0XUB8 | 47 kDa | 12 | 0 |
| Extracellular lipase, putative - Aspergillus fumigatus A1163 | B0YAB3 | 61 kDa | 0 | 11 |
| Tyrosinase, putative - Aspergillus fumigatus A1163 | B0XX11 | 42 kDa | 0 | 14 |
| Putative uncharacterized protein - Aspergillus fumigatus A1163 | B0XVQ9 | 20 kDa | 0 | 15 |
| Oxalate decarboxylase, putative - Aspergillus fumigatus (Sartorya fumigata) | Q4X060 | 49 kDa | 11 | 0 |
| Alpha-L-rhamnosidase C, putative - Aspergillus fumigatus A1163 | B0Y024 | 88 kDa | 0 | 11 |
| Cellulase family protein - Aspergillus fumigatus A1163 | B0Y2L4 | 43 kDa | 4 | 5 |
| DUF1237 domain protein - Aspergillus fumigatus A1163 | B0XVA1 | 59 kDa | 6 | 5 |
| Asp-hemolysin precursor - Aspergillus fumigatus (Sartorya fumigata) | ASPH | 15 kDa | 13 | 0 |
| NlpC/P60-like cell-wall peptidase, putative - Aspergillus fumigatus A1163 | B0Y269 | 39 kDa | 9 | 0 |
| Putative uncharacterized protein - Aspergillus fumigatus A1163 | B0Y742 | 79 kDa | 9 | 0 |
| ML domain protein - Neosartorya fischeri | A1DH49_NEOFI | 19 kDa | 0 | 8 |
| Metallopeptidase MepB - Aspergillus fumigatus A1163 | B0YB44 | 82 kDa | 0 | 5 |
| Putative uncharacterized protein - Aspergillus fumigatus A1163 | B0Y1A9 | 69 kDa | 0 | 9 |
| Alpha-amylase, putative - Neosartorya fischeri | A1CYB1_NEOFI | 69 kDa | 0 | 10 |
| Isoamyl alcohol oxidase, putative - Aspergillus fumigatus A1163 | B0YBR0 | 112 kDa | 4 | 2 |
| Isoamyl alcohol oxidase - Aspergillus fumigatus A1163 | B0XYQ0 | 66 kDa | 9 | 0 |
| Aldehyde dehydrogenase AldA, putative - Aspergillus fumigatus A1163 | B0Y8I3 | 61 kDa | 0 | 6 |
| Mn superoxide dismutase - Aspergillus fumigatus A1163 | B0Y6Y9 | 25 kDa | 0 | 9 |
| Autophagic serine protease Alp2 - Neosartorya fischeri | A1DER5_NEOFI | 53 kDa | 0 | 10 |
| Cutinase, putative - Aspergillus fumigatus A1163 | B0XRY3 | 22 kDa | 0 | 9 |
| Glycosyl hydrolase, putative - Aspergillus fumigatus A1163 | B0Y4Y2 | 37 kDa | 10 | 0 |
| Gamma-glutamyltranspeptidase - Aspergillus fumigatus A1163 | B0YCI4 | 54 kDa | 9 | 0 |
| 1,3-beta-glucanosyltransferase Gel2 - Aspergillus fumigatus A1163 | B0Y8H9 | 52 kDa | 0 | 9 |
| Putative uncharacterized protein - Aspergillus fumigatus A1163 | B0XZT4 | 9 kDa | 0 | 10 |
| Ribonuclease T2, putative - Neosartorya fischeri | A1D1B0_NEOFI | 32 kDa | 0 | 3 |
| Extracellular cellulase CelA/allergen Asp F7-like, putative - Aspergillus fumigatus A1163 | B0Y3V5 | 36 kDa | 6 | 0 |
| Oligopeptidase family protein - Aspergillus fumigatus A1163 | B0Y9Z2 | 80 kDa | 0 | 5 |
| 1,3-beta-glucanosyltransferase Gel3 - Aspergillus fumigatus A1163 | B0XT09 | 57 kDa | 8 | 0 |
| Xylosidase/arabinosidase, putative - Aspergillus fumigatus A1163 | B0XV35 | 41 kDa | 0 | 5 |
| Aspartic endopeptidase Pep2 - Aspergillus fumigatus A1163 | B0XXK9 | 43 kDa | 0 | 7 |
| Spermidine synthase - Neosartorya fischeri | A1D269_NEOFI | 33 kDa | 0 | 5 |
| NAD-dependent formate dehydrogenase AciA/Fdh - Aspergillus fumigatus A1163 | B0YCV9 | 46 kDa | 0 | 3 |
| Elastinolytic metalloproteinase Mep - Aspergillus fumigatus A1163 | B0Y9E2 | 69 kDa | 0 | 7 |
| Beta-glucosidase, putative - Aspergillus fumigatus A1163 | B0XPE1 | 95 kDa | 6 | 0 |
| Alpha-mannosidase - Aspergillus fumigatus A1163 | B0XYH2 | 124 kDa | 0 | 4 |
| Peptidase, putative - Aspergillus fumigatus A1163 | B0Y766 | 46 kDa | 0 | 5 |
| Putative uncharacterized protein - Aspergillus fumigatus A1163 | B0Y1N8 | 22 kDa | 0 | 6 |
| Alpha-1,2-mannosidase, putative subfamily - Aspergillus fumigatus A1163 | B0XM55 | 87 kDa | 6 | 0 |
| G-protein comlpex beta subunit CpcB - Aspergillus clavatus | A1CIN8_ASPCL | 35 kDa | 0 | 6 |

| Identified Proteins (171) | Accession Number | Molecular Weight | Secretome A. fumigatus (number of spectra detected by MS) | |
|---|---|---|---|---|
| | | | pH-3.5 | pH-7 |
| Alpha-amylase, putative - Aspergillus fumigatus (Sartorya fumigata) | Q4WFV4 | 62 kDa | 0 | 5 |
| Nucleoside diphosphate kinase - Aspergillus fumigatus A1163 | B0Y2U5 | 17 kDa | 0 | 4 |
| Putative uncharacterized protein - Aspergillus fumigatus A1163 | B0Y1K3 | 37 kDa | 0 | 6 |
| Putative uncharacterized protein - Aspergillus fumigatus A1163 | B0Y1Z9 | 51 kDa | 4 | 0 |
| Phosphatidylglycerol specific phospholipase, putative - Aspergillus fumigatus A1163 | B0XWP7 | 54 kDa | 0 | 4 |
| Class III chitinase ChiA1 - Aspergillus fumigatus A1163 | B0Y2Y2 | 87 kDa | 0 | 6 |
| Penicillolysin/deuterolysin metalloprotease, putative - Aspergillus fumigatus A1163 | B0Y4X9 | 39 kDa | 0 | 5 |
| Beta-glucosidase, putative - Aspergillus fumigatus A1163 | B0XPB8 | 83 kDa | 3 | 0 |
| Feruloyl esterase, putative - Aspergillus fumigatus A1163 | B0Y7U1 | 58 kDa | 4 | 0 |
| Carboxypeptidase S1, putative - Aspergillus fumigatus A1163 | B0Y3N9 | 68 kDa | 5 | 0 |
| Cellulase, putative - Neosartorya fischeri | A1DGM6_NEOFI | 45 kDa | 5 | 0 |
| Aspartyl aminopeptidase - Aspergillus clavatus | A1CJU9_ASPCL | 54 kDa | 0 | 3 |
| Ser/Thr protein phosphatase family - Aspergillus fumigatus A1163 | B0XZB5 | 71 kDa | 0 | 2 |
| Putative uncharacterized protein - Aspergillus fumigatus A1163 | B0Y221 | 29 kDa | 0 | 3 |
| Putative uncharacterized protein - Aspergillus fumigatus A1163 | B0XT52 | 21 kDa | 0 | 3 |
| Putative uncharacterized protein - Neosartorya fischeri | A1DEN1_NEOFI | 32 kDa | 2 | 0 |
| Mannitol-1-phosphate dehydrogenase - Neosartorya fischeri | A1DGY9_NEOFI | 43 kDa | 0 | 4 |
| Beta-mannosidase - Aspergillus fumigatus A1163 | B0Y7S2 | 104 kDa | 4 | 0 |
| Alpha-1,2-mannosidase family protein - Aspergillus fumigatus (Sartorya fumigata) | Q4WV22 | 89 kDa | 3 | 0 |
| Prolidase pepP, putative - Aspergillus fumigatus A1163 | B0XW47 | 52 kDa | 0 | 3 |
| Alpha-1,2-mannosidase, putative - Aspergillus fumigatus A1163 | B0YCI0 | 88 kDa | 3 | 0 |
| Cu,Zn superoxide dismutase SOD1 - Aspergillus fumigatus A1163 | B0Y476 | 16 kDa | 0 | 4 |
| Major allergen Asp f 2 precursor - Aspergillus fumigatus (Sartorya fumigata) | ALL2 | 33 kDa | 0 | 3 |
| Pectate lyase A - Aspergillus fumigatus A1163 | B0XT32 | 34 kDa | 0 | 4 |
| Putative uncharacterized protein - Neosartorya fischeri | A1D462_NEOFI | 75 kDa | 4 | 0 |
| Lipase, putative - Aspergillus fumigatus A1163 | B0YCB0 | 49 kDa | 2 | 0 |
| BYS1 domain protein, putative - Aspergillus fumigatus A1163 | B0Y209 | 16 kDa | 0 | 3 |
| Carboxypeptidase CpyA/Prc1, putative - Neosartorya fischeri | A1DP75_NEOFI | 61 kDa | 0 | 3 |
| Endo-1,3(4)-beta-glucanase, putative - Aspergillus fumigatus A1163 | B0XM89 | 31 kDa | 0 | 2 |
| Acid phosphatase, putative - Aspergillus fumigatus A1163 | B0YF50 | 30 kDa | 2 | 0 |
| Extracellular lipase, putative - Aspergillus fumigatus A1163 | B0Y0A5 | 47 kDa | 3 | 0 |
| Putative uncharacterized protein - Aspergillus fumigatus A1163 | B0YCY3 | 23 kDa | 0 | 3 |
| Alpha-L-rhamnosidase B, putative - Aspergillus fumigatus A1163 | B0XPH6 | 75 kDa | 2 | 0 |
| Acid phosphatase AphA - Aspergillus fumigatus A1163 | B0Y1M6 | 67 kDa | 2 | 0 |
| GPI anchored protein, putative - Aspergillus fumigatus A1163 | B0XQH8 | 39 kDa | 2 | 0 |
| Aminotransferase, class V, putative - Aspergillus fumigatus A1163 | B0XQ69 | 42 kDa | 0 | 2 |
| Hydrolase, putative - Aspergillus fumigatus A1163 | B0XU31 | 72 kDa | 0 | 2 |
| Cell wall glucanase, putative - Aspergillus fumigatus A1163 | B0Y7N9 | 46 kDa | 3 | 0 |
| Putative uncharacterized protein - Aspergillus fumigatus A1163 | B0XYL8 | 23 kDa | 0 | 2 |
| Pectin methylesterase, putative - Aspergillus fumigatus A1163 | B0Y9F9 | 35 kDa | 2 | 0 |

| Identified Proteins (171) | Accession Number | Molecular Weight | Secretome A. fumigatus (number of spectra detected by MS) | |
|---|---|---|---|---|
| | | | pH-3,5 | pH-7 |
| WSC domain protein, putative - Aspergillus fumigatus A1163 | B0Y4W9 | 31 kDa | 2 | 0 |
| Vacuolar aspartyl aminopeptidase Lap4, putative - Aspergillus clavatus | A1C4U0_ASPCL (+4) | 55 kDa | 0 | 2 |
| Putative uncharacterized protein - Neosartorya fischeri | A1CYA5_NEOFI | 37 kDa | 0 | 2 |
| Pectin lyase, putative - Aspergillus fumigatus A1163 | B0Y0N7 | 40 kDa | 2 | 0 |
| GPI anchored protein, putative - Aspergillus fumigatus A1163 | B0Y935 | 44 kDa | 0 | 2 |
| Dihydrolipoyl dehydrogenase - Neosartorya fischeri | A1CYQ9_NEOFI | 55 kDa | 0 | 2 |
| Putative uncharacterized protein - Aspergillus fumigatus A1163 | B0YDZ8 | 12 kDa | 0 | 2 |
| N,O-diacetyl muramidase, putative - Aspergillus fumigatus A1163 | B0Y858 | 25 kDa | 2 | 0 |
| Conidial hydrophobin RodB - Neosartorya fischeri | A1D142_NEOFI | 14 kDa | 0 | 2 |
| Hydroxyacylglutathione hydrolase, putative - Neosartorya fischeri | A1DDQ5_NEOFI | 28 kDa | 0 | 2 |

## Supplementary Table 5

| Peptides generated from NPY degradation and detected on MS | z charge | Theoritical mass | Mass detected | Delta |
|---|---|---|---|---|
| SKPDNPGEDAPAEDMARYYSALRHYINLITRQRY (NPY3-36) | 3 | 1337.9915 | 1337.9955 | -0.004 |
| | 4 | 1003.7454 | 1003.7503 | -0.0049 |
| | 5 | 803.1978 | 803.2006 | -0.0028 |
| | 6 | 669.4994 | 669.5016 | -0.0022 |
| | 7 | 574.0005 | 574.0027 | -0.0022 |
| DNPGEDAPAEDMARYYSALRHYINLITRQRY (NPY6-36) | 4 | 925.7005 | 925.7052 | -0.0047 |
| | 5 | 740.7619 | 740.765 | -0.0031 |
| | 6 | 617.4694 | 617.4723 | -0.0029 |
| GEDAPAEDMARYYSALRHYINLITRQRY (NPY9-36) | 3 | 1125.224 | 1125.2304 | -0.0064 |
| | 4 | 844.1699 | 844.1734 | -0.0035 |
| | 5 | 675.5373 | 675.5398 | -0.0025 |
| | 6 | 563.1157 | 563.1184 | -0.0027 |
| AEDMARYYSALRHYINLITRQRY (NPY14-36) | 3 | 968.8304 | 968.8364 | -0.006 |
| | 4 | 726.8746 | 726.8778 | -0.0032 |
| | 5 | 581.7012 | 581.7042 | -0.003 |
| | 6 | 484.9188 | 484.9216 | -0.0028 |
| YPSKPDNPGEDAPAEDMARYYSALRHYINLITRQRY (NPY1-36) | 3 | 1424.6969 | 1424.7073 | -0.0104 |
| | 4 | 1068.7745 | 1068.7804 | -0.0059 |
| | 5 | 855.221 | 855.2258 | -0.0048 |
| | 6 | 712.8521 | 712.8568 | -0.0047 |
| | 7 | 611.164 | 611.16 | 0.004 |
| YPSKPDNP | 1 | 917.4363 | 917.4372 | -0.0009 |
| | 2 | 459.2218 | 459.2228 | -0.001 |

| Peptides generated from 3-36 NPY degradation and detected on MS | z charge | Theoritical mass | Mass detected | Delta |
|---|---|---|---|---|
| YPS | 1 | 366.166 | 366.1676 | -0.0016 |
| KPD | 1 | 359.1925 | 359.1941 | -0.0016 |
| NPG | 1 | 287.135 | 287.1363 | -0.0013 |
| SKP | 1 | 331.1976 | 331.1985 | -0.0009 |
| DNP | 1 | 345.1405 | 345.1411 | -0.0006 |
| SKPDNP | 1 | 657.3202 | 657.3216 | -0.0014 |
| GED | 1 | 320.1088 | 320.1096 | -0.0008 |
| GEDAP | 1 | 488.1987 | 488.1995 | -0.0008 |
| AP | 1 | 187.1077 | 187.108 | -0.0003 |
| APA | 1 | 258.1448 | 258.1453 | -0.0005 |
| EDM | 1 | 394.1279 | 394.1288 | -0.0009 |
| ARY | 2 | 205.1133 | 205.1137 | -0.0004 |
| YSA | 1 | 340.1503 | 340.151 | -0.0007 |
| LRH | 1 | 425.2619 | 425.2628 | -0.0009 |
| YIN | 1 | 409.2082 | 409.2107 | -0.0025 |
| LIT | 1 | 346.2336 | 346.2343 | -0.0007 |
| RQR | 1 | 459.2786 | 459.2797 | -0.0011 |
| Y | 1 | 182.0812 | 182.0814 | -0.0002 |
| AED | 1 | 334.1245 | 334.1256 | -0.0011 |
| MAR | 1 | 377.1966 | 377.1973 | -0.0007 |
| YYS | 1 | 432.1765 | 432.1775 | -0.001 |
| ALR | 1 | 359.2401 | 359.2405 | -0.0004 |
| HYI | 1 | 432.2241 | 432.225 | -0.0009 |
| NLI | 1 | 359.2289 | 359.2302 | -0.0013 |
| TRQ | 1 | 404.2252 | 404.2261 | -0.0009 |
| RY | 1 | 338.1823 | 338.1829 | -0.0006 |

## Supplementary Table 6

| Peptides generated from the designed peptide degradation and detected on MS | Charge z | Theoritical mass | Mass detected | Delta | ppm |
|---|---|---|---|---|---|
| **KDKRSFPFF** | **1** | **1171.6259** | **1171.624** | **0.0019** | **1.62** |
| **KDKRSFPFF** | **2** | **586.3166** | **586.3157** | **0.0009** | **1.54** |
| **KDKRSFPFF** | **3** | **391.2135** | **391.2125** | **0.001** | **2.56** |
| **KDK** | **1** | **390.2347** | **390.2344** | **0.0003** | **0.77** |
| **KDK** | **2** | **195.621** | **195.6208** | **0.0002** | **1.02** |
| **RSFP** | **1** | **506.2722** | **506.2712** | **0.001** | **1.98** |
| **RSFP** | **2** | **253.6397** | **253.6391** | **0.0006** | **2.37** |
| **FF** | **1** | **313.1547** | **313.1541** | **0.0006** | **1.92** |
| **KDKRSFP** | **2** | **439.2482** | **439.2474** | **0.0008** | **1.82** |
| **RSFPFF** | **1** | **800.409** | **800.4078** | **0.0012** | **1.50** |
| **RSFPFF** | **2** | **400.7081** | **400.7076** | **0.0005** | **1.25** |

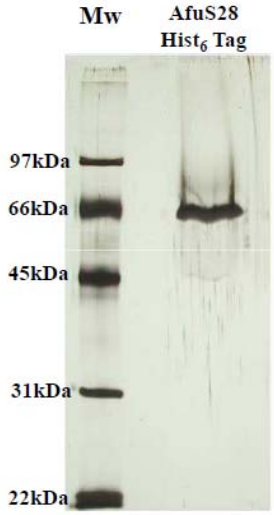
## Supplementary Table 7

| Peptides generated from Apelin-13 degradation and detected on MS | Charge z | Theoritical mass | Mass detected | Delta | ppm |
|---|---|---|---|---|---|
| **QRPRLSHKGPMPF** | **2** | **775.9223** | **775.9207** | **0.0016** | **2.06** |
| **QRPRLSHKGPMPF** | **3** | **517.6173** | **517.6163** | **0.001** | **1.93** |
| **QRPRLSHKGPMPF** | **4** | **388.4648** | **388.4661** | **-0.0013** | **-3.35** |
| **QRP** | **1** | **400.2303** | **400.2292** | **0.0011** | **2.75** |
| **QRP** | **2** | **200.6188** | **200.6183** | **0.0005** | **2.49** |
| **RLS** | **1** | **375.235** | **375.234** | **0.001** | **2.66** |
| **HKGP** | **1** | **438.2439** | **438.2448** | **-0.0009** | **-2.05** |
| **HKGP** | **2** | **219.6266** | **219.626** | **0.0006** | **2.73** |
| **MP** | **1** | **247.1111** | **247.1104** | **0.0007** | **2.83** |
| **MPF** | **1** | **349.1795** | **349.1785** | **0.001** | **2.86** |
| **F** | **1** | **166.0863** | **166.0859** | **0.0004** | **2.41** |
| **RLSHKGPMPF** | **2** | **585.316** | **585.3146** | **0.0014** | **2.39** |
| **RLSHKGP** | **1** | **794.4631** | **794.4609** | **0.0022** | **2.77** |
| **RLSHKGP** | **2** | **397.7352** | **397.7342** | **0.001** | **2.51** |

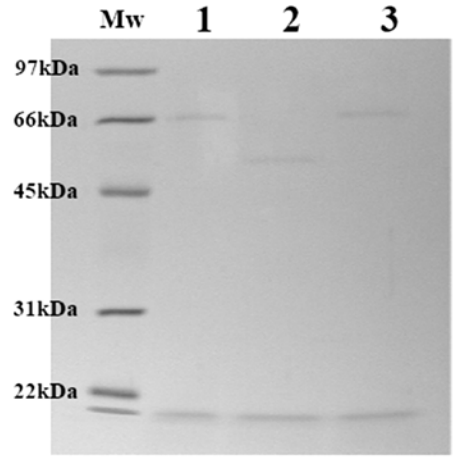
## Supplementary Table 8

| Peptides generated from Dynorphin A degradation and detected on MS | Charge z | Theoritical mass | Mass detected | Delta | ppm |
|---|---|---|---|---|---|
| **GGFLRRIRPKLKWDNQ** | **2** | **992.5712** | **992.5687** | **0.0025** | **2.52** |
| **GGFLRRIRPKLKWDNQ** | **3** | **662.0499** | **662.0486** | **0.0013** | **1.96** |
| **GGFLRRIRPKLKWDNQ** | **4** | **496.7892** | **496.7882** | **0.001** | **2.01** |
| **GGFLRRIRPKLKWDNQ** | **5** | **397.6329** | **397.6319** | **0.001** | **2.51** |
| **GGFLRRIRP** | **2** | **536.3303** | **536.329** | **0.0013** | **2.42** |
| **GGFLRRIRP** | **3** | **357.8893** | **357.8884** | **0.0009** | **2.51** |
| **GGF** | **1** | **280.1292** | **280.1286** | **0.0006** | **2.14** |
| **LRR** | **1** | **444.3041** | **444.3031** | **0.001** | **2.25** |
| **LRR** | **2** | **222.6557** | **222.6554** | **0.0003** | **1.35** |
| **IRP** | **1** | **385.2558** | **385.2549** | **0.0009** | **2.34** |
| **IRP** | **2** | **193.1315** | **193.1311** | **0.0004** | **2.07** |
| **KLK** | **1** | **388.2918** | **388.2909** | **0.0009** | **2.32** |
| **KLK** | **2** | **194.6496** | **194.6491** | **0.0005** | **2.57** |
| **WDN** | **1** | **434.167** | **434.1662** | **0.0008** | **1.84** |
| **LRRIRPKLKWDNQ** | **3** | **575.0128** | **575.0118** | **0.001** | **1.74** |
| **LRRIRPKLKWDNQ** | **4** | **431.5114** | **431.5111** | **0.0003** | **0.70** |
| **IRPKLKWDNQ** | **2** | **649.3724** | **649.3714** | **0.001** | **1.54** |
| **IRPKLKWDNQ** | **3** | **433.2507** | **433.25** | **0.0007** | **1.62** |
| **IRPKLKWDNQ** | **4** | **325.1898** | **325.1893** | **0.0005** | **1.54** |
| **KLKWDNQ** | **1** | **931.4996** | **931.4974** | **0.0022** | **2.36** |
| **KLKWDNQ** | **2** | **466.2534** | **466.2526** | **0.0008** | **1.72** |
| **KLKWDNQ** | **3** | **311.1714** | **311.1707** | **0.0007** | **2.25** |

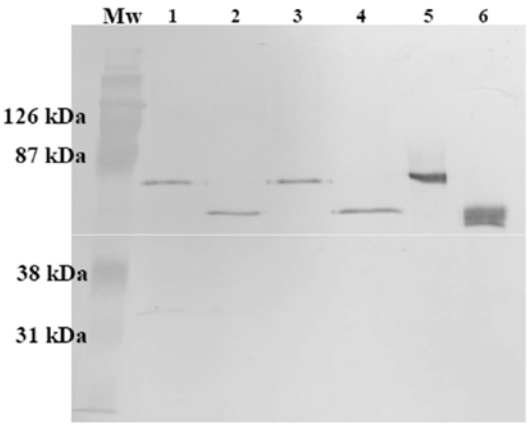
## Supplementary Figure 1



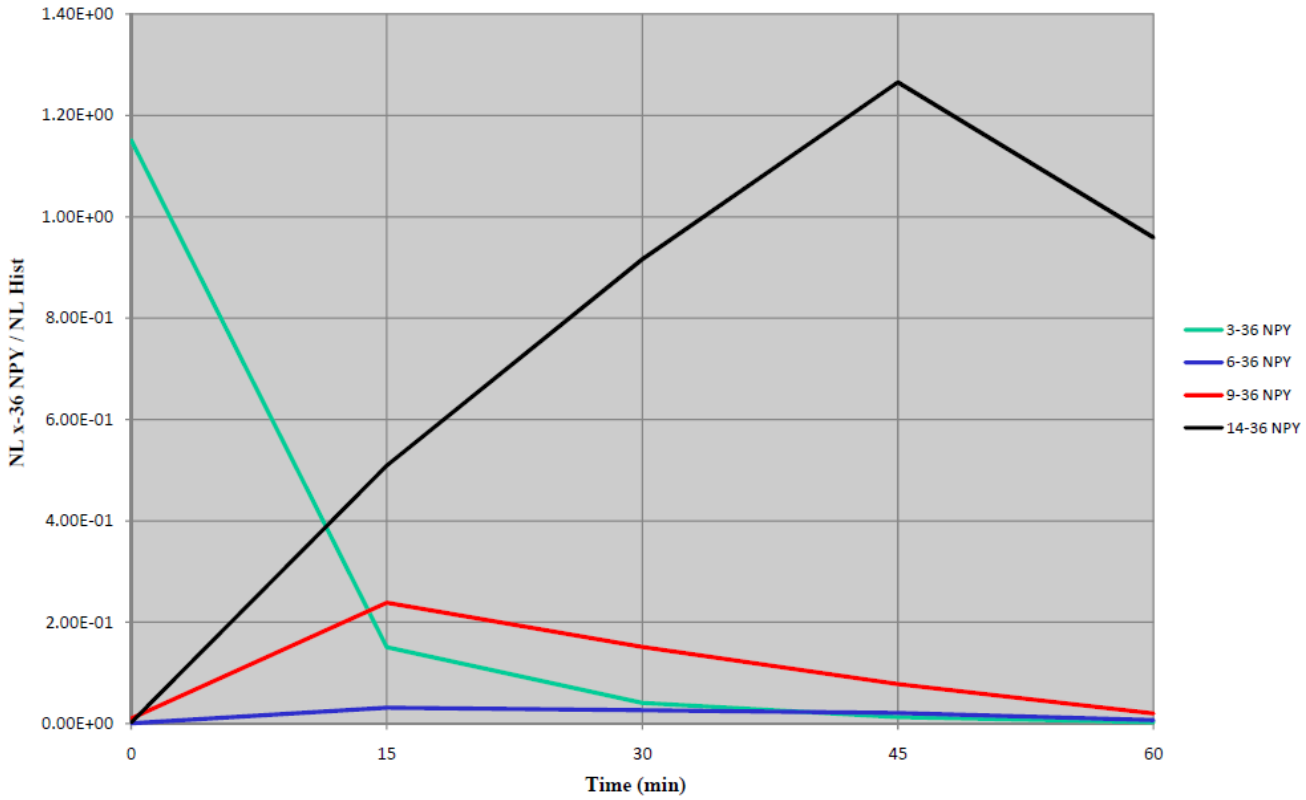

## Supplementary Figure 2



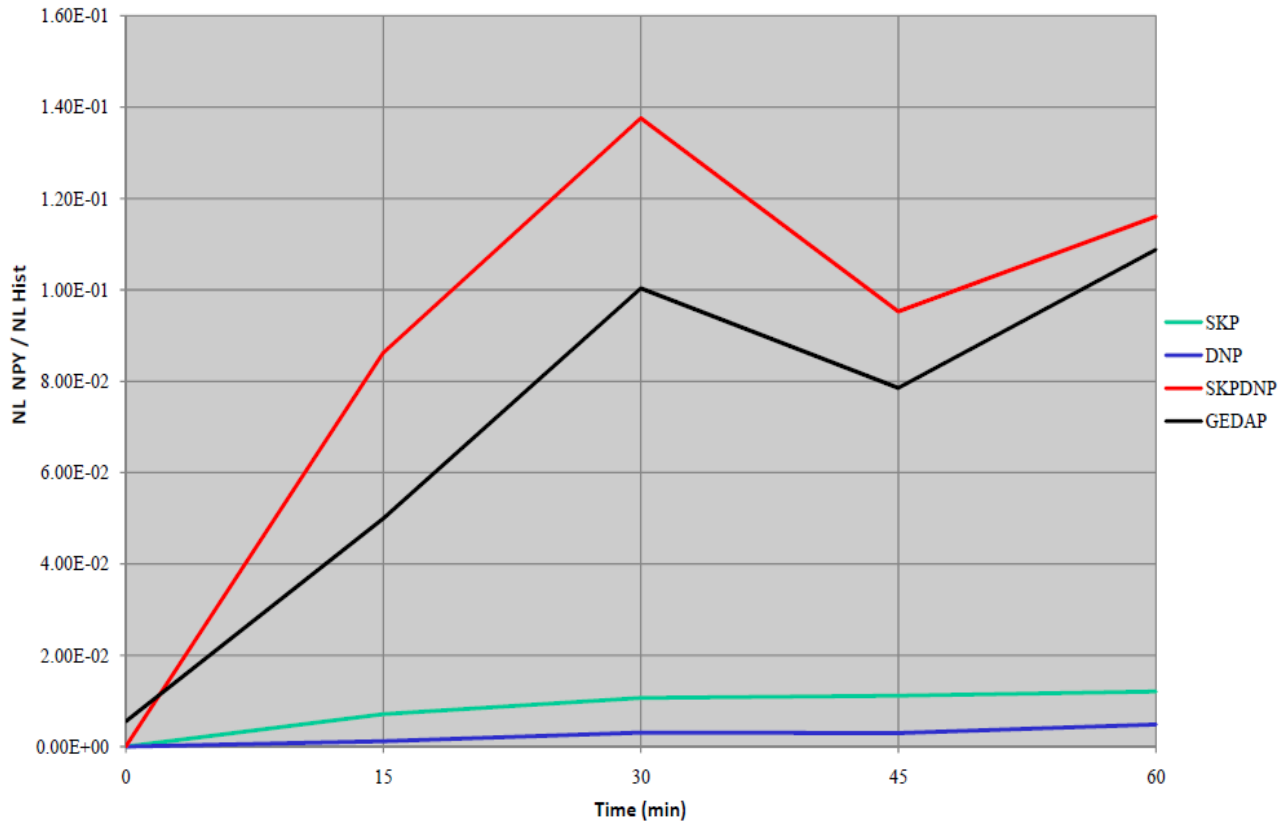

## Supplementary Figure 3



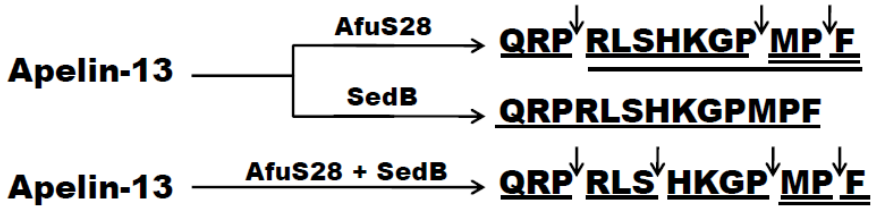
## Supplementary Figure 4a



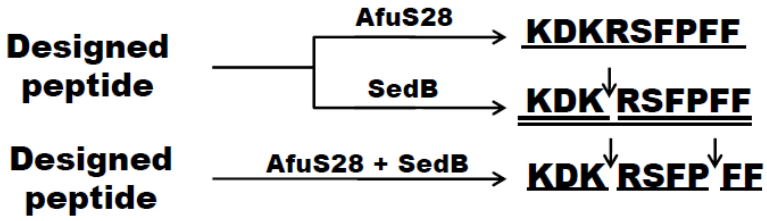

## Supplementary Figure 4b



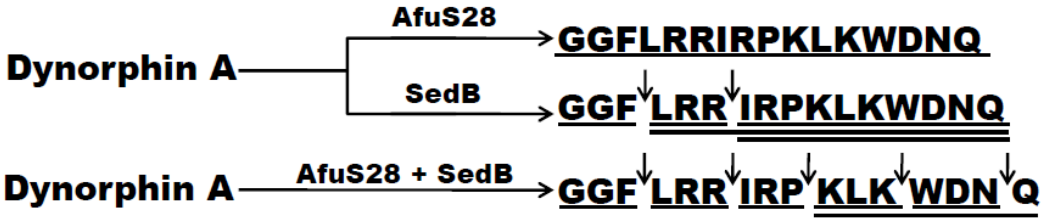
**Supplementary Figure 5**




**Supplementary Figure 6**




**Supplementary Figure 7**



## Supplementary Figures and Legends for the Part 2.

**FIG. S1**: Amino acid sequence alignment of *A. fumigatus* GprA (MER107323) with glutamic peptidases from *Talaromyces emersonii* (Teme_Gpr, MER020134), *A. niger* (aspergillopepsin II, MER001321), *Botryotinia fuckeliana* (Bfuc_Gpr, MER054828), and *Scytalidium lignicolum* (SGP, MER001320). The alignment was optimized by introducing gaps using the CLUSTALW multiple sequence alignment program (41). Identical and similar residues are black and grey shaded, respectively. Vertical arrows indicate potential signal peptidase cleavage sites and cleavage sites in the proprotein generating the mature enzyme. Active site residues Gln and Glu forming the catalytic diad are indicated by solid triangles.

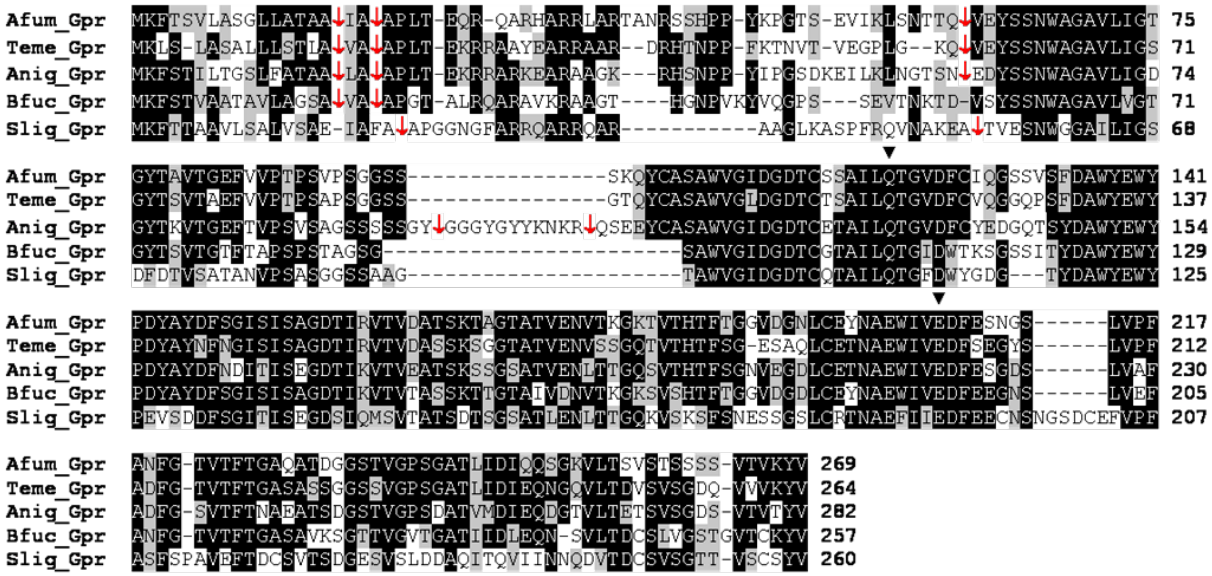


**Table 1.** Primers used in this study*

| Primer | Oligonucleotide sequence§ | Location | PCR product size with cloning sites |
|---|---|---|---|
| P1<br>P2 | 5'-TATAT***GCGGCCGC***CCATATTCGTCTGGCTCACC-3'<br>5'-ATATTA***CCCGGGGGCCTGAGTGGCC***CTTGACGCTGTTCTGTGAGG-3' | Upstream sequence in *AfugprA*<br>Complement of *AfugprA, N-terminal region* | 1192 bp (*Nflr* fragment)<br>*Not*I----***Sfi*I**-*Xma*I |
| P3<br>P4 | 5'-ATATTA***AAGCTTGGCCATCTAGGCC***AGCGGCAAGGTTTTGACTT-3'<br>5'-TATCG***TTAATTAA***ATGAACCGCCTTCAGAGCTA-3' | Downstream sequence in *AfugprA*<br>Complement of *AfugprA, C-terminal region* | 1219 bp (*Cflr* fragment)<br>*Hin*DIII-***Sfi*I**----*Pac*I |
| P5<br>P6 | 5'-GGGCTGAAGGAAGAATACCC-3'<br>5'-AGGTGATATCGGCCTGAGTG-3' | Upstream sequence in *AfugprA*<br>pSK397 | 1343 bp. |
| P7<br>P8 | 5'-CGTTTACCCAGAATGCACAG-3'<br>5'-GCACAGTGATATCGGACGAC-3' | Downstram sequence in *AfugprA*<br>pSK397 | 1350 bp. |
| P9<br>P10 | 5'-GTT***CTCGAG***CCATCGCTGCTCCCCTCACA-3'<br>5'-GTT***GGATCC***TTAGACATACTTAACAGTGAC-3' | *AfugprA*<br>Complement of *AfugprA* | 782 bp.<br>*Xho*I----*Bam*HI |

* Primers P1 to P4 were used to construct *Nflr* and *Cflr* fragments. Primers P5 to P8 were used for screening *A. fumigatus gprA*Δ mutants. For location of those primers in the *A. fumigatus gprA*Δ mutant compare Fig.1. Primers P9 and P10 were used for *AfugprA* gene expression in *P. pastoris*.

§ Italicized and boldfaced nucleotides represent cloning sites.

## Supplementary Tables Legends for the Part 3.

**Supplementary Table 1a: MS Protein report of *M. canis* secretome in SP medium at pH 4.0 and pH 7.0.**

Database Set: 1 Database

Database Name: the uniprot-microsporum-AND-canis database

Taxonomy: All Entries

Number of Proteins: 8805

Search Engine Set: 1 Search Engine

Search Engine: Mascot

Version: Mascot

Fragment Tolerance: 0.50 Da (Monoisotopic)

Parent Tolerance: 10.0 PPM (Monoisotopic)

Fixed Modifications: +57 on C (Carbamidomethyl)

Variable Modifications: +1 on NQ (Deamidation), +16 on M (Oxidation), +42 on n (Acetyl)

Database: the uniprot-microsporum-AND-canis database (unknown version, 8805 entries)

Digestion Enzyme: Trypsin

Max Missed Cleavages: 1

Scaffold Version: Scaffold_3_00_03

Peptide Thresholds: 90.0 % minimum

Protein Thresholds: 95.0 % minimum and 1 peptides minimum

| Biological sample name | Protein name | Protein accession numbers | Protein molecular weight (Da) | Number of unique peptides | Number of unique spectra | Number of total spectra | Percentage of total spectra | Percentage sequence coverage |
|---|---|---|---|---|---|---|---|---|
| Mcanis-pH4-a | Probable neutral protease 2 MCYG_05201 | NPIIA_NANOT | 40'199.70 | 8 | 9 | 12 | 0.37% | 28.20% |
| Mcanis-pH4-a | FAD binding domain-containing protein MCYG_08167 | C5FZP5_NANOT | 54'189.10 | 1 | 1 | 1 | 0.03% | 2.20% |
| Mcanis-pH4-a | Carboxypeptidase S1 SCPB | SCPB_NANOT | 72'773.80 | 10 | 13 | 15 | 0.46% | 24.90% |
| Mcanis-pH4-a | Subtilisin-like protease SUB7 | SUB7_NANOT | 41'362.20 | 2 | 2 | 2 | 0.06% | 10.00% |
| Mcanis-pH4-a | Alkaline serine protease MCYG_02748 | C5FGP4_NANOT | 40'782.50 | 2 | 2 | 2 | 0.06% | 12.90% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Mcanis-pH4-a | Mucin MCYG_07854 | C5FXJ5_NANOT | 76'937.60 | 5 | 5 | 5 | 0.15% | 10.10% |
| Mcanis-pH4-a | LysM domain-containing protein MCYG_04103 | C5FN48_NANOT | 47'704.30 | 2 | 3 | 4 | 0.12% | 6.14% |
| Mcanis-pH4-a | Phosphoglycerate mutase family protein MCYG_08379 | C5G0A7_NANOT | 36'614.60 | 6 | 6 | 7 | 0.21% | 23.50% |
| Mcanis-pH4-a | Dipeptidase 1 MCYG_02918 | C5FK77_NANOT | 47'224.20 | 2 | 2 | 2 | 0.06% | 7.26% |
| Mcanis-pH4-a | FAD binding domain-containing proteinMCYG_04968 | C5FQJ6_NANOT | 55'314.40 | 19 | 27 | 42 | 1.28% | 49.80% |
| Mcanis-pH4-a | Putative uncharacterized protein MCYG_05599 | C5FSC7_NANOT | 20'561.90 | 5 | 9 | 55 | 1.67% | 49.50% |
| Mcanis-pH4-a | Ecm33 MCYG_01225 | C5FEL3_NANOT | 41'117.10 | 10 | 13 | 35 | 1.07% | 32.90% |
| Mcanis-pH4-a | DUF1237 domain-containing protein MCYG_03876 | C5FMF4_NANOT | 60'979.20 | 4 | 4 | 4 | 0.12% | 10.90% |
| Mcanis-pH4-a | Beta-1,3-glucanosyltransferase 3 MCYG_03246 | C5FL55_NANOT | 56'747.70 | 14 | 20 | 29 | 0.88% | 38.40% |
| Mcanis-pH4-a | Subtilisin-like protease SUB3 | SUB3_MICCA,SUB3_NANOT | 40'837.10 | 13 | 21 | 59 | 1.80% | 59.20% |
| Mcanis-pH4-a | Tripeptidyl-peptidase SED2 SED2 | SED2_NANOT | 64'826.30 | 21 | 33 | 157 | 4.78% | 49.50% |
| Mcanis-pH4-a | Chitinase 3 MCYG_07133 | C5FWN0_NANOT | 24'184.50 | 2 | 2 | 2 | 0.06% | 10.90% |
| Mcanis-pH4-a | LysM domain-containing protein MCYG_08445 | C5G0H3_NANOT | 41'770.40 | 2 | 2 | 6 | 0.18% | 6.43% |
| Mcanis-pH4-a | Ser/Thr protein phosphataseMCYG_06525 | C5FUX2_NANOT | 71'499.90 | 16 | 24 | 41 | 1.25% | 30.00% |
| Mcanis-pH4-a | LysM domain-containing protein MCYG_02074 | C5FII6_NANOT | 21'919.30 | 2 | 3 | 4 | 0.12% | 17.30% |
| Mcanis-pH4-a | Putative uncharacterized protein MCYG_00562 | C5FCZ0_NANOT | 26'376.90 | 10 | 11 | 13 | 0.40% | 45.30% |
| Mcanis-pH4-a | Carboxypeptidase cpdS MCYG_07753 | C5FX94_NANOT | 58'407.20 | 14 | 19 | 36 | 1.10% | 37.20% |
| Mcanis-pH4-a | Carboxylesterase MCYG_01781 | C5FHY2_NANOT | 55'467.40 | 8 | 8 | 8 | 0.24% | 26.10% |
| Mcanis-pH4-a | Gamma-glutamyltranspeptidase MCYG_05524 | C5FS52_NANOT | 62'565.80 | 4 | 5 | 5 | 0.15% | 12.50% |
| Mcanis-pH4-a | Carbohydrate-binding protein MCYG_01090 | C5FEG8_NANOT | 41'707.10 | 6 | 9 | 10 | 0.30% | 26.50% |
| Mcanis-pH4-a | Beta-glucosidase 4 MCYG_01527 | C5FHG8_NANOT | 33'218.80 | 19 | 28 | 99 | 3.01% | 68.40% |
| Mcanis-pH4-a | 1,2-alpha-D-mannosidase MCYG_00423 | C5FCK1_NANOT | 55'930.00 | 20 | 25 | 38 | 1.16% | 55.60% |
| Mcanis-pH4-a | Actin MCYG_05457 | C5FRY5_NANOT | 41'706.40 | 2 | 2 | 4 | 0.12% | 7.73% |
| Mcanis-pH4-a | Extracellular metalloproteinase 3 MEP3 | MEP3_MICCA,MEP3_NANOT | 69'360.10 | 10 | 15 | 22 | 0.67% | 28.40% |
| Mcanis-pH4-a | Peptidase M28 MCYG_04217 | C5FP82_NANOT | 55'810.30 | 13 | 15 | 19 | 0.58% | 32.10% |
| Mcanis-pH4-a | Carboxypeptidase S1 SCPA | SPCA_NANOT | 72'179.60 | 14 | 18 | 33 | 1.00% | 31.50% |
| Mcanis-pH4-a | Sulphydryl oxidase Sox MCYG_02499 | C5FFZ5_NANOT | 44'024.60 | 11 | 13 | 28 | 0.85% | 33.70% |
| Mcanis-pH4-a | 6-hydroxy-D-nicotine oxidase MCYG_04909 | C5FQD7_NANOT | 55'997.50 | 7 | 10 | 10 | 0.30% | 23.90% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Mcanis-pH4-a | Extracellular matrix protein MCYG_07613 | C5FWV4_NANOT | 23'404.60 | 3 | 3 | 6 | 0.18% | 23.20% |
| Mcanis-pH4-a | Dipeptidyl-peptidase 5 DPP5 | DPP5_NANOT | 80'058.80 | 26 | 35 | 52 | 1.58% | 49.30% |
| Mcanis-pH4-a | Feruloyl esterase B MCYG_06844 | C5FVU1_NANOT | 57'917.20 | 3 | 3 | 3 | 0.09% | 7.81% |
| Mcanis-pH4-a | Putative uncharacterized protein MCYG_04944 | C5FQH2_NANOT | 18'929.80 | 2 | 2 | 2 | 0.06% | 15.30% |
| Mcanis-pH4-a | Alpha-glucosidase MCYG_00092 | C5FBM0_NANOT | 100'911.70 | 4 | 4 | 4 | 0.12% | 6.71% |
| Mcanis-pH4-a | Extracellular cell wall glucanase Crf1 MCYG_04874 | C5FQA2_NANOT | 44'996.20 | 12 | 19 | 56 | 1.70% | 39.50% |
| Mcanis-pH4-a | 1,3(4)-beta-glucanase MCYG_05925 | C5FTA3_NANOT | 48'684.20 | 10 | 14 | 18 | 0.55% | 34.90% |
| Mcanis-pH4-a | Putative uncharacterized protein MCYG_04152 | C5FN97_NANOT | 21'011.20 | 6 | 8 | 12 | 0.37% | 28.70% |
| Mcanis-pH4-a | Putative uncharacterized protein MCYG_02207 | C5FJ58_NANOT | 61'443.50 | 13 | 14 | 28 | 0.85% | 35.50% |
| Mcanis-pH4-a | Laccase-1 MCYG_08571 | C5G0U9_NANOT | 64'927.40 | 12 | 20 | 44 | 1.34% | 37.90% |
| Mcanis-pH4-a | 6-hydroxy-D-nicotine oxidase MCYG_04127 | C5FN72_NANOT | 62'598.10 | 10 | 11 | 11 | 0.34% | 27.50% |
| Mcanis-pH4-a | Phytase MCYG_02670 | C5FGG6_NANOT | 51'413.20 | 13 | 17 | 19 | 0.58% | 40.10% |
| Mcanis-pH4-a | GDSL Lipase/Acylhydrolase family protein MCYG_04422 | C5FNI9_NANOT | 27'702.80 | 3 | 4 | 4 | 0.12% | 21.00% |
| Mcanis-pH4-a | Ribonuclease T2-like protein MCYG_02204 | C5FJ55_NANOT | 42'269.30 | 5 | 5 | 5 | 0.15% | 16.70% |
| Mcanis-pH4-a | Putative uncharacterized protein MCYG_07328 | C5FYB1_NANOT | 20'833.50 | 11 | 22 | 168 | 5.11% | 72.50% |
| Mcanis-pH4-a | Peptidase S41 family proteinMCYG_03610 | C5FM69_NANOT | 76'977.00 | 21 | 23 | 44 | 1.34% | 39.70% |
| Mcanis-pH4-a | Probable leucine aminopeptidase MCYG_04170 | LAP5_NANOT | 38'208.00 | 2 | 3 | 8 | 0.24% | 9.80% |
| Mcanis-pH4-a | Metallocarboxypeptidase MCPA | MCPA_NANOT | 47'079.60 | 10 | 18 | 44 | 1.34% | 33.90% |
| Mcanis-pH4-a | Tripeptidyl-peptidase SED4 | SED4_NANOT | 65'895.80 | 14 | 25 | 153 | 4.66% | 34.60% |
| Mcanis-pH4-a | Glucoamylase MCYG_06367 | C5FUG4_NANOT | 67'893.70 | 24 | 31 | 35 | 1.07% | 50.10% |
| Mcanis-pH4-a | Metallocarboxypeptidase A-like protein MCYG_01475 | MCPAL_NANOT | 45'896.90 | 13 | 22 | 117 | 3.56% | 55.30% |
| Mcanis-pH4-a | Oxalate decarboxylase oxdD MCYG_04379 | C5FNE6_NANOT | 44'218.50 | 9 | 12 | 17 | 0.52% | 33.90% |
| Mcanis-pH4-a | Putative uncharacterized protein MCYG_00366 | C5FC55_NANOT | 61'787.00 | 7 | 7 | 11 | 0.34% | 14.80% |
| Mcanis-pH4-a | Phosphatidylinositol transfer protein MCYG_02226 | C5FFG0_NANOT | 18'642.00 | 5 | 5 | 6 | 0.18% | 42.90% |
| Mcanis-pH4-a | Glycosyl hydrolase MCYG_03093 | C5FKQ2_NANOT | 87'712.10 | 7 | 7 | 7 | 0.21% | 14.10% |
| Mcanis-pH4-a | Putative uncharacterized protein MCYG_03713 | C5FJN3_NANOT | 25'502.60 | 9 | 16 | 43 | 1.31% | 45.00% |
| Mcanis-pH4-a | Putative uncharacterized protein MCYG_03189 | C5FKZ8_NANOT | 46'783.50 | 7 | 7 | 7 | 0.21% | 24.70% |
| Mcanis-pH4-a | IgE-binding protein MCYG_03081 | C5FKP0_NANOT | 20'333.70 | 9 | 12 | 43 | 1.31% | 54.10% |
| Mcanis-pH4-a | Putative uncharacterized protein MCYG_08572 | C5G0V0_NANOT | 33'320.40 | 7 | 9 | 18 | 0.55% | 38.30% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Mcanis-pH4-a | N,O-diacetylmuramidase MCYG_02645 | C5FGE1_NANOT | 25'288.00 | 4 | 4 | 9 | 0.27% | 26.70% |
| Mcanis-pH4-a | Putative uncharacterized protein MCYG_04701 | C5FP29_NANOT | 26'644.10 | 4 | 5 | 6 | 0.18% | 24.80% |
| Mcanis-pH4-a | Carboxypeptidase Y MCYG_05486 | C5FS14_NANOT | 66'387.20 | 8 | 13 | 16 | 0.49% | 23.80% |
| Mcanis-pH4-a | FAD binding domain-containing protein MCYG_06815 | C5FVR2_NANOT | 63'164.50 | 8 | 10 | 11 | 0.34% | 23.10% |
| Mcanis-pH4-a | Probable neutral protease 2 homolog MCYG_07155 | NPIID_NANOT | 38'232.20 | 9 | 15 | 201 | 6.12% | 37.40% |
| Mcanis-pH4-a | Extracellular metalloproteinase MEP5 | MEP5_MICCA,MEP5_NANOT | 69'529.10 | 10 | 11 | 21 | 0.64% | 27.20% |
| Mcanis-pH4-a | Aspartic endopeptidase PEP1 | PEPF_NANOT | 41'148.90 | 7 | 11 | 206 | 6.27% | 35.50% |
| Mcanis-pH4-a | Putative uncharacterized protein MCYG_03822 | C5FMA0_NANOT | 56'426.10 | 2 | 2 | 2 | 0.06% | 6.24% |
| Mcanis-pH4-a | Putative uncharacterized protein MCYG_03637 | C5FM96_NANOT | 24'982.00 | 4 | 5 | 9 | 0.27% | 32.40% |
| Mcanis-pH4-a | Copper radical oxidase MCYG_08682 | C5G160_NANOT | 96'394.50 | 2 | 2 | 4 | 0.12% | 3.45% |
| Mcanis-pH4-a | Cell wall protein PhiA MCYG_00375 | C5FC64_NANOT | 19'726.70 | 6 | 6 | 9 | 0.27% | 50.50% |
| Mcanis-pH4-a | Predicted protein MCYG_01508 | C5FHE9_NANOT | 19'477.60 | 3 | 4 | 4 | 0.12% | 27.20% |
| Mcanis-pH4-a | Acid trehalase MCYG_02758 | C5FGQ4_NANOT | 114'531.50 | 3 | 3 | 3 | 0.09% | 3.43% |
| Mcanis-pH4-a | Aspartic protease MCYG_05372 | C5FRQ0_NANOT | 50'249.10 | 9 | 11 | 12 | 0.37% | 35.90% |
| Mcanis-pH4-a | Polysaccharide deacetylase family protein MCYG_07538 | C5FYX1_NANOT | 32'868.80 | 8 | 12 | 41 | 1.25% | 37.90% |
| Mcanis-pH4-a | Exo-beta-1,3-glucanase Exg0 MCYG_06600 | C5FV47_NANOT | 33'625.30 | 6 | 6 | 8 | 0.24% | 36.50% |
| Mcanis-pH4-a | Lcc2 MCYG_02578 | C5FG74_NANOT | 73'949.30 | 8 | 8 | 8 | 0.24% | 19.90% |
| Mcanis-pH4-a | Acid phosphatase MCYG_07729 | C5FX70_NANOT | 30'625.90 | 10 | 11 | 12 | 0.37% | 65.20% |
| Mcanis-pH4-a | Extracellular metalloproteinase MEP1 | MEP1_MICCA,MEP1_NANOT | 69'753.70 | 3 | 3 | 4 | 0.12% | 11.60% |
| Mcanis-pH4-a | Carboxylesterase family protein MCYG_00869 | C5FDU7_NANOT | 62'320.30 | 13 | 15 | 22 | 0.67% | 29.20% |
| Mcanis-pH4-a | Lysophospholipase MCYG_02652 | C5FGE8_NANOT | 68'263.40 | 7 | 7 | 9 | 0.27% | 13.50% |
| Mcanis-pH4-a | Putative uncharacterized protein MCYG_00150 | C5FBS8_NANOT | 18'683.60 | 2 | 3 | 4 | 0.12% | 23.70% |
| Mcanis-pH4-b | Probable neutral protease 2 homolog MCYG_05201 | NPIIA_NANOT | 40'199.70 | 2 | 2 | 3 | 0.17% | 7.24% |
| Mcanis-pH4-b | Putative uncharacterized protein MCYG_04951 | C5FQH9_NANOT | 15'009.70 | 7 | 10 | 24 | 1.37% | 55.20% |
| Mcanis-pH4-b | FAD binding domain-containing protein MCYG_04968 | C5FQJ6_NANOT | 55'314.40 | 9 | 9 | 12 | 0.69% | 24.60% |
| Mcanis-pH4-b | Polysaccharide deacetylase family protein MCYG_07538 | C5FYX1_NANOT | 32'868.80 | 2 | 2 | 3 | 0.17% | 7.19% |
| Mcanis-pH4-b | Extracellular cell wall glucanase Crf1 MCYG_04874 | C5FQA2_NANOT | 44'996.20 | 7 | 10 | 18 | 1.03% | 20.70% |
| Mcanis-pH4-b | FAD binding domain-containing protein MCYG_06815 | C5FVR2_NANOT | 63'164.50 | 11 | 12 | 12 | 0.69% | 29.40% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Mcanis-pH4-b | Aspartic endopeptidase PEP1 | PEPF_NANOT | 41'148.90 | 3 | 5 | 19 | 1.09% | 13.40% |
| Mcanis-pH4-b | Sulphydryl oxidase Sox MCYG_02499 | C5FFZ5_NANOT | 44'024.60 | 8 | 10 | 19 | 1.09% | 27.60% |
| Mcanis-pH4-b | Ecm33 MCYG_01225 | C5FEL3_NANOT | 41'117.10 | 4 | 4 | 8 | 0.46% | 10.60% |
| Mcanis-pH4-b | DUF1237 domain-containing protein MCYG_03876 | C5FMF4_NANOT | 60'979.20 | 3 | 3 | 3 | 0.17% | 6.01% |
| Mcanis-pH4-b | Subtilisin-like protease SUB3 | SUB3_MICCA,SUB3_NANOT | 40'837.10 | 5 | 6 | 6 | 0.34% | 17.90% |
| Mcanis-pH4-b | Tripeptidyl-peptidase SED2 | SED2_NANOT | 64'826.30 | 19 | 29 | 221 | 12.60% | 48.70% |
| Mcanis-pH4-b | Ser/Thr protein phosphatase MCYG_06525 | C5FUX2_NANOT | 71'499.90 | 8 | 9 | 14 | 0.80% | 13.90% |
| Mcanis-pH4-b | Carboxypeptidase cpdS MCYG_07753 | C5FX94_NANOT | 58'407.20 | 13 | 16 | 36 | 2.06% | 31.60% |
| Mcanis-pH4-b | Putative uncharacterized protein MCYG_03713 | C5FJN3_NANOT | 25'502.60 | 8 | 11 | 59 | 3.37% | 43.70% |
| Mcanis-pH4-b | Carbohydrate-binding protein MCYG_01090 | C5FEG8_NANOT | 41'707.10 | 4 | 6 | 6 | 0.34% | 15.90% |
| Mcanis-pH4-b | Lcc2 MCYG_02578 | C5FG74_NANOT | 73'949.30 | 2 | 3 | 3 | 0.17% | 3.06% |
| Mcanis-pH4-b | Beta-glucosidase 4 MCYG_01527 | C5FHG8_NANOT | 33'218.80 | 14 | 18 | 92 | 5.26% | 57.70% |
| Mcanis-pH4-b | 1,2-alpha-D-mannosidase MCYG_00423 | C5FCK1_NANOT | 55'930.00 | 11 | 11 | 13 | 0.74% | 31.30% |
| Mcanis-pH4-b | Extracellular metalloproteinase MEP3 | MEP3_MICCA,MEP3_NANOT | 69'360.10 | 2 | 2 | 2 | 0.11% | 4.90% |
| Mcanis-pH4-b | Peptidase M28 MCYG_04217 | C5FP82_NANOT | 55'810.30 | 13 | 13 | 16 | 0.92% | 29.30% |
| Mcanis-pH4-b | Carboxypeptidase S1 SCPA | SPCA_NANOT | 72'179.60 | 14 | 17 | 27 | 1.54% | 26.00% |
| Mcanis-pH4-b | 6-hydroxy-D-nicotine oxidase MCYG_04909 | C5FQD7_NANOT | 55'997.50 | 7 | 8 | 15 | 0.86% | 18.60% |
| Mcanis-pH4-b | Putative uncharacterized protein MCYG_04944 | C5FQH2_NANOT | 18'929.80 | 3 | 3 | 4 | 0.23% | 26.00% |
| Mcanis-pH4-b | Lysophospholipase MCYG_02652 | C5FGE8_NANOT | 68'263.40 | 4 | 4 | 4 | 0.23% | 6.92% |
| Mcanis-pH4-b | 1,3(4)-beta-glucanase MCYG_05925 | C5FTA3_NANOT | 48'684.20 | 3 | 3 | 5 | 0.29% | 7.56% |
| Mcanis-pH4-b | Laccase-1 MCYG_08571 | C5G0U9_NANOT | 64'927.40 | 13 | 17 | 31 | 1.77% | 31.10% |
| Mcanis-pH4-b | Ribonuclease T2-like protein MCYG_02204 | C5FJ55_NANOT | 42'269.30 | 3 | 3 | 6 | 0.34% | 9.85% |
| Mcanis-pH4-b | Peptidase S41 family protein MCYG_03610 | C5FM69_NANOT | 76'977.00 | 20 | 22 | 53 | 3.03% | 33.80% |
| Mcanis-pH4-b | Glycosyl hydrolase family 3 N terminal MCYG_07820 | C5FXG1_NANOT | 40'034.60 | 3 | 3 | 4 | 0.23% | 8.29% |
| Mcanis-pH4-b | Metallocarboxypeptidase MCPA | MCPA_NANOT | 47'079.60 | 6 | 9 | 13 | 0.74% | 22.70% |
| Mcanis-pH4-b | Tripeptidyl-peptidase SED4 | SED4_NANOT | 65'895.80 | 14 | 22 | 128 | 7.32% | 35.40% |
| Mcanis-pH4-b | Metallocarboxypeptidase A-like protein MCYG_01475 | MCPAL_NANOT | 45'896.90 | 3 | 3 | 6 | 0.34% | 9.62% |
| Mcanis-pH4-b | Hydrophobic surface binding protein B MCYG_01969 | C5FIQ9_NANOT | 19'243.20 | 2 | 2 | 2 | 0.11% | 14.30% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Mcanis-pH4-b | Phosphatidylinositol transfer protein MCYG_02226 | C5FFG0_NANOT | 18'642.00 | 6 | 6 | 13 | 0.74% | 38.90% |
| Mcanis-pH4-b | Glycosyl hydrolase MCYG_03093 | C5FKQ2_NANOT | 87'712.10 | 8 | 8 | 8 | 0.46% | 13.80% |
| Mcanis-pH4-b | IgE-binding protein MCYG_03081 | C5FKP0_NANOT | 20'333.70 | 7 | 9 | 44 | 2.52% | 41.80% |
| Mcanis-pH4-b | Putative uncharacterized protein MCYG_08572 | C5G0V0_NANOT | 33'320.40 | 2 | 2 | 2 | 0.11% | 9.73% |
| Mcanis-pH4-b | N,O-diacetylmuramidase MCYG_02645 | C5FGE1_NANOT | 25'288.00 | 3 | 3 | 4 | 0.23% | 19.50% |
| Mcanis-pH4-b | Carboxypeptidase Y MCYG_05486 | C5FS14_NANOT | 66'387.20 | 8 | 9 | 13 | 0.74% | 17.60% |
| Mcanis-pH4-b | HMF1 MCYG_06048 | C5FTM6_NANOT | 13'129.00 | 2 | 2 | 2 | 0.11% | 26.40% |
| Mcanis-pH4-b | Probable neutral protease 2 homolog MCYG_07155 | NPIID_NANOT | 38'232.20 | 6 | 8 | 61 | 3.49% | 17.80% |
| Mcanis-pH4-b | Putative uncharacterized protein MCYG_05599 | C5FSC7_NANOT | 20'561.90 | 4 | 8 | 71 | 4.06% | 36.50% |
| Mcanis-pH4-b | Putative uncharacterized protein MCYG_03637 | C5FM96_NANOT | 24'982.00 | 1 | 2 | 2 | 0.11% | 5.53% |
| Mcanis-pH4-b | Cell wall protein PhiA MCYG_00375 | C5FC64_NANOT | 19'726.70 | 7 | 8 | 19 | 1.09% | 52.70% |
| Mcanis-pH4-b | Predicted protein MCYG_01508 | C5FHE9_NANOT | 19'477.60 | 2 | 2 | 2 | 0.11% | 19.60% |
| Mcanis-pH4-b | Carboxypeptidase S1 SCPB | SCPB_NANOT | 72'773.80 | 2 | 2 | 2 | 0.11% | 3.97% |
| Mcanis-pH4-b | Aspartic protease MCYG_05372 | C5FRQ0_NANOT | 50'249.10 | 5 | 5 | 5 | 0.29% | 13.40% |
| Mcanis-pH4-b | Exo-beta-1,3-glucanase Exg0 MCYG_06600 | C5FV47_NANOT | 33'625.30 | 5 | 5 | 7 | 0.40% | 28.30% |
| Mcanis-pH4-b | Acid phosphatase MCYG_07729 | C5FX70_NANOT | 30'625.90 | 3 | 3 | 4 | 0.23% | 17.70% |
| Mcanis-pH4-c | Putative uncharacterized protein MCYG_00562 | C5FCZ0_NANOT | 26'376.90 | 8 | 9 | 12 | 0.30% | 44.00% |
| Mcanis-pH4-c | ThiJ/PfpI family protein MCYG_02762 | C5FGQ8_NANOT | 24'934.80 | 3 | 4 | 5 | 0.12% | 17.00% |
| Mcanis-pH4-c | Probable neutral protease 2 homolog MCYG_05201 | NPIIA_NANOT | 40'199.70 | 2 | 2 | 4 | 0.10% | 4.02% |
| Mcanis-pH4-c | Putative uncharacterized protein MCYG_04951 | C5FQH9_NANOT | 15'009.70 | 3 | 4 | 5 | 0.12% | 37.90% |
| Mcanis-pH4-c | FAD binding domain-containing protein MCYG_08167 | C5FZP5_NANOT | 54'189.10 | 3 | 4 | 4 | 0.10% | 7.20% |
| Mcanis-pH4-c | Carboxypeptidase S1 SCPB | SCPB_NANOT | 72'773.80 | 9 | 11 | 12 | 0.30% | 18.00% |
| Mcanis-pH4-c | Phosphoglycerate mutase family protein MCYG_08379 | C5G0A7_NANOT | 36'614.60 | 8 | 8 | 15 | 0.37% | 33.20% |
| Mcanis-pH4-c | FAD binding domain-containing protein MCYG_04968 | C5FQJ6_NANOT | 55'314.40 | 19 | 25 | 68 | 1.68% | 53.50% |
| Mcanis-pH4-c | Putative uncharacterized protein MCYG_06096 | C5FTS4_NANOT | 57'065.30 | 2 | 2 | 2 | 0.05% | 4.76% |
| Mcanis-pH4-c | Glucoamylase MCYG_06367 | C5FUG4_NANOT | 67'893.70 | 23 | 27 | 53 | 1.31% | 42.10% |
| Mcanis-pH4-c | Beta-glucosidase 1 MCYG_00928 | C5FE06_NANOT | 93'578.70 | 12 | 13 | 13 | 0.32% | 22.70% |
| Mcanis-pH4-c | Putative uncharacterized protein MCYG_03637 | C5FM96_NANOT | 24'982.00 | 2 | 2 | 2 | 0.05% | 13.40% |
| Mcanis-pH4-c | DUF1237 domain-containing protein MCYG_03876 | C5FMF4_NANOT | 60'979.20 | 9 | 11 | 18 | 0.44% | 25.30% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Mcanis-pH4-c | Putative uncharacterized protein MCYG_06727 | C5FVH4_NANOT | 36'564.80 | 5 | 6 | 9 | 0.22% | 17.50% |
| Mcanis-pH4-c | Dipeptidyl peptidase DPPIV | A0S5V9_MICCA,DPP4_NANOT | 87'901.80 | 5 | 5 | 5 | 0.12% | 6.97% |
| Mcanis-pH4-c | Copper radical oxidase MCYG_08682 | C5G160_NANOT | 96'394.50 | 2 | 2 | 3 | 0.07% | 2.67% |
| Mcanis-pH4-c | Beta-1,3-glucanosyltransferase 3 MCYG_03246 | C5FL55_NANOT | 56'747.70 | 9 | 13 | 18 | 0.44% | 20.70% |
| Mcanis-pH4-c | Subtilisin-like protease SUB3 | SUB3_MICCA,SUB3_NANOT | 40'837.10 | 15 | 23 | 100 | 2.46% | 62.20% |
| Mcanis-pH4-c | Tripeptidyl-peptidase SED2 | SED2_NANOT | 64'826.30 | 19 | 30 | 247 | 6.09% | 48.70% |
| Mcanis-pH4-c | Predicted protein MCYG_04621 | C5FNU9_NANOT | 35'543.40 | 2 | 2 | 2 | 0.05% | 8.49% |
| Mcanis-pH4-c | Ser/Thr protein phosphatase MCYG_06525 | C5FUX2_NANOT | 71'499.90 | 13 | 14 | 32 | 0.79% | 24.30% |
| Mcanis-pH4-c | Aldose 1-epimerase MCYG_00654 | C5FD82_NANOT | 46'289.40 | 2 | 2 | 2 | 0.05% | 4.45% |
| Mcanis-pH4-c | Carboxypeptidase cpdS MCYG_07753 | C5FX94_NANOT | 58'407.20 | 12 | 19 | 56 | 1.38% | 30.10% |
| Mcanis-pH4-c | Carboxylesterase MCYG_01781 | C5FHY2_NANOT | 55'467.40 | 9 | 10 | 12 | 0.30% | 23.70% |
| Mcanis-pH4-c | Glutaminase GtaA MCYG_00814 | C5FDF2_NANOT | 94'824.40 | 8 | 8 | 11 | 0.27% | 12.60% |
| Mcanis-pH4-c | Gamma-glutamyltranspeptidase MCYG_05524 | C5FS52_NANOT | 62'565.80 | 10 | 15 | 25 | 0.62% | 25.40% |
| Mcanis-pH4-c | Carbohydrate-binding protein MCYG_01090 | C5FEG8_NANOT | 41'707.10 | 7 | 14 | 30 | 0.74% | 30.50% |
| Mcanis-pH4-c | Lcc2 MCYG_02578 | C5FG74_NANOT | 73'949.30 | 5 | 6 | 6 | 0.15% | 12.40% |
| Mcanis-pH4-c | Beta-glucosidase 4 MCYG_01527 | C5FHG8_NANOT | 33'218.80 | 13 | 16 | 90 | 2.22% | 49.20% |
| Mcanis-pH4-c | 1,2-alpha-D-mannosidase MCYG_00423 | C5FCK1_NANOT | 55'930.00 | 19 | 24 | 54 | 1.33% | 48.90% |
| Mcanis-pH4-c | Extracellular metalloproteinase MEP3 | MEP3_MICCA,MEP3_NANOT | 69'360.10 | 12 | 19 | 50 | 1.23% | 37.80% |
| Mcanis-pH4-c | Peptidase M28 MCYG_04217 | C5FP82_NANOT | 55'810.30 | 16 | 20 | 44 | 1.08% | 41.30% |
| Mcanis-pH4-c | Putative uncharacterized protein MCYG_03515 | C5FLX4_NANOT | 22'660.00 | 4 | 4 | 5 | 0.12% | 21.00% |
| Mcanis-pH4-c | 6-hydroxy-D-nicotine oxidase MCYG_04909 | C5FQD7_NANOT | 55'997.50 | 8 | 12 | 20 | 0.49% | 22.50% |
| Mcanis-pH4-c | Dipeptidyl-peptidase DPP5 | DPP5_NANOT | 80'058.80 | 29 | 43 | 61 | 1.50% | 54.40% |
| Mcanis-pH4-c | Feruloyl esterase B MCYG_06844 | C5FVU1_NANOT | 57'917.20 | 4 | 4 | 4 | 0.10% | 10.00% |
| Mcanis-pH4-c | Polysaccharide deacetylase family protein MCYG_07538 | C5FYX1_NANOT | 32'868.80 | 7 | 13 | 73 | 1.80% | 36.30% |
| Mcanis-pH4-c | Alpha-glucosidase MCYG_00092 | C5FBM0_NANOT | 100'911.70 | 4 | 4 | 4 | 0.10% | 6.49% |
| Mcanis-pH4-c | 2,6-dihydropseudooxynicotine hydrolase MCYG_02065 | C5FIH7_NANOT | 48'680.60 | 2 | 2 | 4 | 0.10% | 6.38% |
| Mcanis-pH4-c | Putative uncharacterized protein MCYG_04944 | C5FQH2_NANOT | 18'929.80 | 4 | 4 | 5 | 0.12% | 33.90% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Mcanis-pH4-c | 1,3(4)-beta-glucanase MCYG_05925 | C5FTA3_NANOT | 48'684.20 | 5 | 8 | 11 | 0.27% | 20.70% |
| Mcanis-pH4-c | Putative uncharacterized protein MCYG_04152 | C5FN97_NANOT | 21'011.20 | 4 | 7 | 12 | 0.30% | 19.00% |
| Mcanis-pH4-c | Carboxy-cis,cis-muconate cyclase MCYG_00595 | C5FD23_NANOT | 41'885.60 | 8 | 8 | 12 | 0.30% | 35.60% |
| Mcanis-pH4-c | Putative uncharacterized protein MCYG_02207 | C5FJ58_NANOT | 61'443.50 | 15 | 20 | 55 | 1.36% | 37.70% |
| Mcanis-pH4-c | Laccase-1 MCYG_08571 | C5G0U9_NANOT | 64'927.40 | 16 | 25 | 56 | 1.38% | 38.70% |
| Mcanis-pH4-c | 6-hydroxy-D-nicotine oxidase MCYG_04127 | C5FN72_NANOT | 62'598.10 | 8 | 10 | 19 | 0.47% | 18.80% |
| Mcanis-pH4-c | Phytase MCYG_02670 | C5FGG6_NANOT | 51'413.20 | 11 | 15 | 17 | 0.42% | 32.20% |
| Mcanis-pH4-c | Tripeptidyl-peptidase SED3 | SED3_NANOT | 65'310.40 | 5 | 5 | 5 | 0.12% | 13.60% |
| Mcanis-pH4-c | Ribonuclease T2-like protein MCYG_02204 | C5FJ55_NANOT | 42'269.30 | 6 | 6 | 10 | 0.25% | 14.90% |
| Mcanis-pH4-c | FAD binding domain-containing protein MCYG_07459 | C5FYP2_NANOT | 54'460.30 | 2 | 2 | 3 | 0.07% | 4.81% |
| Mcanis-pH4-c | Probable neutral protease 2 homolog MCYG_07155 | NPIID_NANOT | 38'232.20 | 8 | 10 | 152 | 3.75% | 19.80% |
| Mcanis-pH4-c | Putative uncharacterized protein MCYG_00150 | C5FBS8_NANOT | 18'683.60 | 2 | 2 | 2 | 0.05% | 16.70% |
| Mcanis-pH4-c | Putative uncharacterized protein MCYG_07328 | C5FYB1_NANOT | 20'833.50 | 9 | 18 | 148 | 3.65% | 63.70% |
| Mcanis-pH4-c | Peptidase S41 family protein MCYG_03610 | C5FM69_NANOT | 76'977.00 | 29 | 37 | 94 | 2.32% | 46.50% |
| Mcanis-pH4-c | Probable leucine aminopeptidase MCYG_04170 | LAP5_NANOT | 38'208.00 | 2 | 2 | 5 | 0.12% | 9.80% |
| Mcanis-pH4-c | Putative uncharacterized protein MCYG_05202 | C5FR80_NANOT | 19'295.60 | 3 | 3 | 3 | 0.07% | 23.00% |
| Mcanis-pH4-c | Metallocarboxypeptidase MCPA | MCPA_NANOT | 47'079.60 | 9 | 15 | 39 | 0.96% | 32.00% |
| Mcanis-pH4-c | Alkaline serine protease MCYG_02748 | C5FGP4_NANOT | 40'782.50 | 3 | 3 | 3 | 0.07% | 12.40% |
| Mcanis-pH4-c | Tripeptidyl-peptidase SED4 | SED4_NANOT | 65'895.80 | 12 | 21 | 113 | 2.79% | 28.30% |
| Mcanis-pH4-c | Glucan 1,3-beta-glucosidase MCYG_05874 | C5FT52_NANOT | 45'774.50 | 2 | 2 | 2 | 0.05% | 4.59% |
| Mcanis-pH4-c | Alkaline proteinase MCYG_01476 | C5FH27_NANOT | 41'484.80 | 4 | 5 | 6 | 0.15% | 16.50% |
| Mcanis-pH4-c | ATP-dependent permease MCYG_02170 | C5FJ21_NANOT | 107'299.40 | 2 | 2 | 2 | 0.05% | 2.99% |
| Mcanis-pH4-c | Mucin MCYG_07854 | C5FXJ5_NANOT | 76'937.60 | 13 | 15 | 16 | 0.39% | 24.10% |
| Mcanis-pH4-c | Ecm33 MCYG_01225 | C5FEL3_NANOT | 41'117.10 | 8 | 9 | 32 | 0.79% | 21.00% |
| Mcanis-pH4-c | Metallocarboxypeptidase A-like protein MCYG_01475 | MCPAL_NANOT | 45'896.90 | 10 | 16 | 84 | 2.07% | 43.00% |
| Mcanis-pH4-c | Oxalate decarboxylase oxdD MCYG_04379 | C5FNE6_NANOT | 44'218.50 | 9 | 11 | 29 | 0.72% | 33.90% |
| Mcanis-pH4-c | Glycerophosphoryl diester phosphodiesterase MCYG_05364 | C5FRP2_NANOT | 44'497.00 | 4 | 4 | 4 | 0.10% | 11.50% |
| Mcanis-pH4-c | Putative uncharacterized protein MCYG_00366 | C5FC55_NANOT | 61'787.00 | 8 | 9 | 16 | 0.39% | 18.80% |
| Mcanis-pH4-c | Endo-beta-1,3-glucanase MCYG_06694 | C5FVE1_NANOT | 73'890.20 | 3 | 3 | 3 | 0.07% | 5.31% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Mcanis-pH4-c | Sulphydryl oxidase Sox MCYG_02499 | C5FFZ5_NANOT | 44'024.60 | 15 | 21 | 81 | 2.00% | 43.30% |
| Mcanis-pH4-c | Phosphatidylinositol transfer protein MCYG_02226 | C5FFG0_NANOT | 18'642.00 | 3 | 4 | 5 | 0.12% | 27.40% |
| Mcanis-pH4-c | Glycosyl hydrolase MCYG_03093 | C5FKQ2_NANOT | 87'712.10 | 10 | 11 | 12 | 0.30% | 20.90% |
| Mcanis-pH4-c | GDSL Lipase/Acylhydrolase family protein MCYG_04422 | C5FNI9_NANOT | 27'702.80 | 5 | 7 | 7 | 0.17% | 36.70% |
| Mcanis-pH4-c | Putative uncharacterized protein MCYG_03189 | C5FKZ8_NANOT | 46'783.50 | 4 | 6 | 6 | 0.15% | 14.40% |
| Mcanis-pH4-c | Beta-hexosaminidase MCYG_02200 | C5FJ51_NANOT | 67'781.60 | 6 | 6 | 6 | 0.15% | 13.20% |
| Mcanis-pH4-c | IgE-binding protein MCYG_03081 | C5FKP0_NANOT | 20'333.70 | 6 | 8 | 29 | 0.72% | 41.80% |
| Mcanis-pH4-c | Putative uncharacterized protein MCYG_08572 | C5G0V0_NANOT | 33'320.40 | 7 | 9 | 27 | 0.67% | 38.30% |
| Mcanis-pH4-c | N,O-diacetylmuramidase MCYG_02645 | C5FGE1_NANOT | 25'288.00 | 5 | 7 | 13 | 0.32% | 26.70% |
| Mcanis-pH4-c | Carboxypeptidase S1 homolog SCPA | SPCA_NANOT | 72'179.60 | 15 | 23 | 47 | 1.16% | 29.00% |
| Mcanis-pH4-c | Carboxypeptidase Y MCYG_05486 | C5FS14_NANOT | 66'387.20 | 7 | 10 | 15 | 0.37% | 16.40% |
| Mcanis-pH4-c | Aminopeptidase Y LAP1 | LAP1_NANOT | 52'839.50 | 2 | 2 | 2 | 0.05% | 5.25% |
| Mcanis-pH4-c | Endo/exonuclease/phosphatase family MCYG_03887 | C5FMG5_NANOT | 33'384.70 | 1 | 1 | 1 | 0.02% | 2.67% |
| Mcanis-pH4-c | FAD binding domain-containing protein MCYG_06815 | C5FVR2_NANOT | 63'164.50 | 14 | 19 | 22 | 0.54% | 38.40% |
| Mcanis-pH4-c | Glycosyl hydrolase family 3 N terminal MCYG_07820 | C5FXG1_NANOT | 40'034.60 | 7 | 7 | 7 | 0.17% | 21.10% |
| Mcanis-pH4-c | Extracellular metalloproteinase MEP5 | MEP5_MICCA,MEP5_NANOT | 69'529.10 | 11 | 12 | 34 | 0.84% | 28.50% |
| Mcanis-pH4-c | Aspartic endopeptidase PEP1 | PEPF_NANOT | 41'148.90 | 3 | 6 | 97 | 2.39% | 13.40% |
| Mcanis-pH4-c | Putative uncharacterized protein MCYG_05599 | C5FSC7_NANOT | 20'561.90 | 3 | 6 | 63 | 1.55% | 31.20% |
| Mcanis-pH4-c | Extracellular cell wall glucanase Crf1MCYG_04874 | C5FQA2_NANOT | 44'996.20 | 9 | 14 | 40 | 0.99% | 31.00% |
| Mcanis-pH4-c | Putative uncharacterized protein MCYG_03822 | C5FMA0_NANOT | 56'426.10 | 6 | 6 | 6 | 0.15% | 16.80% |
| Mcanis-pH4-c | Leucine aminopeptidase LAP2 | LAP2_NANOT | 40'524.20 | 2 | 2 | 2 | 0.05% | 6.17% |
| Mcanis-pH4-c | Amidase MCYG_00475 | C5FCQ3_NANOT | 61'861.80 | 7 | 8 | 12 | 0.30% | 15.60% |
| Mcanis-pH4-c | Cell wall protein PhiA MCYG_00375 | C5FC64_NANOT | 19'726.70 | 5 | 6 | 14 | 0.35% | 37.10% |
| Mcanis-pH4-c | Predicted protein MCYG_01508 | C5FHE9_NANOT | 19'477.60 | 2 | 4 | 5 | 0.12% | 27.20% |
| Mcanis-pH4-c | Acid trehalase MCYG_02758 | C5FGQ4_NANOT | 114'531.50 | 7 | 7 | 7 | 0.17% | 8.86% |
| Mcanis-pH4-c | Proteinase inhibitor I4 MCYG_03085 | C5FKP4_NANOT | 39'549.80 | 3 | 3 | 3 | 0.07% | 12.10% |
| Mcanis-pH4-c | Aspartic protease MCYG_05372 | C5FRQ0_NANOT | 50'249.10 | 8 | 10 | 21 | 0.52% | 28.80% |
| Mcanis-pH4-c | Putative uncharacterized protein MCYG_01908 | C5FIA9_NANOT | 96'835.90 | 3 | 3 | 3 | 0.07% | 4.76% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Mcanis-pH4-c | Exo-beta-1,3-glucanase Exg0 MCYG_06600 | C5FV47_NANOT | 33'625.30 | 7 | 8 | 14 | 0.35% | 40.40% |
| Mcanis-pH4-c | Putative uncharacterized protein MCYG_03713 | C5FJN3_NANOT | 25'502.60 | 8 | 12 | 60 | 1.48% | 43.70% |
| Mcanis-pH4-c | Acid phosphatase MCYG_07729 | C5FX70_NANOT | 30'625.90 | 5 | 9 | 19 | 0.47% | 31.10% |
| Mcanis-pH4-c | Extracellular metalloproteinase MEP1 | MEP1_MICCA,MEP1_NANOT | 69'753.70 | 5 | 6 | 8 | 0.20% | 18.40% |
| Mcanis-pH4-c | Carboxylesterase family protein MCYG_00869 | C5FDU7_NANOT | 62'320.30 | 8 | 8 | 12 | 0.30% | 15.80% |
| Mcanis-pH4-c | Lysophospholipase MCYG_02652 | C5FGE8_NANOT | 68'263.40 | 8 | 8 | 13 | 0.32% | 12.60% |
| Mcanis-pH7-a | Putative uncharacterized protein MCYG_08685 | C5G163_NANOT | 79'189.30 | 2 | 2 | 2 | 0.06% | 4.58% |
| Mcanis-pH7-a | Alkaline phosphatase MCYG_04625 | C5FNV3_NANOT | 56'302.20 | 30 | 56 | 358 | 10.00% | 72.50% |
| Mcanis-pH7-a | 4-hydroxyphenylpyruvate dioxygenase MCYG_00524 | C5FCV2_NANOT | 45'157.10 | 5 | 6 | 8 | 0.22% | 19.00% |
| Mcanis-pH7-a | Putative uncharacterized protein MCYG_01574 | C5FHL5_NANOT | 27'690.30 | 10 | 14 | 20 | 0.56% | 42.80% |
| Mcanis-pH7-a | Putative uncharacterized protein MCYG_04556 | C5FNN4_NANOT | 50'223.20 | 4 | 4 | 4 | 0.11% | 13.50% |
| Mcanis-pH7-a | Subtilisin-like protease SUB7 | SUB7_NANOT | 41'362.20 | 6 | 9 | 21 | 0.59% | 28.50% |
| Mcanis-pH7-a | LysM domain-containing protein MCYG_04646 | C5FNX4_NANOT | 28'076.50 | 5 | 7 | 29 | 0.81% | 29.20% |
| Mcanis-pH7-a | Glycerophosphoryl diester phosphodiesterase MCYG_05364 | C5FRP2_NANOT | 44'497.00 | 3 | 3 | 3 | 0.08% | 11.50% |
| Mcanis-pH7-a | Phosphoglycerate mutase family protein MCYG_08379 | C5G0A7_NANOT | 36'614.60 | 6 | 6 | 9 | 0.25% | 25.90% |
| Mcanis-pH7-a | Secreted protein MCYG_00341 | C5FC30_NANOT | 22'728.10 | 7 | 10 | 27 | 0.76% | 54.50% |
| Mcanis-pH7-a | Dipeptidase 1 MCYG_02918 | C5FK77_NANOT | 47'224.20 | 11 | 14 | 15 | 0.42% | 40.00% |
| Mcanis-pH7-a | Putative uncharacterized protein MCYG_06096 | C5FTS4_NANOT | 57'065.30 | 14 | 15 | 15 | 0.42% | 36.80% |
| Mcanis-pH7-a | Putative uncharacterized protein MCYG_07164 | C5FXU7_NANOT | 48'942.20 | 7 | 7 | 10 | 0.28% | 21.90% |
| Mcanis-pH7-a | Probable neutral protease 2 homolog MCYG_05201 | NPIIA_NANOT | 40'199.70 | 4 | 4 | 6 | 0.17% | 16.40% |
| Mcanis-pH7-a | Dihydrolipoamide dehydrogenase MCYG_08327 | C5G055_NANOT | 46'342.40 | 3 | 3 | 4 | 0.11% | 10.70% |
| Mcanis-pH7-a | Beta-glucosidase 1 MCYG_00928 | C5FE06_NANOT | 93'578.70 | 22 | 28 | 40 | 1.12% | 36.30% |
| Mcanis-pH7-a | Sulphydryl oxidase Sox MCYG_02499 | C5FFZ5_NANOT | 44'024.60 | 2 | 2 | 4 | 0.11% | 5.82% |
| Mcanis-pH7-a | DUF1237 domain-containing protein MCYG_03876 | C5FMF4_NANOT | 60'979.20 | 5 | 6 | 9 | 0.25% | 13.70% |
| Mcanis-pH7-a | Ecm33 MCYG_01225 | C5FEL3_NANOT | 41'117.10 | 8 | 10 | 13 | 0.36% | 23.60% |
| Mcanis-pH7-a | HypA MCYG_01872 | C5FI73_NANOT | 51'308.20 | 5 | 6 | 6 | 0.17% | 15.60% |
| Mcanis-pH7-a | Beta-1,3-glucanosyltransferase 3 MCYG_03246 | C5FL55_NANOT | 56'747.70 | 5 | 5 | 6 | 0.17% | 13.50% |
| Mcanis-pH7-a | Subtilisin-like protease SUB3 | SUB3_MICCA,SUB3_NANOT | 40'837.10 | 16 | 22 | 169 | 4.74% | 63.20% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Mcanis-pH7-a | Putative uncharacterized protein MCYG_00150 | C5FBS8_NANOT | 18'683.60 | 3 | 6 | 20 | 0.56% | 30.10% |
| Mcanis-pH7-a | Extracellular metalloproteinase MEP4 | MEP4_MICCA,MEP4_NANOT | 71'233.30 | 12 | 13 | 37 | 1.04% | 23.30% |
| Mcanis-pH7-a | Fumarate reductase flavoprotein subunit MCYG_02604 | C5FGA0_NANOT | 67'964.70 | 5 | 5 | 6 | 0.17% | 9.18% |
| Mcanis-pH7-a | Lactoylglutathione lyase MCYG_08655 | C5G133_NANOT | 36'259.70 | 1 | 1 | 1 | 0.03% | 3.47% |
| Mcanis-pH7-a | Carbohydrate-binding protein MCYG_01090 | C5FEG8_NANOT | 41'707.10 | 4 | 5 | 5 | 0.14% | 18.60% |
| Mcanis-pH7-a | Alpha-mannosidase MCYG_06176 | C5FTX3_NANOT | 123'001.50 | 33 | 38 | 72 | 2.02% | 37.10% |
| Mcanis-pH7-a | Metallocarboxypeptidase MCPA | MCPA_NANOT | 47'079.60 | 3 | 5 | 8 | 0.22% | 11.10% |
| Mcanis-pH7-a | Beta-glucosidase 4 MCYG_01527 | C5FHG8_NANOT | 33'218.80 | 9 | 12 | 29 | 0.81% | 43.30% |
| Mcanis-pH7-a | Arabinan endo-1,5-alpha-L-arabinosidase A MCYG_02777 | C5FGS3_NANOT | 36'492.80 | 4 | 4 | 8 | 0.22% | 16.40% |
| Mcanis-pH7-a | N-carbamoyl-L-amino acid hydrolase MCYG_03207 | C5FL16_NANOT | 50'422.80 | 2 | 2 | 2 | 0.06% | 5.84% |
| Mcanis-pH7-a | Tripeptidyl-peptidase SED2 | SED2_NANOT | 64'826.30 | 2 | 2 | 2 | 0.06% | 4.87% |
| Mcanis-pH7-a | Gamma-glutamyltranspeptidase MCYG_05524 | C5FS52_NANOT | 62'565.80 | 3 | 3 | 3 | 0.08% | 9.04% |
| Mcanis-pH7-a | Peptidase S8 and S53 MCYG_01699 | C5FHQ0_NANOT | 75'193.00 | 1 | 1 | 1 | 0.03% | 2.59% |
| Mcanis-pH7-a | Carboxypeptidase 2 MCPB | MCPB_NANOT | 59'765.20 | 24 | 35 | 64 | 1.79% | 54.20% |
| Mcanis-pH7-a | Putative uncharacterized protein MCYG_01528 | C5FHG9_NANOT | 250'822.20 | 1 | 1 | 1 | 0.03% | 0.49% |
| Mcanis-pH7-a | 1,2-alpha-D-mannosidase MCYG_00423 | C5FCK1_NANOT | 55'930.00 | 13 | 13 | 15 | 0.42% | 37.60% |
| Mcanis-pH7-a | Extracellular metalloproteinase MEP3 | MEP3_MICCA,MEP3_NANOT | 69'360.10 | 12 | 16 | 38 | 1.07% | 36.30% |
| Mcanis-pH7-a | Dipeptidyl peptidase DPPIV | A0S5V9_MICCA,DPP4_NANOT | 87'901.80 | 28 | 37 | 54 | 1.51% | 46.20% |
| Mcanis-pH7-a | Glutamate carboxypeptidase 2 MCYG_07431 | C5FYL4_NANOT | 78'351.90 | 11 | 16 | 22 | 0.62% | 22.60% |
| Mcanis-pH7-a | Neutral ceramidase MCYG_06637 | C5FV84_NANOT | 83'246.50 | 16 | 22 | 25 | 0.70% | 32.10% |
| Mcanis-pH7-a | Predicted protein MCYG_08251 | C5FZX9_NANOT | 17'591.50 | 2 | 2 | 2 | 0.06% | 13.80% |
| Mcanis-pH7-a | Dienelactone hydrolase family protein MCYG_02976 | C5FKD5_NANOT | 32'377.30 | 3 | 3 | 3 | 0.08% | 10.70% |
| Mcanis-pH7-a | Putative uncharacterized protein MCYG_03515 | C5FLX4_NANOT | 22'660.00 | 3 | 3 | 3 | 0.08% | 18.50% |
| Mcanis-pH7-a | Endochitinase 1 MCYG_00676 | C5FDA4_NANOT | 44'249.10 | 14 | 16 | 34 | 0.95% | 50.90% |
| Mcanis-pH7-a | Superoxide dismutase [Cu-Zn] MCYG_02077 | C5FII9_NANOT | 15'965.90 | 6 | 10 | 18 | 0.50% | 61.70% |
| Mcanis-pH7-a | Exochitinase 1 MCYG_05745 | C5FSS3_NANOT | 53'464.50 | 2 | 3 | 3 | 0.08% | 6.85% |
| Mcanis-pH7-a | Lysophospholipase MCYG_02652 | C5FGE8_NANOT | 68'263.40 | 3 | 3 | 3 | 0.08% | 5.82% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Mcanis-pH7-a | Putative uncharacterized protein MCYG_03989 | C5FMR7_NANOT | 45'583.40 | 5 | 5 | 5 | 0.14% | 19.00% |
| Mcanis-pH7-a | Alpha-glucosidase MCYG_00092 | C5FBM0_NANOT | 100'911.70 | 8 | 9 | 9 | 0.25% | 12.90% |
| Mcanis-pH7-a | Aconitase MCYG_03299 | C5FLA8_NANOT | 84'103.20 | 3 | 3 | 4 | 0.11% | 4.52% |
| Mcanis-pH7-a | Copper radical oxidase MCYG_08682 | C5G160_NANOT | 96'394.50 | 24 | 32 | 56 | 1.57% | 42.60% |
| Mcanis-pH7-a | Superoxide dismutase MCYG_06348 | C5FUE5_NANOT | 25'212.90 | 4 | 5 | 9 | 0.25% | 27.30% |
| Mcanis-pH7-a | G-protein complex beta subunit CpcB MCYG_01069 | C5FEE7_NANOT | 35'071.00 | 7 | 7 | 9 | 0.25% | 24.70% |
| Mcanis-pH7-a | Class II aldolase/adducin domain MCYG_00391 | C5FC80_NANOT | 31'482.70 | 3 | 3 | 3 | 0.08% | 12.90% |
| Mcanis-pH7-a | Putative uncharacterized protein MCYG_04152 | C5FN97_NANOT | 21'011.20 | 5 | 6 | 9 | 0.25% | 19.50% |
| Mcanis-pH7-a | Aspartylglucosaminidase family protein MCYG_04957 | C5FQI5_NANOT | 44'887.40 | 5 | 6 | 6 | 0.17% | 18.80% |
| Mcanis-pH7-a | Beta-mannosidase MCYG_06102 | C5FTT0_NANOT | 97'855.30 | 2 | 2 | 3 | 0.08% | 2.22% |
| Mcanis-pH7-a | Glutaminase GtaA MCYG_00814 | C5FDF2_NANOT | 94'824.40 | 7 | 7 | 9 | 0.25% | 13.40% |
| Mcanis-pH7-a | GH17932p MCYG_04430 | C5FNJ7_NANOT | 22'552.00 | 4 | 4 | 5 | 0.14% | 28.50% |
| Mcanis-pH7-a | 6-hydroxy-D-nicotine oxidase MCYG_04127 | C5FN72_NANOT | 62'598.10 | 18 | 24 | 43 | 1.21% | 48.80% |
| Mcanis-pH7-a | Cutinase MCYG_05629 | C5FSF7_NANOT | 28'464.90 | 5 | 5 | 7 | 0.20% | 28.50% |
| Mcanis-pH7-a | Secretory phospholipase A2 MCYG_00583 | C5FD11_NANOT | 16'687.00 | 3 | 3 | 3 | 0.08% | 27.30% |
| Mcanis-pH7-a | Putative uncharacterized protein MCYG_07025 | C5FWC2_NANOT | 32'427.90 | 3 | 3 | 3 | 0.08% | 13.20% |
| Mcanis-pH7-a | Putative uncharacterized protein MCYG_07766 | C5FXA7_NANOT | 23'998.90 | 3 | 3 | 5 | 0.14% | 13.30% |
| Mcanis-pH7-a | Peptidase S41 family protein MCYG_03610 | C5FM69_NANOT | 76'977.00 | 4 | 4 | 5 | 0.14% | 8.37% |
| Mcanis-pH7-a | Chitinase 4 MCYG_08403 | C5G0D1_NANOT | 38'958.30 | 2 | 3 | 3 | 0.08% | 10.90% |
| Mcanis-pH7-a | Glycosyl hydrolase family 3 N terminal MCYG_07820 | C5FXG1_NANOT | 40'034.60 | 2 | 2 | 2 | 0.06% | 8.82% |
| Mcanis-pH7-a | Aspartyl aminopeptidase MCYG_06746 | C5FVJ3_NANOT | 73'706.30 | 3 | 3 | 3 | 0.08% | 5.05% |
| Mcanis-pH7-a | Aminopeptidase P MCYG_01718 | C5FHR9_NANOT | 69'181.80 | 3 | 3 | 4 | 0.11% | 6.41% |
| Mcanis-pH7-a | Oxalate decarboxylase MCYG_02705 | C5FGK1_NANOT | 43'946.20 | 3 | 3 | 3 | 0.08% | 20.10% |
| Mcanis-pH7-a | Glycoprotein X MCYG_08739 | C5G1B7_NANOT | 98'352.10 | 3 | 3 | 3 | 0.08% | 4.14% |
| Mcanis-pH7-a | Tripeptidyl-peptidase SED4 | SED4_NANOT | 65'895.80 | 2 | 2 | 4 | 0.11% | 4.16% |
| Mcanis-pH7-a | Glucoamylase MCYG_06367 | C5FUG4_NANOT | 67'893.70 | 34 | 51 | 388 | 10.90% | 63.50% |
| Mcanis-pH7-a | ATP-dependent permease MCYG_02170 | C5FJ21_NANOT | 107'299.40 | 2 | 2 | 3 | 0.08% | 3.92% |
| Mcanis-pH7-a | Mucin MCYG_07854 | C5FXJ5_NANOT | 76'937.60 | 28 | 39 | 78 | 2.19% | 50.30% |
| Mcanis-pH7-a | PBSP domain-containing protein MCYG_01682 | C5FHE4_NANOT | 32'863.60 | 1 | 1 | 2 | 0.06% | 3.95% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Mcanis-pH7-a | Oxalate decarboxylase oxdD MCYG_04379 | C5FNE6_NANOT | 44'218.50 | 10 | 13 | 19 | 0.53% | 42.60% |
| Mcanis-pH7-a | Thimet oligopeptidase MCYG_02264 | C5FFJ8_NANOT | 81'672.10 | 2 | 2 | 2 | 0.06% | 2.79% |
| Mcanis-pH7-a | Hydrophobic surface binding protein B MCYG_01969 | C5FIQ9_NANOT | 19'243.20 | 6 | 9 | 17 | 0.48% | 48.40% |
| Mcanis-pH7-a | Phosphatidylinositol transfer protein MCYG_02226 | C5FFG0_NANOT | 18'642.00 | 6 | 10 | 46 | 1.29% | 38.90% |
| Mcanis-pH7-a | Cyanate hydratase MCYG_03856 | C5FMD4_NANOT | 17'890.20 | 4 | 4 | 4 | 0.11% | 36.30% |
| Mcanis-pH7-a | Beta-hexosaminidase MCYG_02200 | C5FJ51_NANOT | 67'781.60 | 13 | 15 | 16 | 0.45% | 33.30% |
| Mcanis-pH7-a | IgE-binding protein MCYG_03081 | C5FKP0_NANOT | 20'333.70 | 5 | 5 | 11 | 0.31% | 27.80% |
| Mcanis-pH7-a | Citrate synthase MCYG_03094 | C5FKQ3_NANOT | 43'799.80 | 3 | 3 | 4 | 0.11% | 10.70% |
| Mcanis-pH7-a | Extracellular serine-threonine rich protein MCYG_04810 | C5FQ38_NANOT | 92'970.60 | 3 | 3 | 4 | 0.11% | 4.31% |
| Mcanis-pH7-a | Aminopeptidase Y LAP1 | LAP1_NANOT | 52'839.50 | 27 | 42 | 156 | 4.37% | 57.80% |
| Mcanis-pH7-a | Probable neutral protease 2 homolog MCYG_07155 | NPIID_NANOT | 38'232.20 | 5 | 6 | 12 | 0.34% | 19.30% |
| Mcanis-pH7-a | Aspartic endopeptidase PEP1 | PEPF_NANOT | 41'148.90 | 3 | 5 | 6 | 0.17% | 13.40% |
| Mcanis-pH7-a | Nucleoside diphosphate kinase MCYG_01839 | C5FI40_NANOT | 16'989.90 | 5 | 6 | 14 | 0.39% | 39.90% |
| Mcanis-pH7-a | Leucine aminopeptidase LAP2 | LAP2_NANOT | 40'524.20 | 6 | 8 | 10 | 0.28% | 24.40% |
| Mcanis-pH7-a | Amidase MCYG_00475 | C5FCQ3_NANOT | 61'861.80 | 22 | 34 | 81 | 2.27% | 54.60% |
| Mcanis-pH7-a | Putative uncharacterized protein MCYG_00366 | C5FC55_NANOT | 61'787.00 | 5 | 5 | 5 | 0.14% | 13.90% |
| Mcanis-pH7-a | LysM domain-containing protein MCYG_02074 | C5FII6_NANOT | 21'919.30 | 2 | 4 | 5 | 0.14% | 17.30% |
| Mcanis-pH7-a | Putative uncharacterized protein MCYG_03637 | C5FM96_NANOT | 24'982.00 | 2 | 2 | 4 | 0.11% | 19.00% |
| Mcanis-pH7-a | Predicted protein MCYG_04553 | C5FNN1_NANOT | 27'810.60 | 6 | 8 | 17 | 0.48% | 33.50% |
| Mcanis-pH7-a | Metallocarboxypeptidase A-like protein MCYG_01475 | MCPAL_NANOT | 45'896.90 | 7 | 11 | 33 | 0.93% | 24.50% |
| Mcanis-pH7-a | FAD binding domain-containing protein MCYG_04968 | C5FQJ6_NANOT | 55'314.40 | 19 | 27 | 75 | 2.10% | 50.80% |
| Mcanis-pH7-a | Glutathione S-transferase Gst3 MCYG_07941 | C5FXT2_NANOT | 40'867.60 | 5 | 5 | 5 | 0.14% | 16.00% |
| Mcanis-pH7-a | Polysaccharide deacetylase family protein MCYG_07538 | C5FYX1_NANOT | 32'868.80 | 8 | 12 | 78 | 2.19% | 37.90% |
| Mcanis-pH7-a | Exo-beta-1,3-glucanase Exg0 MCYG_06600 | C5FV47_NANOT | 33'625.30 | 5 | 5 | 10 | 0.28% | 23.10% |
| Mcanis-pH7-a | Multicopper oxidase MCYG_01191 | C5FF09_NANOT | 74'572.90 | 7 | 7 | 7 | 0.20% | 15.30% |
| Mcanis-pH7-a | Alanine-glyoxylate aminotransferase MCYG_08492 | C5G0M0_NANOT | 41'392.90 | 2 | 2 | 2 | 0.06% | 7.07% |
| Mcanis-pH7-a | Putative uncharacterized protein MCYG_05599 | C5FSC7_NANOT | 20'561.90 | 3 | 5 | 9 | 0.25% | 31.20% |
| Mcanis-pH7-a | Uricase MCYG_00197 | C5FBX5_NANOT | 33'902.40 | 2 | 2 | 2 | 0.06% | 11.10% |
| Mcanis-pH7-a | Fumarylacetoacetase MCYG_00527 | C5FCV5_NANOT | 47'213.90 | 11 | 13 | 27 | 0.76% | 39.10% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Mcanis-pH7-b | Polysaccharide deacetylase family protein MCYG_04253 | C5FPB8_NANOT | 30'019.90 | 2 | 2 | 4 | 0.14% | 9.25% |
| Mcanis-pH7-b | Putative uncharacterized protein MCYG_08685 | C5G163_NANOT | 79'189.30 | 3 | 3 | 3 | 0.10% | 6.60% |
| Mcanis-pH7-b | Alkaline phosphatase MCYG_04625 | C5FNV3_NANOT | 56'302.20 | 22 | 26 | 64 | 2.18% | 65.30% |
| Mcanis-pH7-b | 4-hydroxyphenylpyruvate dioxygenase MCYG_00524 | C5FCV2_NANOT | 45'157.10 | 3 | 4 | 7 | 0.24% | 9.25% |
| Mcanis-pH7-b | Probable neutral protease 2 homolog MCYG_05201 | NPIIA_NANOT | 40'199.70 | 3 | 3 | 8 | 0.27% | 9.38% |
| Mcanis-pH7-b | Putative uncharacterized protein MCYG_04556 | C5FNN4_NANOT | 50'223.20 | 3 | 3 | 7 | 0.24% | 6.43% |
| Mcanis-pH7-b | LysM domain-containing protein MCYG_04646 | C5FNX4_NANOT | 28'076.50 | 3 | 4 | 15 | 0.51% | 12.80% |
| Mcanis-pH7-b | Glycerophosphoryl diester phosphodiesterase MCYG_05364 | C5FRP2_NANOT | 44'497.00 | 3 | 3 | 3 | 0.10% | 10.00% |
| Mcanis-pH7-b | Phosphoglycerate mutase family protein MCYG_08379 | C5G0A7_NANOT | 36'614.60 | 5 | 5 | 8 | 0.27% | 20.70% |
| Mcanis-pH7-b | Metallocarboxypeptidase A-like protein MCYG_01475 | MCPAL_NANOT | 45'896.90 | 3 | 3 | 4 | 0.14% | 9.62% |
| Mcanis-pH7-b | Dipeptidase 1 MCYG_02918 | C5FK77_NANOT | 47'224.20 | 12 | 15 | 21 | 0.72% | 36.80% |
| Mcanis-pH7-b | FAD binding domain-containing protein MCYG_04968 | C5FQJ6_NANOT | 55'314.40 | 16 | 18 | 50 | 1.71% | 40.40% |
| Mcanis-pH7-b | Putative uncharacterized protein MCYG_06096 | C5FTS4_NANOT | 57'065.30 | 5 | 5 | 6 | 0.21% | 13.10% |
| Mcanis-pH7-b | Carboxypeptidase cpdS MCYG_07753 | C5FX94_NANOT | 58'407.20 | 2 | 2 | 2 | 0.07% | 6.13% |
| Mcanis-pH7-b | Putative uncharacterized protein MCYG_07164 | C5FXU7_NANOT | 48'942.20 | 2 | 2 | 2 | 0.07% | 5.42% |
| Mcanis-pH7-b | Dihydrolipoamide dehydrogenase MCYG_08327 | C5G055_NANOT | 46'342.40 | 3 | 4 | 4 | 0.14% | 8.86% |
| Mcanis-pH7-b | Beta-glucosidase 1 MCYG_00928 | C5FE06_NANOT | 93'578.70 | 16 | 22 | 25 | 0.85% | 23.10% |
| Mcanis-pH7-b | Secreted protein MCYG_00341 | C5FC30_NANOT | 22'728.10 | 3 | 3 | 6 | 0.21% | 15.30% |
| Mcanis-pH7-b | Sulphydryl oxidase Sox MCYG_02499 | C5FFZ5_NANOT | 44'024.60 | 2 | 2 | 5 | 0.17% | 5.82% |
| Mcanis-pH7-b | Ecm33 MCYG_01225 | C5FEL3_NANOT | 41'117.10 | 3 | 3 | 7 | 0.24% | 8.55% |
| Mcanis-pH7-b | Fumarylacetoacetase MCYG_00527 | C5FCV5_NANOT | 47'213.90 | 8 | 9 | 21 | 0.72% | 24.60% |
| Mcanis-pH7-b | Beta-glucosidase MCYG_00545 | C5FCX3_NANOT | 135'337.50 | 3 | 3 | 3 | 0.10% | 3.33% |
| Mcanis-pH7-b | Dipeptidyl peptidase DPPIV | A0S5V9_MICCA,DPP4_NANOT | 87'901.80 | 17 | 20 | 39 | 1.33% | 23.60% |
| Mcanis-pH7-b | HypA MCYG_01872 | C5FI73_NANOT | 51'308.20 | 3 | 3 | 4 | 0.14% | 6.46% |
| Mcanis-pH7-b | Beta-1,3-glucanosyltransferase 3 MCYG_03246 | C5FL55_NANOT | 56'747.70 | 1 | 1 | 2 | 0.07% | 2.66% |
| Mcanis-pH7-b | Subtilisin-like protease SUB3 | SUB3_MICCA,SUB3_NANOT | 40'837.10 | 14 | 18 | 59 | 2.01% | 39.30% |
| Mcanis-pH7-b | Putative uncharacterized protein MCYG_00150 | C5FBS8_NANOT | 18'683.60 | 2 | 4 | 11 | 0.38% | 16.70% |
| Mcanis-pH7-b | Aldose 1-epimerase MCYG_00654 | C5FD82_NANOT | 46'289.40 | 2 | 2 | 2 | 0.07% | 4.45% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Mcanis-pH7-b | Oxalate decarboxylase MCYG_02705 | C5FGK1_NANOT | 43'946.20 | 4 | 4 | 5 | 0.17% | 16.60% |
| Mcanis-pH7-b | Extracellular metalloproteinase MEP4 | MEP4_MICCA,MEP4_NANOT | 71'233.30 | 11 | 13 | 45 | 1.53% | 20.20% |
| Mcanis-pH7-b | Stress responsive A/B barrel domain MCYG_02249 | C5FFI3_NANOT | 12'020.40 | 1 | 2 | 2 | 0.07% | 13.20% |
| Mcanis-pH7-b | Fumarate reductase flavoprotein subunit MCYG_02604 | C5FGA0_NANOT | 67'964.70 | 4 | 4 | 5 | 0.17% | 6.49% |
| Mcanis-pH7-b | Predicted protein MCYG_04553 | C5FNN1_NANOT | 27'810.60 | 3 | 4 | 16 | 0.55% | 16.50% |
| Mcanis-pH7-b | Peroxisomal matrix protein MCYG_00580 | C5FD08_NANOT | 18'035.80 | 1 | 1 | 1 | 0.03% | 10.70% |
| Mcanis-pH7-b | Subtilisin-like protease SUB4 | SUB4_NANOT | 42'052.90 | 1 | 2 | 2 | 0.07% | 9.52% |
| Mcanis-pH7-b | Alpha-mannosidase MCYG_06176 | C5FTX3_NANOT | 123'001.50 | 8 | 8 | 13 | 0.44% | 8.29% |
| Mcanis-pH7-b | Metallocarboxypeptidase MCPA | MCPA_NANOT | 47'079.60 | 1 | 1 | 1 | 0.03% | 1.90% |
| Mcanis-pH7-b | Beta-glucosidase 4 MCYG_01527 | C5FHG8_NANOT | 33'218.80 | 8 | 11 | 40 | 1.36% | 33.60% |
| Mcanis-pH7-b | Arabinan endo-1,5-alpha-L-arabinosidase A MCYG_02777 | C5FGS3_NANOT | 36'492.80 | 8 | 8 | 9 | 0.31% | 31.60% |
| Mcanis-pH7-b | Leucine aminopeptidase LAP2 | LAP2_NANOT | 40'524.20 | 8 | 11 | 24 | 0.82% | 31.10% |
| Mcanis-pH7-b | Gamma-glutamyltranspeptidase MCYG_05524 | C5FS52_NANOT | 62'565.80 | 12 | 12 | 18 | 0.61% | 28.30% |
| Mcanis-pH7-b | Chitinase MCYG_04741 | C5FPW9_NANOT | 130'394.50 | 3 | 3 | 3 | 0.10% | 3.00% |
| Mcanis-pH7-b | Chitinase MCYG_04644 | C5FNX2_NANOT | 159'340.70 | 3 | 3 | 4 | 0.14% | 2.64% |
| Mcanis-pH7-b | Carboxypeptidase 2 MCPB | MCPB_NANOT | 59'765.20 | 18 | 20 | 51 | 1.74% | 32.80% |
| Mcanis-pH7-b | Glucan 1,3-beta-glucosidase MCYG_05874 | C5FT52_NANOT | 45'774.50 | 4 | 4 | 7 | 0.24% | 11.60% |
| Mcanis-pH7-b | Extracellular metalloproteinase MEP3 | MEP3_MICCA,MEP3_NANOT | 69'360.10 | 6 | 9 | 33 | 1.13% | 17.20% |
| Mcanis-pH7-b | Glutamate carboxypeptidase 2 MCYG_07431 | C5FYL4_NANOT | 78'351.90 | 7 | 8 | 12 | 0.41% | 14.60% |
| Mcanis-pH7-b | Neutral ceramidase MCYG_06637 | C5FV84_NANOT | 83'246.50 | 10 | 11 | 18 | 0.61% | 17.60% |
| Mcanis-pH7-b | Predicted protein MCYG_08251 | C5FZX9_NANOT | 17'591.50 | 2 | 2 | 2 | 0.07% | 14.40% |
| Mcanis-pH7-b | Dienelactone hydrolase family protein MCYG_02976 | C5FKD5_NANOT | 32'377.30 | 2 | 2 | 3 | 0.10% | 5.50% |
| Mcanis-pH7-b | Putative uncharacterized protein MCYG_02350 | C5FJB0_NANOT | 16'434.60 | 3 | 3 | 4 | 0.14% | 26.00% |
| Mcanis-pH7-b | Exochitinase 1 MCYG_05745 | C5FSS3_NANOT | 53'464.50 | 2 | 2 | 4 | 0.14% | 6.64% |
| Mcanis-pH7-b | Putative uncharacterized protein MCYG_03989 | C5FMR7_NANOT | 45'583.40 | 3 | 3 | 3 | 0.10% | 10.70% |
| Mcanis-pH7-b | G-protein complex beta subunit CpcB MCYG_01069 | C5FEE7_NANOT | 35'071.00 | 4 | 4 | 5 | 0.17% | 12.30% |
| Mcanis-pH7-b | Alpha-glucosidase MCYG_00092 | C5FBM0_NANOT | 100'911.70 | 10 | 11 | 14 | 0.48% | 11.70% |
| Mcanis-pH7-b | Calnexin MCYG_01115 | C5FEJ3_NANOT | 62'059.80 | 1 | 1 | 1 | 0.03% | 1.78% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Mcanis-pH7-b | Aconitase MCYG_03299 | C5FLA8_NANOT | 84'103.20 | 13 | 13 | 16 | 0.55% | 18.10% |
| Mcanis-pH7-b | Copper radical oxidase MCYG_08682 | C5G160_NANOT | 96'394.50 | 16 | 17 | 32 | 1.09% | 24.60% |
| Mcanis-pH7-b | Superoxide dismutase MCYG_06348 | C5FUE5_NANOT | 25'212.90 | 2 | 2 | 2 | 0.07% | 12.10% |
| Mcanis-pH7-b | Class II aldolase/adducin domain MCYG_00391 | C5FC80_NANOT | 31'482.70 | 3 | 4 | 5 | 0.17% | 11.80% |
| Mcanis-pH7-b | Putative uncharacterized protein MCYG_04152 | C5FN97_NANOT | 21'011.20 | 3 | 3 | 4 | 0.14% | 13.30% |
| Mcanis-pH7-b | Aspartylglucosaminidase family protein MCYG_04957 | C5FQI5_NANOT | 44'887.40 | 4 | 5 | 7 | 0.24% | 14.90% |
| Mcanis-pH7-b | Beta-mannosidase MCYG_06102 | C5FTT0_NANOT | 97'855.30 | 4 | 4 | 5 | 0.17% | 4.33% |
| Mcanis-pH7-b | Glutaminase GtaA MCYG_00814 | C5FDF2_NANOT | 94'824.40 | 3 | 3 | 4 | 0.14% | 4.27% |
| Mcanis-pH7-b | GH17932p MCYG_04430 | C5FNJ7_NANOT | 22'552.00 | 2 | 3 | 4 | 0.14% | 14.00% |
| Mcanis-pH7-b | Peptidyl-prolyl cis-trans isomerase D MCYG_08316 | C5G044_NANOT | 38'036.00 | 1 | 1 | 1 | 0.03% | 2.59% |
| Mcanis-pH7-b | Cutinase MCYG_05629 | C5FSF7_NANOT | 28'464.90 | 3 | 3 | 4 | 0.14% | 12.70% |
| Mcanis-pH7-b | Dipeptidyl-peptidase DPP5 | DPP5_NANOT | 80'058.80 | 7 | 7 | 11 | 0.38% | 13.20% |
| Mcanis-pH7-b | 1,2-alpha-D-mannosidase MCYG_00423 | C5FCK1_NANOT | 55'930.00 | 7 | 7 | 7 | 0.24% | 18.20% |
| Mcanis-pH7-b | Putative uncharacterized protein MCYG_07025 | C5FWC2_NANOT | 32'427.90 | 2 | 2 | 2 | 0.07% | 8.11% |
| Mcanis-pH7-b | Putative uncharacterized protein MCYG_07766 | C5FXA7_NANOT | 23'998.90 | 2 | 2 | 3 | 0.10% | 10.40% |
| Mcanis-pH7-b | Putative uncharacterized protein MCYG_07328 | C5FYB1_NANOT | 20'833.50 | 4 | 4 | 12 | 0.41% | 39.90% |
| Mcanis-pH7-b | Peptidase S41 family protein MCYG_03610 | C5FM69_NANOT | 76'977.00 | 8 | 9 | 13 | 0.44% | 15.20% |
| Mcanis-pH7-b | Putative uncharacterized protein MCYG_03515 | C5FLX4_NANOT | 22'660.00 | 3 | 3 | 5 | 0.17% | 17.00% |
| Mcanis-pH7-b | Aminopeptidase P MCYG_01718 | C5FHR9_NANOT | 69'181.80 | 4 | 4 | 4 | 0.14% | 8.49% |
| Mcanis-pH7-b | Alkaline serine protease MCYG_02748 | C5FGP4_NANOT | 40'782.50 | 7 | 8 | 8 | 0.27% | 23.70% |
| Mcanis-pH7-b | Tripeptidyl-peptidase SED4 | SED4_NANOT | 65'895.80 | 3 | 3 | 7 | 0.24% | 7.15% |
| Mcanis-pH7-b | Glucoamylase MCYG_06367 | C5FUG4_NANOT | 67'893.70 | 29 | 37 | 174 | 5.93% | 45.50% |
| Mcanis-pH7-b | Esterase MCYG_01130 | C5FEU8_NANOT | 59'920.10 | 3 | 4 | 4 | 0.14% | 6.13% |
| Mcanis-pH7-b | Mucin MCYG_07854 | C5FXJ5_NANOT | 76'937.60 | 22 | 30 | 62 | 2.11% | 35.90% |
| Mcanis-pH7-b | PBSP domain-containing protein MCYG_01682 | C5FHE4_NANOT | 32'863.60 | 1 | 1 | 3 | 0.10% | 3.95% |
| Mcanis-pH7-b | Oxalate decarboxylase oxdD MCYG_04379 | C5FNE6_NANOT | 44'218.50 | 6 | 6 | 16 | 0.55% | 19.80% |
| Mcanis-pH7-b | Putative uncharacterized protein MCYG_00366 | C5FC55_NANOT | 61'787.00 | 6 | 7 | 20 | 0.68% | 11.70% |
| Mcanis-pH7-b | Hydrophobic surface binding protein B MCYG_01969 | C5FIQ9_NANOT | 19'243.20 | 6 | 8 | 27 | 0.92% | 39.00% |
| Mcanis-pH7-b | Phosphatidylinositol transfer protein MCYG_02226 | C5FFG0_NANOT | 18'642.00 | 3 | 5 | 27 | 0.92% | 21.70% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Mcanis-pH7-b | Glycosyl hydrolase MCYG_03093 | C5FKQ2_NANOT | 87'712.10 | 4 | 4 | 4 | 0.14% | 6.91% |
| Mcanis-pH7-b | Putative uncharacterized protein MCYG_03713 | C5FJN3_NANOT | 25'502.60 | 2 | 2 | 8 | 0.27% | 9.46% |
| Mcanis-pH7-b | Putative uncharacterized protein MCYG_01574 | C5FHL5_NANOT | 27'690.30 | 4 | 4 | 5 | 0.17% | 18.30% |
| Mcanis-pH7-b | Beta-hexosaminidase MCYG_02200 | C5FJ51_NANOT | 67'781.60 | 6 | 6 | 9 | 0.31% | 10.70% |
| Mcanis-pH7-b | IgE-binding protein MCYG_03081 | C5FKP0_NANOT | 20'333.70 | 4 | 5 | 8 | 0.27% | 22.70% |
| Mcanis-pH7-b | Carboxypeptidase S1 SCPA | SPCA_NANOT | 72'179.60 | 3 | 3 | 4 | 0.14% | 5.84% |
| Mcanis-pH7-b | Aminopeptidase Y LAP1 | LAP1_NANOT | 52'839.50 | 24 | 32 | 301 | 10.30% | 50.30% |
| Mcanis-pH7-b | 6-hydroxy-D-nicotine oxidase MCYG_04127 | C5FN72_NANOT | 62'598.10 | 11 | 14 | 33 | 1.13% | 20.80% |
| Mcanis-pH7-b | Probable neutral protease 2 homolog MCYG_07155 | NPIID_NANOT | 38'232.20 | 2 | 2 | 12 | 0.41% | 5.95% |
| Mcanis-pH7-b | Nucleoside diphosphate kinase MCYG_01839 | C5FI40_NANOT | 16'989.90 | 5 | 6 | 22 | 0.75% | 40.50% |
| Mcanis-pH7-b | Putative uncharacterized protein MCYG_06389 | C5FUI6_NANOT | 38'003.10 | 2 | 2 | 2 | 0.07% | 5.71% |
| Mcanis-pH7-b | Amidase MCYG_00475 | C5FCQ3_NANOT | 61'861.80 | 19 | 26 | 79 | 2.69% | 34.20% |
| Mcanis-pH7-b | Proteinase inhibitor I4 MCYG_03085 | C5FKP4_NANOT | 39'549.80 | 3 | 3 | 3 | 0.10% | 10.20% |
| Mcanis-pH7-b | Secretory phospholipase A2 MCYG_00583 | C5FD11_NANOT | 16'687.00 | 2 | 2 | 2 | 0.07% | 10.70% |
| Mcanis-pH7-b | Amidohydrolase MCYG_03185 | C5FKZ4_NANOT | 32'541.60 | 4 | 4 | 4 | 0.14% | 17.20% |
| Mcanis-pH7-b | Glutathione S-transferase Gst3 MCYG_07941 | C5FXT2_NANOT | 40'867.60 | 1 | 1 | 1 | 0.03% | 5.06% |
| Mcanis-pH7-b | Endochitinase 1 MCYG_00676 | C5FDA4_NANOT | 44'249.10 | 5 | 6 | 14 | 0.48% | 22.20% |
| Mcanis-pH7-b | Polysaccharide deacetylase family protein MCYG_07538 | C5FYX1_NANOT | 32'868.80 | 5 | 6 | 18 | 0.61% | 25.80% |
| Mcanis-pH7-b | Multicopper oxidase MCYG_01191 | C5FF09_NANOT | 74'572.90 | 3 | 4 | 7 | 0.24% | 6.65% |
| Mcanis-pH7-b | Putative uncharacterized protein MCYG_07611 | C5FWV2_NANOT | 29'064.80 | 6 | 6 | 8 | 0.27% | 27.20% |
| Mcanis-pH7-b | Uricase MCYG_00197 | C5FBX5_NANOT | 33'902.40 | 4 | 4 | 7 | 0.24% | 19.00% |
| Mcanis-pH7-b | Lysophospholipase MCYG_02652 | C5FGE8_NANOT | 68'263.40 | 4 | 4 | 7 | 0.24% | 7.55% |
| Mcanis-pH7-c | Putative uncharacterized protein MCYG_07611 | C5FWV2_NANOT | 29'064.80 | 7 | 7 | 12 | 0.16% | 27.20% |
| Mcanis-pH7-c | Putative uncharacterized protein MCYG_08076 | C5FZF4_NANOT | 33'917.80 | 2 | 2 | 3 | 0.04% | 6.67% |
| Mcanis-pH7-c | Oxalate decarboxylase oxdD MCYG_04379 | C5FNE6_NANOT | 44'218.50 | 7 | 9 | 21 | 0.28% | 22.80% |
| Mcanis-pH7-c | 4-hydroxyphenylpyruvate dioxygenase MCYG_00524 | C5FCV2_NANOT | 45'157.10 | 5 | 6 | 7 | 0.09% | 15.50% |
| Mcanis-pH7-c | Putative uncharacterized protein MCYG_03989 | C5FMR7_NANOT | 45'583.40 | 5 | 7 | 7 | 0.09% | 19.00% |
| Mcanis-pH7-c | LysM domain-containing protein MCYG_04646 | C5FNX4_NANOT | 28'076.50 | 3 | 4 | 20 | 0.27% | 12.80% |
| Mcanis-pH7-c | Glutathione S-transferase Gst3 MCYG_07941 | C5FXT2_NANOT | 40'867.60 | 5 | 5 | 5 | 0.07% | 16.90% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Mcanis-pH7-c | Carbohydrate-binding protein MCYG_01090 | C5FEG8_NANOT | 41'707.10 | 12 | 20 | 66 | 0.89% | 55.40% |
| Mcanis-pH7-c | Secretory phospholipase A2 MCYG_00583 | C5FD11_NANOT | 16'687.00 | 2 | 2 | 4 | 0.05% | 16.00% |
| Mcanis-pH7-c | Aspartyl aminopeptidase MCYG_06746 | C5FVJ3_NANOT | 73'706.30 | 4 | 4 | 4 | 0.05% | 7.43% |
| Mcanis-pH7-c | Alpha-mannosidase MCYG_06176 | C5FTX3_NANOT | 123'001.50 | 30 | 34 | 102 | 1.37% | 34.70% |
| Mcanis-pH7-c | Putative uncharacterized protein MCYG_00150 | C5FBS8_NANOT | 18'683.60 | 3 | 6 | 18 | 0.24% | 30.10% |
| Mcanis-pH7-c | Alkaline proteinase MCYG_01476 | C5FH27_NANOT | 41'484.80 | 4 | 5 | 7 | 0.09% | 16.20% |
| Mcanis-pH7-c | Putative uncharacterized protein MCYG_07025 | C5FWC2_NANOT | 32'427.90 | 8 | 10 | 18 | 0.24% | 28.00% |
| Mcanis-pH7-c | Putative uncharacterized protein MCYG_07328 | C5FYB1_NANOT | 20'833.50 | 2 | 3 | 5 | 0.07% | 21.80% |
| Mcanis-pH7-c | Dienelactone hydrolase family protein MCYG_02976 | C5FKD5_NANOT | 32'377.30 | 1 | 1 | 1 | 0.01% | 2.75% |
| Mcanis-pH7-c | Fumarate reductase flavoprotein subunit MCYG_02604 | C5FGA0_NANOT | 67'964.70 | 5 | 5 | 5 | 0.07% | 8.39% |
| Mcanis-pH7-c | Alkaline phosphatase MCYG_04625 | C5FNV3_NANOT | 56'302.20 | 29 | 53 | 377 | 5.08% | 73.10% |
| Mcanis-pH7-c | G-protein complex beta subunit CpcB MCYG_01069 | C5FEE7_NANOT | 35'071.00 | 7 | 7 | 10 | 0.14% | 31.60% |
| Mcanis-pH7-c | Secreted protein MCYG_06024 | C5FTK2_NANOT | 21'961.50 | 2 | 2 | 2 | 0.03% | 16.70% |
| Mcanis-pH7-c | Putative uncharacterized protein MCYG_07164 | C5FXU7_NANOT | 48'942.20 | 10 | 12 | 26 | 0.35% | 27.10% |
| Mcanis-pH7-c | Putative uncharacterized protein MCYG_01574 | C5FHL5_NANOT | 27'690.30 | 7 | 9 | 13 | 0.18% | 43.60% |
| Mcanis-pH7-c | Polysaccharide deacetylase family protein MCYG_04253 | C5FPB8_NANOT | 30'019.90 | 6 | 7 | 14 | 0.19% | 35.60% |
| Mcanis-pH7-c | DUF1237 domain-containing protein MCYG_03876 | C5FMF4_NANOT | 60'979.20 | 8 | 10 | 13 | 0.18% | 20.60% |
| Mcanis-pH7-c | Carboxylesterase family protein MCYG_00869 | C5FDU7_NANOT | 62'320.30 | 2 | 2 | 2 | 0.03% | 4.75% |
| Mcanis-pH7-c | Probable neutral protease 2 homolog MCYG_05201 | NPIIA_NANOT | 40'199.70 | 1 | 1 | 2 | 0.03% | 3.49% |
| Mcanis-pH7-c | Sulphydryl oxidase Sox MCYG_02499 | C5FFZ5_NANOT | 44'024.60 | 5 | 5 | 13 | 0.18% | 17.50% |
| Mcanis-pH7-c | Putative uncharacterized protein MCYG_05599 | C5FSC7_NANOT | 20'561.90 | 3 | 3 | 7 | 0.09% | 31.20% |
| Mcanis-pH7-c | Class II aldolase/adducin domain MCYG_00391 | C5FC80_NANOT | 31'482.70 | 5 | 5 | 7 | 0.09% | 26.10% |
| Mcanis-pH7-c | Beta-glucosidase MCYG_00545 | C5FCX3_NANOT | 135'337.50 | 6 | 6 | 6 | 0.08% | 6.99% |
| Mcanis-pH7-c | Glycosyl hydrolase MCYG_03093 | C5FKQ2_NANOT | 87'712.10 | 2 | 2 | 2 | 0.03% | 2.89% |
| Mcanis-pH7-c | Cell wall protein PhiA MCYG_00375 | C5FC64_NANOT | 19'726.70 | 4 | 4 | 9 | 0.12% | 28.00% |
| Mcanis-pH7-c | Cutinase MCYG_05629 | C5FSF7_NANOT | 28'464.90 | 4 | 5 | 12 | 0.16% | 21.00% |
| Mcanis-pH7-c | GH17932p MCYG_04430 | C5FNJ7_NANOT | 22'552.00 | 3 | 3 | 3 | 0.04% | 23.70% |
| Mcanis-pH7-c | Uricase MCYG_00197 | C5FBX5_NANOT | 33'902.40 | 3 | 3 | 3 | 0.04% | 18.00% |
| Mcanis-pH7-c | Phosphoglycerate mutase family protein MCYG_08379 | C5G0A7_NANOT | 36'614.60 | 7 | 8 | 11 | 0.15% | 28.40% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Mcanis-pH7-c | Predicted protein MCYG_04553 | C5FNN1_NANOT | 27'810.60 | 5 | 8 | 52 | 0.70% | 33.10% |
| Mcanis-pH7-c | Carboxypeptidase 2 MCPB | MCPB_NANOT | 59'765.20 | 26 | 33 | 127 | 1.71% | 54.00% |
| Mcanis-pH7-c | Tripeptidyl-peptidase SED4 | SED4_NANOT | 65'895.80 | 4 | 4 | 15 | 0.20% | 9.82% |
| Mcanis-pH7-c | Alanine-glyoxylate aminotransferase MCYG_08492 | C5G0M0_NANOT | 41'392.90 | 3 | 3 | 3 | 0.04% | 10.70% |
| Mcanis-pH7-c | Putative uncharacterized protein MCYG_00366 | C5FC55_NANOT | 61'787.00 | 11 | 13 | 27 | 0.36% | 27.00% |
| Mcanis-pH7-c | 1,2-alpha-D-mannosidase MCYG_00423 | C5FCK1_NANOT | 55'930.00 | 14 | 20 | 50 | 0.67% | 32.10% |
| Mcanis-pH7-c | Aminopeptidase P MCYG_01718 | C5FHR9_NANOT | 69'181.80 | 2 | 2 | 4 | 0.05% | 4.65% |
| Mcanis-pH7-c | Aspartylglucosaminidase family protein MCYG_04957 | C5FQI5_NANOT | 44'887.40 | 6 | 8 | 15 | 0.20% | 24.60% |
| Mcanis-pH7-c | Ecm33 MCYG_01225 | C5FEL3_NANOT | 41'117.10 | 4 | 4 | 11 | 0.15% | 10.60% |
| Mcanis-pH7-c | Multicopper oxidase MCYG_01191 | C5FF09_NANOT | 74'572.90 | 9 | 12 | 25 | 0.34% | 16.20% |
| Mcanis-pH7-c | Subtilisin-like protease SUB4 | SUB4_NANOT | 42'052.90 | 1 | 1 | 1 | 0.01% | 6.52% |
| Mcanis-pH7-c | Tripeptidyl-peptidase SED2 | SED2_NANOT | 64'826.30 | 7 | 7 | 19 | 0.26% | 18.30% |
| Mcanis-pH7-c | Extracellular metalloproteinase MEP3 | MEP3_MICCA,MEP3_NANOT | 69'360.10 | 11 | 23 | 120 | 1.62% | 36.00% |
| Mcanis-pH7-c | ATP-dependent permease MCYG_02170 | C5FJ21_NANOT | 107'299.40 | 3 | 4 | 8 | 0.11% | 3.92% |
| Mcanis-pH7-c | IgE-binding protein MCYG_03081 | C5FKP0_NANOT | 20'333.70 | 3 | 3 | 7 | 0.09% | 16.50% |
| Mcanis-pH7-c | Peptidase S8 and S53 MCYG_01699 | C5FHQ0_NANOT | 75'193.00 | 3 | 4 | 7 | 0.09% | 7.19% |
| Mcanis-pH7-c | Glycerophosphoryl diester phosphodiesterase MCYG_05364 | C5FRP2_NANOT | 44'497.00 | 6 | 6 | 11 | 0.15% | 16.40% |
| Mcanis-pH7-c | FAD binding domain-containing protein MCYG_06815 | C5FVR2_NANOT | 63'164.50 | 6 | 6 | 6 | 0.08% | 14.60% |
| Mcanis-pH7-c | Anthranilate synthase multifunctional enzyme MCYG_01660 | C5FHC2_NANOT | 82'113.70 | 1 | 1 | 1 | 0.01% | 1.57% |
| Mcanis-pH7-c | Proteinase inhibitor I4 MCYG_03085 | C5FKP4_NANOT | 39'549.80 | 9 | 10 | 13 | 0.18% | 33.90% |
| Mcanis-pH7-c | Extracellular metalloproteinase MEP1 | MEP1_MICCA,MEP1_NANOT | 69'753.70 | 3 | 3 | 3 | 0.04% | 10.10% |
| Mcanis-pH7-c | Beta-glucosidase 1 MCYG_00928 | C5FE06_NANOT | 93'578.70 | 21 | 35 | 75 | 1.01% | 31.70% |
| Mcanis-pH7-c | Putative uncharacterized protein MCYG_04951 | C5FQH9_NANOT | 15'009.70 | 3 | 5 | 11 | 0.15% | 37.90% |
| Mcanis-pH7-c | Phosphoribosylglycinamide formyltransferase MCYG_05959 | C5FTD7_NANOT | 24'230.10 | 2 | 2 | 2 | 0.03% | 9.22% |
| Mcanis-pH7-c | Gamma-glutamyltranspeptidase MCYG_05524 | C5FS52_NANOT | 62'565.80 | 4 | 4 | 4 | 0.05% | 8.87% |
| Mcanis-pH7-c | Glyoxalase family protein MCYG_00690 | C5FDK6_NANOT | 17'236.10 | 4 | 4 | 6 | 0.08% | 20.90% |
| Mcanis-pH7-c | Ribonuclease T2-like protein MCYG_02204 | C5FJ55_NANOT | 42'269.30 | 2 | 2 | 3 | 0.04% | 6.31% |
| Mcanis-pH7-c | Fumarylacetoacetase MCYG_00527 | C5FCV5_NANOT | 47'213.90 | 11 | 14 | 39 | 0.53% | 35.20% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Mcanis-pH7-c | Secreted protein MCYG_00341 | C5FC30_NANOT | 22'728.10 | 7 | 8 | 15 | 0.20% | 50.20% |
| Mcanis-pH7-c | Superoxide dismutase [Cu-Zn] MCYG_02077 | C5FII9_NANOT | 15'965.90 | 3 | 3 | 3 | 0.04% | 35.10% |
| Mcanis-pH7-c | HypA MCYG_01872 | C5FI73_NANOT | 51'308.20 | 5 | 5 | 7 | 0.09% | 13.10% |
| Mcanis-pH7-c | Citrate synthase MCYG_03094 | C5FKQ3_NANOT | 43'799.80 | 1 | 1 | 3 | 0.04% | 2.99% |
| Mcanis-pH7-c | Putative uncharacterized protein MCYG_03189 | C5FKZ8_NANOT | 46'783.50 | 6 | 7 | 8 | 0.11% | 18.50% |
| Mcanis-pH7-c | FAD binding domain-containing protein MCYG_04968 | C5FQJ6_NANOT | 55'314.40 | 19 | 27 | 95 | 1.28% | 53.50% |
| Mcanis-pH7-c | Copper radical oxidase MCYG_08682 | C5G160_NANOT | 96'394.50 | 20 | 29 | 92 | 1.24% | 32.70% |
| Mcanis-pH7-c | Putative uncharacterized protein MCYG_01908 | C5FIA9_NANOT | 96'835.90 | 2 | 2 | 2 | 0.03% | 3.51% |
| Mcanis-pH7-c | Predicted protein MCYG_08251 | C5FZX9_NANOT | 17'591.50 | 2 | 2 | 3 | 0.04% | 14.40% |
| Mcanis-pH7-c | Proline iminopeptidase MCYG_04539 | C5FNL7_NANOT | 50'855.50 | 7 | 8 | 9 | 0.12% | 22.60% |
| Mcanis-pH7-c | Arabinan endo-1,5-alpha-L-arabinosidase A MCYG_02777 | C5FGS3_NANOT | 36'492.80 | 6 | 7 | 15 | 0.20% | 23.40% |
| Mcanis-pH7-c | Dipeptidase 1 MCYG_02918 | C5FK77_NANOT | 47'224.20 | 11 | 16 | 38 | 0.51% | 39.30% |
| Mcanis-pH7-c | Aminopeptidase Y LAP1 | LAP1_NANOT | 52'839.50 | 31 | 47 | 798 | 10.70% | 61.80% |
| Mcanis-pH7-c | Probable neutral protease 2 homolog MCYG_07155 | NPIID_NANOT | 38'232.20 | 3 | 4 | 9 | 0.12% | 10.80% |
| Mcanis-pH7-c | Hydrophobic surface binding protein B MCYG_01969 | C5FIQ9_NANOT | 19'243.20 | 6 | 10 | 64 | 0.86% | 48.40% |
| Mcanis-pH7-c | Metallocarboxypeptidase A-like protein MCYG_01475 | MCPAL_NANOT | 45'896.90 | 6 | 9 | 22 | 0.30% | 24.00% |
| Mcanis-pH7-c | Beta-hexosaminidase MCYG_02200 | C5FJ51_NANOT | 67'781.60 | 13 | 14 | 17 | 0.23% | 30.90% |
| Mcanis-pH7-c | Neutral ceramidase MCYG_06637 | C5FV84_NANOT | 83'246.50 | 15 | 21 | 67 | 0.90% | 29.40% |
| Mcanis-pH7-c | Dipeptidyl peptidase DPPIV | A0S5V9_MICCA,DPP4_NANOT | 87'901.80 | 27 | 36 | 90 | 1.21% | 47.70% |
| Mcanis-pH7-c | Glucoamylase MCYG_06367 | C5FUG4_NANOT | 67'893.70 | 34 | 54 | 662 | 8.91% | 59.60% |
| Mcanis-pH7-c | Putative uncharacterized protein MCYG_02350 | C5FJB0_NANOT | 16'434.60 | 7 | 7 | 10 | 0.14% | 47.30% |
| Mcanis-pH7-c | Ser/Thr protein phosphatase MCYG_02775 | C5FGS1_NANOT | 68'425.90 | 6 | 6 | 7 | 0.09% | 11.60% |
| Mcanis-pH7-c | Endochitinase 1 MCYG_00676 | C5FDA4_NANOT | 44'249.10 | 15 | 18 | 75 | 1.01% | 59.40% |
| Mcanis-pH7-c | Aldose 1-epimerase MCYG_00654 | C5FD82_NANOT | 46'289.40 | 2 | 2 | 2 | 0.03% | 4.45% |
| Mcanis-pH7-c | FAD binding domain-containing protein MCYG_07459 | C5FYP2_NANOT | 54'460.30 | 2 | 2 | 2 | 0.03% | 5.01% |
| Mcanis-pH7-c | Putative uncharacterized protein MCYG_07766 | C5FXA7_NANOT | 23'998.90 | 2 | 2 | 7 | 0.09% | 10.40% |
| Mcanis-pH7-c | Putative uncharacterized protein MCYG_04556 | C5FNN4_NANOT | 50'223.20 | 6 | 6 | 11 | 0.15% | 22.00% |
| Mcanis-pH7-c | Amidohydrolase MCYG_03185 | C5FKZ4_NANOT | 32'541.60 | 4 | 4 | 7 | 0.09% | 19.50% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Mcanis-pH7-c | Beta-1,3-glucanosyltransferase 3 MCYG_03246 | C5FL55_NANOT | 56'747.70 | 3 | 3 | 4 | 0.05% | 8.94% |
| Mcanis-pH7-c | Chitinase 4 MCYG_08403 | C5G0D1_NANOT | 38'958.30 | 3 | 4 | 4 | 0.05% | 13.10% |
| Mcanis-pH7-c | Exochitinase 1 MCYG_05745 | C5FSS3_NANOT | 53'464.50 | 5 | 7 | 13 | 0.18% | 13.70% |
| Mcanis-pH7-c | Nucleoside diphosphate kinase MCYG_01839 | C5FI40_NANOT | 16'989.90 | 4 | 6 | 15 | 0.20% | 34.60% |
| Mcanis-pH7-c | Putative uncharacterized protein MCYG_03515 | C5FLX4_NANOT | 22'660.00 | 4 | 4 | 14 | 0.19% | 21.00% |
| Mcanis-pH7-c | Cyanate hydratase MCYG_03856 | C5FMD4_NANOT | 17'890.20 | 3 | 4 | 5 | 0.07% | 25.00% |
| Mcanis-pH7-c | Phosphatidylinositol transfer protein MCYG_02226 | C5FFG0_NANOT | 18'642.00 | 6 | 9 | 31 | 0.42% | 38.90% |
| Mcanis-pH7-c | Dihydroorotase MCYG_02534 | C5FG30_NANOT | 38'833.00 | 6 | 6 | 8 | 0.11% | 18.20% |
| Mcanis-pH7-c | Subtilisin-like protease SUB7 | SUB7_NANOT | 41'362.20 | 3 | 4 | 19 | 0.26% | 10.50% |
| Mcanis-pH7-c | FAD binding domain-containing protein MCYG_08167 | C5FZP5_NANOT | 54'189.10 | 2 | 3 | 3 | 0.04% | 5.20% |
| Mcanis-pH7-c | Alkaline phosphatase MCYG_05903 | C5FT81_NANOT | 66'451.10 | 5 | 6 | 7 | 0.09% | 10.30% |
| Mcanis-pH7-c | Polysaccharide deacetylase family protein MCYG_07538 | C5FYX1_NANOT | 32'868.80 | 7 | 12 | 142 | 1.91% | 36.30% |
| Mcanis-pH7-c | Beta-mannosidase MCYG_06102 | C5FTT0_NANOT | 97'855.30 | 9 | 9 | 14 | 0.19% | 11.00% |
| Mcanis-pH7-c | Extracellular metalloproteinase MEP5 | MEP5_MICCA,MEP5_NANOT | 69'529.10 | 1 | 1 | 1 | 0.01% | 1.90% |
| Mcanis-pH7-c | Putative uncharacterized protein MCYG_08685 | C5G163_NANOT | 79'189.30 | 5 | 5 | 5 | 0.07% | 11.10% |
| Mcanis-pH7-c | Putative uncharacterized protein MCYG_07934 | C5FXS5_NANOT | 33'703.60 | 1 | 1 | 1 | 0.01% | 2.95% |
| Mcanis-pH7-c | Alpha-glucosidase MCYG_00092 | C5FBM0_NANOT | 100'911.70 | 11 | 13 | 17 | 0.23% | 14.20% |
| Mcanis-pH7-c | Exo-beta-1,3-glucanase Exg0 MCYG_06600 | C5FV47_NANOT | 33'625.30 | 6 | 6 | 17 | 0.23% | 38.40% |
| Mcanis-pH7-c | Peptidase M28 MCYG_04217 | C5FP82_NANOT | 55'810.30 | 5 | 5 | 5 | 0.07% | 10.40% |
| Mcanis-pH7-c | Glutamate carboxypeptidase 2 MCYG_07431 | C5FYL4_NANOT | 78'351.90 | 10 | 17 | 39 | 0.53% | 22.10% |
| Mcanis-pH7-c | Superoxide dismutase MCYG_06348 | C5FUE5_NANOT | 25'212.90 | 4 | 6 | 7 | 0.09% | 29.40% |
| Mcanis-pH7-c | Peptidase S41 family protein MCYG_03610 | C5FM69_NANOT | 76'977.00 | 10 | 12 | 26 | 0.35% | 18.20% |
| Mcanis-pH7-c | Putative uncharacterized protein MCYG_04152 | C5FN97_NANOT | 21'011.20 | 3 | 3 | 3 | 0.04% | 13.30% |
| Mcanis-pH7-c | Beta-glucosidase 4 MCYG_01527 | C5FHG8_NANOT | 33'218.80 | 12 | 17 | 93 | 1.25% | 47.20% |
| Mcanis-pH7-c | Alkaline serine protease MCYG_02748 | C5FGP4_NANOT | 40'782.50 | 2 | 2 | 2 | 0.03% | 14.70% |
| Mcanis-pH7-c | Cysteinyl-tRNA synthetase MCYG_01772 | C5FHX3_NANOT | 90'189.90 | 2 | 2 | 2 | 0.03% | 2.99% |
| Mcanis-pH7-c | 6-hydroxy-D-nicotine oxidase MCYG_04127 | C5FN72_NANOT | 62'598.10 | 16 | 24 | 82 | 1.10% | 38.40% |
| Mcanis-pH7-c | Aconitase MCYG_03299 | C5FLA8_NANOT | 84'103.20 | 3 | 3 | 3 | 0.04% | 4.77% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Mcanis-pH7-c | Chitinase MCYG_04644 | C5FNX2_NANOT | 159'340.70 | 4 | 4 | 6 | 0.08% | 3.32% |
| Mcanis-pH7-c | Phosphoserine aminotransferase MCYG_05388 | C5FRR6_NANOT | 47'253.40 | 2 | 2 | 4 | 0.05% | 6.02% |
| Mcanis-pH7-c | Amidase MCYG_00475 | C5FCQ3_NANOT | 61'861.80 | 26 | 40 | 211 | 2.84% | 58.10% |
| Mcanis-pH7-c | Mucin MCYG_07854 | C5FXJ5_NANOT | 76'937.60 | 26 | 42 | 150 | 2.02% | 43.80% |
| Mcanis-pH7-c | Lysophospholipase MCYG_02652 | C5FGE8_NANOT | 68'263.40 | 3 | 3 | 3 | 0.04% | 5.50% |
| Mcanis-pH7-c | Extracellular metalloproteinase MEP4 | MEP4_MICCA,MEP4_NANOT | 71'233.30 | 13 | 20 | 148 | 1.99% | 27.90% |
| Mcanis-pH7-c | Metallocarboxypeptidase MCPA | MCPA_NANOT | 47'079.60 | 5 | 6 | 10 | 0.14% | 17.80% |
| Mcanis-pH7-c | Putative uncharacterized protein MCYG_06096 | C5FTS4_NANOT | 57'065.30 | 13 | 15 | 24 | 0.32% | 36.80% |
| Mcanis-pH7-c | Thimet oligopeptidase MCYG_02264 | C5FFJ8_NANOT | 81'672.10 | 3 | 3 | 3 | 0.04% | 5.45% |
| Mcanis-pH7-c | Leucine aminopeptidase LAP2 | LAP2_NANOT | 40'524.20 | 9 | 13 | 26 | 0.35% | 36.70% |
| Mcanis-pH7-c | Oxalate decarboxylase MCYG_02705 | C5FGK1_NANOT | 43'946.20 | 3 | 3 | 5 | 0.07% | 17.90% |
| Mcanis-pH7-c | Glutaminase GtaA MCYG_00814 | C5FDF2_NANOT | 94'824.40 | 10 | 10 | 23 | 0.31% | 14.60% |
| Mcanis-pH7-c | Subtilisin-like protease SUB3 | SUB3_MICCA,SUB3_NANOT | 40'837.10 | 18 | 30 | 202 | 2.72% | 64.20% |
| Mcanis-pH7-c | Serine proteinase MCYG_02070 | C5FII2_NANOT | 52'462.10 | 2 | 2 | 3 | 0.04% | 3.88% |

**Supplementary Table 1b: MS Protein report of *A. benhamiae* secretome in SP medium at pH 4.0 and pH 7.0.**

Experiment: Abenhamiae-pH4-7_ 3 shotguns

Database Name: the arthroderma_benhamiae_cbs_112371_proteins database

Number of Proteins: 7980

Search Engine: Mascot

Version: Mascot

Samples: All Samples

Fragment Tolerance: 0.50 Da (Monoisotopic)

Parent Tolerance: 10.0 PPM (Monoisotopic)

Fixed Modifications: +57 on C (Carbamidomethyl)

Variable Modifications: +1 on NQ (Deamidation), +16 on M (Oxidation), +42 on n (Acetyl)

Database: the arthroderma_benhamiae_cbs_112371_proteins database (unknown version, 7980 entries)

Digestion Enzyme: Trypsin

Max Missed Cleavages: 1

Scaffold Version: Scaffold_3_00_03

Peptide Thresholds: 90.0 % minimum

Protein Thresholds: 95.0 % minimum and 1 peptides minimum

| Biological sample name | Protein name | Protein accession n° | Protein Mw (Da) | Number of unique peptides | Number of unique spectra | Number of total spectra | Percentage of total spectra | Percentage sequence coverage |
|---|---|---|---|---|---|---|---|---|
| Abenh-pH4 a | A. benhamiae CBS 112371 null (401 aa) | ARB_06111 | 41'702.10 | 3 | 3 | 3 | 0.07% | 9.25% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (607 aa) | ARB_04499 | 66'514.90 | 3 | 3 | 3 | 0.07% | 6.27% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (417 aa) | ARB_03789 | 45'801.80 | 9 | 13 | 17 | 0.41% | 37.30% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (250 aa) | ARB_04619 | 27'122.70 | 5 | 7 | 8 | 0.20% | 32.90% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (512 aa) | ARB_07056 | 55'227.80 | 8 | 9 | 12 | 0.29% | 23.50% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Abenh-pH4 a | A. benhamiae CBS 112371 null (213 aa) | ARB_06108 | 24'150.70 | 2 | 3 | 9 | 0.22% | 16.00% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (644 aa) | ARB_05919 | 68'907.10 | 8 | 9 | 11 | 0.27% | 18.50% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (413 aa) | ARB_01347 | 44'166.80 | 7 | 7 | 8 | 0.20% | 24.50% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (131 aa) | ARB_07026 | 14'085.60 | 5 | 8 | 33 | 0.80% | 73.80% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (243 aa) | ARB_07186 | 26'586.30 | 4 | 4 | 6 | 0.15% | 19.40% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (546 aa) | ARB_07487 | 58'808.10 | 4 | 4 | 4 | 0.10% | 10.80% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (172 aa) | ARB_00344 | 18'201.10 | 7 | 11 | 263 | 6.41% | 59.10% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (503 aa) | ARB_05319 | 54'495.60 | 17 | 21 | 46 | 1.12% | 50.80% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (223 aa) | ARB_03106 | 25'133.60 | 7 | 11 | 31 | 0.76% | 42.80% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (134 aa) | ARB_05536 | 15'101.60 | 7 | 9 | 18 | 0.44% | 60.90% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (623 aa) | ARB_05535 | 70'472.60 | 12 | 15 | 31 | 0.76% | 25.40% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (351 aa) | ARB_03276 | 38'765.00 | 5 | 5 | 6 | 0.15% | 20.00% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (315 aa) | ARB_06966 | 34'730.00 | 8 | 9 | 12 | 0.29% | 31.80% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (336 aa) | ARB_03491 | 37'530.20 | 9 | 9 | 11 | 0.27% | 34.90% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (866 aa) | ARB_02101 | 98'073.30 | 4 | 4 | 4 | 0.10% | 6.59% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (653 aa) | ARB_04046 | 71'688.10 | 13 | 20 | 83 | 2.02% | 28.70% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (566 aa) | ARB_02478 | 61'777.10 | 5 | 5 | 5 | 0.12% | 13.10% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (634 aa) | ARB_06472 | 69'823.90 | 9 | 9 | 11 | 0.27% | 25.60% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (206 aa) | ARB_05304 | 21'463.10 | 9 | 12 | 33 | 0.80% | 54.10% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (175 aa) | ARB_01325 | 18'707.50 | 3 | 5 | 11 | 0.27% | 24.70% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (231 aa) | ARB_01545 | 21'449.70 | 1 | 1 | 3 | 0.07% | 10.90% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (1029 aa) | ARB_02327 | 113'054.40 | 13 | 14 | 15 | 0.37% | 18.90% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Abenh-pH4 a | A. benhamiae CBS 112371 null (238 aa) | ARB_07027 | 26'853.40 | 3 | 7 | 14 | 0.34% | 20.30% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (440 aa) | ARB_05253 | 44'989.10 | 9 | 11 | 52 | 1.27% | 26.90% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (226 aa) | ARB_01979 | 25'408.90 | 6 | 8 | 15 | 0.37% | 28.00% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (756 aa) | ARB_05732 | 81'168.90 | 2 | 2 | 2 | 0.05% | 5.83% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (211 aa) | ARB_05178 | 21'524.00 | 3 | 3 | 3 | 0.07% | 26.20% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (593 aa) | ARB_02965 | 63'994.80 | 8 | 8 | 8 | 0.20% | 18.20% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (187 aa) | ARB_02741 | 18'247.20 | 3 | 6 | 10 | 0.24% | 15.10% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (255 aa) | ARB_03514 | 27'613.80 | 4 | 7 | 9 | 0.22% | 29.90% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (509 aa) | ARB_05721 | 57'153.20 | 6 | 6 | 6 | 0.15% | 29.90% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (883 aa) | ARB_07888 | 99'853.00 | 10 | 12 | 12 | 0.29% | 14.40% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (257 aa) | ARB_03024 | 25'239.30 | 3 | 3 | 4 | 0.10% | 21.50% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (552 aa) | ARB_00083 | 62'360.70 | 14 | 21 | 35 | 0.85% | 39.90% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (408 aa) | ARB_01032 | 42'987.80 | 4 | 4 | 4 | 0.10% | 21.40% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (354 aa) | ARB_00204 | 38'505.10 | 4 | 4 | 5 | 0.12% | 11.00% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (395 aa) | ARB_00047 | 44'081.90 | 3 | 3 | 3 | 0.07% | 13.20% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (491 aa) | ARB_00777 | 52'317.20 | 7 | 8 | 16 | 0.39% | 23.70% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (214 aa) | ARB_05177 | 22'933.00 | 3 | 3 | 3 | 0.07% | 18.30% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (242 aa) | ARB_04889 | 26'743.60 | 7 | 10 | 15 | 0.37% | 44.80% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (395 aa) | ARB_02015 | 42'491.90 | 10 | 10 | 10 | 0.24% | 43.70% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (1055 aa) | ARB_03719 | 114'777.50 | 3 | 3 | 3 | 0.07% | 5.31% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (925 aa) | ARB_07867 | 99'074.10 | 13 | 15 | 18 | 0.44% | 20.90% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (496 aa) | ARB_04170 | 51'840.90 | 4 | 6 | 6 | 0.15% | 14.10% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Abenh-pH4 a | A. benhamiae CBS 112371 null (821 aa) | ARB_05654 | 88'821.60 | 8 | 9 | 12 | 0.29% | 14.40% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (530 aa) | ARB_03264 | 59'162.30 | 11 | 14 | 23 | 0.56% | 31.90% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (374 aa) | ARB_03568 | 40'712.70 | 10 | 16 | 38 | 0.93% | 39.40% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (198 aa) | ARB_03673 | 21'021.70 | 4 | 6 | 18 | 0.44% | 31.00% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (236 aa) | ARB_05911 | 25'146.90 | 6 | 10 | 49 | 1.19% | 40.90% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (196 aa) | ARB_07637 | 20'530.80 | 9 | 12 | 26 | 0.63% | 47.70% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (224 aa) | ARB_03853 | 24'951.00 | 4 | 4 | 9 | 0.22% | 20.60% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (910 aa) | ARB_06590 | 102'291.70 | 6 | 6 | 6 | 0.15% | 8.47% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (910 aa) | ARB_01444 | 98'838.70 | 15 | 16 | 20 | 0.49% | 26.10% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (409 aa) | ARB_03431 | 44'385.60 | 2 | 2 | 2 | 0.05% | 7.60% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (585 aa) | ARB_02921 | 63'752.10 | 6 | 7 | 9 | 0.22% | 17.30% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (441 aa) | ARB_05372 | 47'817.30 | 4 | 6 | 13 | 0.32% | 12.70% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (530 aa) | ARB_06414 | 59'403.00 | 12 | 17 | 45 | 1.10% | 33.80% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (657 aa) | ARB_06019 | 72'360.20 | 7 | 7 | 7 | 0.17% | 15.40% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (771 aa) | ARB_07629 | 85'022.20 | 20 | 21 | 22 | 0.54% | 36.40% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (197 aa) | ARB_00926 | 20'992.40 | 5 | 7 | 12 | 0.29% | 46.40% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (398 aa) | ARB_00701 | 40'996.40 | 13 | 20 | 119 | 2.90% | 55.90% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (426 aa) | ARB_02208 | 46'364.90 | 4 | 4 | 9 | 0.22% | 20.20% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (405 aa) | ARB_04859 | 43'686.00 | 6 | 7 | 14 | 0.34% | 23.00% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (481 aa) | ARB_05360 | 52'728.10 | 17 | 22 | 25 | 0.61% | 58.30% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (354 aa) | ARB_06359 | 37'581.10 | 14 | 16 | 28 | 0.68% | 47.60% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (945 aa) | ARB_06907 | 103'907.50 | 25 | 33 | 41 | 1.00% | 41.60% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Abenh-pH4 a | A. benhamiae CBS 112371 null (501 aa) | ARB_02372 | 54'522.30 | 7 | 7 | 7 | 0.17% | 23.60% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (342 aa) | ARB_07185 | 37'342.80 | 3 | 3 | 3 | 0.07% | 18.80% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (196 aa) | ARB_00595 | 20'913.60 | 7 | 8 | 23 | 0.56% | 64.10% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (508 aa) | ARB_01886 | 56'227.30 | 5 | 5 | 5 | 0.12% | 13.60% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (292 aa) | ARB_03620 | 32'033.60 | 2 | 2 | 2 | 0.05% | 8.93% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (191 aa) | ARB_05566 | 20'524.50 | 3 | 3 | 5 | 0.12% | 24.20% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (157 aa) | ARB_03576 | 16'148.80 | 2 | 2 | 2 | 0.05% | 13.50% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (403 aa) | ARB_03790 | 42'110.30 | 8 | 13 | 18 | 0.44% | 39.60% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (699 aa) | ARB_02220 | 77'510.00 | 36 | 45 | 177 | 4.31% | 55.90% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (727 aa) | ARB_06651 | 80'067.80 | 42 | 67 | 277 | 6.75% | 72.70% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (414 aa) | ARB_04467 | 45'398.30 | 18 | 26 | 48 | 1.17% | 61.70% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (187 aa) | ARB_00075 | 19'791.90 | 8 | 13 | 92 | 2.24% | 61.30% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (667 aa) | ARB_01864 | 75'175.40 | 19 | 27 | 50 | 1.22% | 37.10% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (634 aa) | ARB_05085 | 69'232.20 | 18 | 26 | 96 | 2.34% | 55.50% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (342 aa) | ARB_03699 | 36'255.50 | 9 | 14 | 49 | 1.19% | 52.50% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (505 aa) | ARB_06076 | 53'188.50 | 6 | 10 | 15 | 0.37% | 33.10% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (510 aa) | ARB_00035 | 56'275.40 | 20 | 28 | 48 | 1.17% | 52.50% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (418 aa) | ARB_05392 | 45'903.20 | 16 | 23 | 52 | 1.27% | 61.20% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (879 aa) | ARB_02077 | 93'825.10 | 20 | 27 | 46 | 1.12% | 43.40% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (779 aa) | ARB_06110 | 88'383.60 | 22 | 29 | 41 | 1.00% | 40.90% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (717 aa) | ARB_01345 | 79'290.90 | 18 | 25 | 74 | 1.80% | 36.30% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (388 aa) | ARB_01627 | 41'241.10 | 11 | 12 | 25 | 0.61% | 34.90% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Abenh-pH4 a | A. benhamiae CBS 112371 null (404 aa) | ARB_05728 | 42'961.40 | 13 | 24 | 184 | 4.48% | 49.40% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (597 aa) | ARB_05765 | 64'844.70 | 18 | 25 | 73 | 1.78% | 48.20% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (267 aa) | ARB_00862 | 28'612.50 | 2 | 2 | 2 | 0.05% | 9.77% |
| Abenh-pH4 a | A. benhamiae CBS 112371 null (309 aa) | ARB_02797 | 32'960.20 | 10 | 17 | 101 | 2.46% | 53.60% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (496 aa) | ARB_04170 | 51'840.90 | 9 | 13 | 16 | 0.26% | 27.90% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (401 aa) | ARB_06111 | 41'702.10 | 2 | 2 | 3 | 0.05% | 6.00% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (405 aa) | ARB_06224 | 45'281.70 | 1 | 1 | 1 | 0.02% | 2.72% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (417 aa) | ARB_03789 | 45'801.80 | 5 | 8 | 16 | 0.26% | 21.20% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (512 aa) | ARB_07056 | 55'227.80 | 12 | 13 | 16 | 0.26% | 32.10% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (418 aa) | ARB_07536 | 47'017.10 | 4 | 5 | 5 | 0.08% | 13.20% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (413 aa) | ARB_01347 | 44'166.80 | 9 | 11 | 19 | 0.31% | 22.80% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (546 aa) | ARB_07487 | 58'808.10 | 5 | 5 | 5 | 0.08% | 9.17% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (149 aa) | ARB_04828 | 16'449.50 | 2 | 2 | 2 | 0.03% | 16.90% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (601 aa) | ARB_04101 | 65'387.00 | 4 | 4 | 4 | 0.06% | 8.67% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (223 aa) | ARB_03106 | 25'133.60 | 6 | 10 | 26 | 0.42% | 40.50% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (134 aa) | ARB_05536 | 15'101.60 | 6 | 8 | 21 | 0.34% | 44.40% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (623 aa) | ARB_05535 | 70'472.60 | 13 | 18 | 37 | 0.60% | 26.70% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (351 aa) | ARB_03276 | 38'765.00 | 9 | 11 | 11 | 0.18% | 34.00% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (1029 aa) | ARB_02327 | 113'054.40 | 17 | 19 | 36 | 0.58% | 20.50% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (315 aa) | ARB_06966 | 34'730.00 | 5 | 7 | 10 | 0.16% | 25.80% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (336 aa) | ARB_03491 | 37'530.20 | 8 | 9 | 9 | 0.15% | 33.40% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (866 aa) | ARB_02101 | 98'073.30 | 12 | 13 | 15 | 0.24% | 19.20% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Abenh-pH4 b | A. benhamiae CBS 112371 null (653 aa) | ARB_04046 | 71'688.10 | 12 | 16 | 54 | 0.87% | 27.50% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (566 aa) | ARB_02478 | 61'777.10 | 10 | 11 | 12 | 0.19% | 25.10% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (634 aa) | ARB_06472 | 69'823.90 | 10 | 13 | 16 | 0.26% | 27.20% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (206 aa) | ARB_05304 | 21'463.10 | 10 | 13 | 51 | 0.83% | 54.10% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (175 aa) | ARB_01325 | 18'707.50 | 4 | 4 | 7 | 0.11% | 34.50% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (238 aa) | ARB_07027 | 26'853.40 | 3 | 7 | 21 | 0.34% | 20.30% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (131 aa) | ARB_07026 | 14'085.60 | 5 | 8 | 33 | 0.53% | 50.80% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (508 aa) | ARB_01498 | 55'708.10 | 2 | 2 | 2 | 0.03% | 4.54% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (440 aa) | ARB_05253 | 44'989.10 | 9 | 12 | 56 | 0.91% | 26.90% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (226 aa) | ARB_01979 | 25'408.90 | 9 | 13 | 22 | 0.36% | 50.20% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (388 aa) | ARB_01751 | 42'248.00 | 5 | 6 | 6 | 0.10% | 19.90% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (606 aa) | ARB_07893 | 68'500.30 | 8 | 8 | 9 | 0.15% | 19.80% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (593 aa) | ARB_02965 | 63'994.80 | 17 | 20 | 24 | 0.39% | 39.50% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (243 aa) | ARB_07186 | 26'586.30 | 5 | 5 | 6 | 0.10% | 23.10% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (342 aa) | ARB_07185 | 37'342.80 | 2 | 2 | 3 | 0.05% | 13.80% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (395 aa) | ARB_00047 | 44'081.90 | 3 | 3 | 3 | 0.05% | 10.40% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (191 aa) | ARB_05566 | 20'524.50 | 4 | 8 | 19 | 0.31% | 35.30% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (312 aa) | ARB_02251 | 34'329.90 | 3 | 3 | 3 | 0.05% | 11.30% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (509 aa) | ARB_05721 | 57'153.20 | 9 | 12 | 14 | 0.23% | 29.30% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (883 aa) | ARB_07888 | 99'853.00 | 10 | 12 | 13 | 0.21% | 15.20% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (257 aa) | ARB_03024 | 25'239.30 | 2 | 2 | 2 | 0.03% | 12.50% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (552 aa) | ARB_00083 | 62'360.70 | 20 | 32 | 69 | 1.12% | 60.60% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Abenh-pH4 b | A. benhamiae CBS 112371 null (457 aa) | ARB_05933 | 50'474.40 | 7 | 7 | 7 | 0.11% | 24.60% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (491 aa) | ARB_00777 | 52'317.20 | 2 | 2 | 2 | 0.03% | 8.98% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (242 aa) | ARB_04889 | 26'743.60 | 8 | 11 | 17 | 0.28% | 54.80% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (395 aa) | ARB_02015 | 42'491.90 | 9 | 13 | 15 | 0.24% | 39.30% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (1055 aa) | ARB_03719 | 114'777.50 | 8 | 9 | 9 | 0.15% | 11.60% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (503 aa) | ARB_05319 | 54'495.60 | 20 | 31 | 64 | 1.03% | 63.10% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (925 aa) | ARB_07867 | 99'074.10 | 12 | 15 | 21 | 0.34% | 20.60% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (821 aa) | ARB_05654 | 88'821.60 | 11 | 13 | 17 | 0.28% | 19.00% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (530 aa) | ARB_03264 | 59'162.30 | 13 | 18 | 36 | 0.58% | 36.90% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (669 aa) | ARB_01230 | 71'462.70 | 2 | 2 | 2 | 0.03% | 4.49% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (644 aa) | ARB_05919 | 68'907.10 | 6 | 7 | 7 | 0.11% | 13.20% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (374 aa) | ARB_03568 | 40'712.70 | 11 | 15 | 39 | 0.63% | 46.90% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (198 aa) | ARB_03673 | 21'021.70 | 4 | 6 | 12 | 0.19% | 31.00% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (236 aa) | ARB_05911 | 25'146.90 | 6 | 12 | 93 | 1.50% | 45.50% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (196 aa) | ARB_07637 | 20'530.80 | 5 | 6 | 22 | 0.36% | 34.90% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (224 aa) | ARB_03853 | 24'951.00 | 5 | 7 | 11 | 0.18% | 27.80% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (910 aa) | ARB_06590 | 102'291.70 | 2 | 2 | 2 | 0.03% | 2.75% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (910 aa) | ARB_01444 | 98'838.70 | 16 | 17 | 23 | 0.37% | 23.40% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (409 aa) | ARB_03431 | 44'385.60 | 7 | 7 | 8 | 0.13% | 22.30% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (585 aa) | ARB_02921 | 63'752.10 | 7 | 9 | 13 | 0.21% | 22.10% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (441 aa) | ARB_05372 | 47'817.30 | 6 | 8 | 13 | 0.21% | 17.30% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (530 aa) | ARB_06414 | 59'403.00 | 15 | 23 | 70 | 1.13% | 38.40% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Abenh-pH4 b | A. benhamiae CBS 112371 null (657 aa) | ARB_06019 | 72'360.20 | 5 | 5 | 5 | 0.08% | 11.60% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (771 aa) | ARB_07629 | 85'022.20 | 20 | 24 | 28 | 0.45% | 34.90% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (197 aa) | ARB_00926 | 20'992.40 | 8 | 11 | 16 | 0.26% | 52.00% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (398 aa) | ARB_00701 | 40'996.40 | 13 | 20 | 178 | 2.88% | 56.20% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (426 aa) | ARB_02208 | 46'364.90 | 3 | 4 | 4 | 0.06% | 11.50% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (405 aa) | ARB_04859 | 43'686.00 | 6 | 6 | 11 | 0.18% | 18.60% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (481 aa) | ARB_05360 | 52'728.10 | 16 | 23 | 33 | 0.53% | 56.00% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (538 aa) | ARB_07085 | 57'758.90 | 2 | 2 | 2 | 0.03% | 5.21% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (354 aa) | ARB_06359 | 37'581.10 | 18 | 26 | 59 | 0.95% | 52.40% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (172 aa) | ARB_00344 | 18'201.10 | 5 | 9 | 218 | 3.53% | 42.70% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (945 aa) | ARB_06907 | 103'907.50 | 23 | 31 | 41 | 0.66% | 39.50% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (501 aa) | ARB_02372 | 54'522.30 | 14 | 20 | 27 | 0.44% | 36.20% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (354 aa) | ARB_00204 | 38'505.10 | 2 | 2 | 2 | 0.03% | 6.52% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (196 aa) | ARB_00595 | 20'913.60 | 4 | 6 | 11 | 0.18% | 21.50% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (508 aa) | ARB_01886 | 56'227.30 | 4 | 4 | 4 | 0.06% | 14.20% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (292 aa) | ARB_03620 | 32'033.60 | 4 | 4 | 5 | 0.08% | 17.90% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (250 aa) | ARB_04619 | 27'122.70 | 2 | 3 | 6 | 0.10% | 14.10% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (527 aa) | ARB_02407 | 58'544.90 | 6 | 8 | 8 | 0.13% | 15.20% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (636 aa) | ARB_02406 | 70'044.90 | 3 | 3 | 3 | 0.05% | 13.50% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (403 aa) | ARB_03790 | 42'110.30 | 6 | 9 | 11 | 0.18% | 23.60% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (388 aa) | ARB_01627 | 41'241.10 | 14 | 18 | 75 | 1.21% | 46.50% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (717 aa) | ARB_01345 | 79'290.90 | 18 | 30 | 114 | 1.84% | 35.30% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Abenh-pH4 b | A. benhamiae CBS 112371 null (634 aa) | ARB_05085 | 69'232.20 | 16 | 31 | 142 | 2.30% | 35.10% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (727 aa) | ARB_06651 | 80'067.80 | 48 | 82 | 645 | 10.40% | 76.20% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (309 aa) | ARB_02797 | 32'960.20 | 13 | 20 | 169 | 2.73% | 57.50% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (667 aa) | ARB_01864 | 75'175.40 | 21 | 32 | 113 | 1.83% | 39.90% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (414 aa) | ARB_04467 | 45'398.30 | 18 | 28 | 66 | 1.07% | 56.90% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (779 aa) | ARB_06110 | 88'383.60 | 25 | 31 | 42 | 0.68% | 47.40% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (418 aa) | ARB_05392 | 45'903.20 | 15 | 25 | 52 | 0.84% | 58.00% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (505 aa) | ARB_06076 | 53'188.50 | 2 | 3 | 6 | 0.10% | 8.13% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (342 aa) | ARB_03699 | 36'255.50 | 12 | 21 | 135 | 2.18% | 52.80% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (510 aa) | ARB_00035 | 56'275.40 | 21 | 30 | 46 | 0.74% | 60.10% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (267 aa) | ARB_00862 | 28'612.50 | 2 | 2 | 2 | 0.03% | 9.77% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (496 aa) | ARB_00494 | 53'225.40 | 14 | 16 | 16 | 0.26% | 31.50% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (187 aa) | ARB_00075 | 19'791.90 | 10 | 15 | 112 | 1.81% | 54.80% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (699 aa) | ARB_02220 | 77'510.00 | 43 | 57 | 384 | 6.21% | 59.00% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (597 aa) | ARB_05765 | 64'844.70 | 18 | 29 | 153 | 2.47% | 47.10% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (404 aa) | ARB_05728 | 42'961.40 | 14 | 26 | 273 | 4.41% | 57.80% |
| Abenh-pH4 b | A. benhamiae CBS 112371 null (879 aa) | ARB_02077 | 93'825.10 | 15 | 24 | 51 | 0.83% | 34.60% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (512 aa) | ARB_07056 | 55'227.80 | 12 | 15 | 18 | 0.46% | 42.30% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (238 aa) | ARB_07027 | 26'853.40 | 3 | 7 | 13 | 0.33% | 20.30% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (172 aa) | ARB_00344 | 18'201.10 | 4 | 7 | 193 | 4.92% | 36.80% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (503 aa) | ARB_05319 | 54'495.60 | 18 | 22 | 37 | 0.94% | 52.40% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (223 aa) | ARB_03106 | 25'133.60 | 6 | 10 | 18 | 0.46% | 40.50% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Abenh-pH4 c | A. benhamiae CBS 112371 null (623 aa) | ARB_05535 | 70'472.60 | 10 | 11 | 19 | 0.48% | 22.70% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (1029 aa) | ARB_02327 | 113'054.40 | 16 | 18 | 22 | 0.56% | 15.40% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (206 aa) | ARB_05304 | 21'463.10 | 7 | 9 | 32 | 0.82% | 39.00% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (131 aa) | ARB_07026 | 14'085.60 | 4 | 5 | 7 | 0.18% | 34.60% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (440 aa) | ARB_05253 | 44'989.10 | 9 | 12 | 44 | 1.12% | 26.90% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (593 aa) | ARB_02965 | 63'994.80 | 7 | 7 | 7 | 0.18% | 15.00% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (821 aa) | ARB_05654 | 88'821.60 | 10 | 11 | 15 | 0.38% | 17.40% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (255 aa) | ARB_03514 | 27'613.80 | 4 | 6 | 6 | 0.15% | 27.20% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (552 aa) | ARB_00083 | 62'360.70 | 13 | 18 | 35 | 0.89% | 30.90% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (175 aa) | ARB_01325 | 18'707.50 | 3 | 3 | 6 | 0.15% | 21.80% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (925 aa) | ARB_07867 | 99'074.10 | 13 | 16 | 24 | 0.61% | 21.40% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (530 aa) | ARB_03264 | 59'162.30 | 9 | 14 | 20 | 0.51% | 23.30% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (374 aa) | ARB_03568 | 40'712.70 | 10 | 17 | 41 | 1.04% | 39.40% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (236 aa) | ARB_05911 | 25'146.90 | 6 | 10 | 51 | 1.30% | 45.50% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (196 aa) | ARB_07637 | 20'530.80 | 5 | 7 | 21 | 0.54% | 34.90% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (653 aa) | ARB_04046 | 71'688.10 | 10 | 13 | 46 | 1.17% | 25.20% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (910 aa) | ARB_01444 | 98'838.70 | 11 | 11 | 17 | 0.43% | 14.20% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (530 aa) | ARB_06414 | 59'403.00 | 9 | 14 | 32 | 0.82% | 23.60% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (771 aa) | ARB_07629 | 85'022.20 | 17 | 20 | 22 | 0.56% | 32.10% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (398 aa) | ARB_00701 | 40'996.40 | 8 | 14 | 33 | 0.84% | 39.00% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (405 aa) | ARB_04859 | 43'686.00 | 6 | 6 | 9 | 0.23% | 21.80% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (354 aa) | ARB_06359 | 37'581.10 | 14 | 16 | 29 | 0.74% | 46.50% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Abenh-pH4 c | A. benhamiae CBS 112371 null (945 aa) | ARB_06907 | 103'907.50 | 21 | 25 | 31 | 0.79% | 31.60% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (501 aa) | ARB_02372 | 54'522.30 | 6 | 7 | 10 | 0.26% | 16.60% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (196 aa) | ARB_00595 | 20'913.60 | 4 | 5 | 8 | 0.20% | 21.50% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (226 aa) | ARB_01979 | 25'408.90 | 7 | 9 | 13 | 0.33% | 32.00% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (257 aa) | ARB_03024 | 25'239.30 | 2 | 3 | 4 | 0.10% | 9.38% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (354 aa) | ARB_00204 | 38'505.10 | 1 | 1 | 1 | 0.03% | 3.40% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (315 aa) | ARB_06966 | 34'730.00 | 5 | 6 | 7 | 0.18% | 18.20% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (191 aa) | ARB_05566 | 20'524.50 | 5 | 8 | 15 | 0.38% | 43.70% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (388 aa) | ARB_01627 | 41'241.10 | 10 | 11 | 51 | 1.30% | 22.00% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (634 aa) | ARB_06472 | 69'823.90 | 6 | 7 | 8 | 0.20% | 15.50% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (879 aa) | ARB_02077 | 93'825.10 | 15 | 22 | 39 | 0.99% | 28.90% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (585 aa) | ARB_02921 | 63'752.10 | 4 | 4 | 5 | 0.13% | 8.56% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (566 aa) | ARB_02478 | 61'777.10 | 8 | 8 | 9 | 0.23% | 17.30% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (351 aa) | ARB_03276 | 38'765.00 | 8 | 9 | 9 | 0.23% | 26.00% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (667 aa) | ARB_01864 | 75'175.40 | 17 | 25 | 73 | 1.86% | 31.70% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (527 aa) | ARB_02407 | 58'544.90 | 2 | 2 | 2 | 0.05% | 4.94% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (224 aa) | ARB_03853 | 24'951.00 | 5 | 5 | 9 | 0.23% | 25.10% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (309 aa) | ARB_02797 | 32'960.20 | 11 | 18 | 89 | 2.27% | 54.90% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (699 aa) | ARB_02220 | 77'510.00 | 23 | 27 | 108 | 2.75% | 38.40% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (246 aa) | ARB_07466 | 26'212.40 | 2 | 2 | 2 | 0.05% | 10.60% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (634 aa) | ARB_05828 | 71'535.40 | 1 | 1 | 1 | 0.03% | 2.53% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (312 aa) | ARB_02251 | 34'329.90 | 2 | 2 | 2 | 0.05% | 8.36% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Abenh-pH4 c | A. benhamiae CBS 112371 null (413 aa) | ARB_01347 | 44'166.80 | 8 | 9 | 16 | 0.41% | 25.70% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (417 aa) | ARB_03789 | 45'801.80 | 5 | 5 | 7 | 0.18% | 18.50% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (491 aa) | ARB_00777 | 52'317.20 | 2 | 2 | 2 | 0.05% | 4.90% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (336 aa) | ARB_03491 | 37'530.20 | 7 | 7 | 7 | 0.18% | 27.20% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (342 aa) | ARB_07185 | 37'342.80 | 2 | 2 | 2 | 0.05% | 6.45% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (509 aa) | ARB_05721 | 57'153.20 | 4 | 4 | 4 | 0.10% | 11.40% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (510 aa) | ARB_00035 | 56'275.40 | 17 | 24 | 30 | 0.77% | 42.20% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (404 aa) | ARB_05728 | 42'961.40 | 10 | 14 | 94 | 2.40% | 39.70% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (630 aa) | ARB_06361 | 69'690.80 | 2 | 2 | 2 | 0.05% | 3.97% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (395 aa) | ARB_02015 | 42'491.90 | 7 | 7 | 7 | 0.18% | 25.40% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (457 aa) | ARB_05933 | 50'474.40 | 2 | 2 | 2 | 0.05% | 4.17% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (187 aa) | ARB_02741 | 18'247.20 | 2 | 2 | 3 | 0.08% | 9.14% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (644 aa) | ARB_05919 | 68'907.10 | 8 | 9 | 12 | 0.31% | 19.30% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (214 aa) | ARB_02516 | 23'548.90 | 1 | 1 | 1 | 0.03% | 4.23% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (1055 aa) | ARB_03719 | 114'777.50 | 3 | 3 | 3 | 0.08% | 2.94% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (418 aa) | ARB_05392 | 45'903.20 | 14 | 21 | 37 | 0.94% | 48.00% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (426 aa) | ARB_02208 | 46'364.90 | 3 | 3 | 3 | 0.08% | 15.10% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (606 aa) | ARB_07893 | 68'500.30 | 3 | 3 | 3 | 0.08% | 5.95% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (657 aa) | ARB_06019 | 72'360.20 | 4 | 4 | 4 | 0.10% | 9.45% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (779 aa) | ARB_06110 | 88'383.60 | 17 | 21 | 25 | 0.64% | 30.80% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (134 aa) | ARB_05536 | 15'101.60 | 5 | 7 | 18 | 0.46% | 37.60% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (546 aa) | ARB_07487 | 58'808.10 | 2 | 2 | 2 | 0.05% | 3.67% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Abenh-pH4 c | A. benhamiae CBS 112371 null (717 aa) | ARB_01345 | 79'290.90 | 15 | 19 | 53 | 1.35% | 31.70% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (481 aa) | ARB_05360 | 52'728.10 | 11 | 15 | 18 | 0.46% | 32.30% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (187 aa) | ARB_00075 | 19'791.90 | 9 | 15 | 180 | 4.59% | 65.10% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (342 aa) | ARB_03699 | 36'255.50 | 9 | 14 | 58 | 1.48% | 52.50% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (395 aa) | ARB_00047 | 44'081.90 | 3 | 4 | 4 | 0.10% | 13.50% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (727 aa) | ARB_06651 | 80'067.80 | 37 | 63 | 410 | 10.40% | 62.80% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (198 aa) | ARB_03673 | 21'021.70 | 4 | 6 | 12 | 0.31% | 31.00% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (866 aa) | ARB_02101 | 98'073.30 | 5 | 6 | 7 | 0.18% | 6.82% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (366 aa) | ARB_05022 | 40'280.20 | 2 | 3 | 3 | 0.08% | 8.77% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (418 aa) | ARB_07536 | 47'017.10 | 2 | 2 | 2 | 0.05% | 7.19% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (496 aa) | ARB_04170 | 51'840.90 | 6 | 6 | 6 | 0.15% | 19.00% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (883 aa) | ARB_07888 | 99'853.00 | 13 | 15 | 18 | 0.46% | 18.10% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (243 aa) | ARB_07186 | 26'586.30 | 4 | 4 | 6 | 0.15% | 15.70% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (414 aa) | ARB_04467 | 45'398.30 | 14 | 18 | 35 | 0.89% | 41.90% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (242 aa) | ARB_04889 | 26'743.60 | 4 | 6 | 9 | 0.23% | 25.30% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (597 aa) | ARB_05765 | 64'844.70 | 17 | 24 | 81 | 2.06% | 46.60% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (388 aa) | ARB_01751 | 42'248.00 | 4 | 4 | 4 | 0.10% | 17.30% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (441 aa) | ARB_05372 | 47'817.30 | 5 | 7 | 8 | 0.20% | 15.20% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (496 aa) | ARB_00494 | 53'225.40 | 2 | 2 | 2 | 0.05% | 4.65% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (250 aa) | ARB_04619 | 27'122.70 | 3 | 4 | 7 | 0.18% | 18.10% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (403 aa) | ARB_03790 | 42'110.30 | 6 | 9 | 14 | 0.36% | 24.40% |
| Abenh-pH4 c | A. benhamiae CBS 112371 null (634 aa) | ARB_05085 | 69'232.20 | 13 | 20 | 61 | 1.55% | 41.90% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Abenh-pH4 c | A. benhamiae CBS 112371 null (197 aa) | ARB_00926 | 20'992.40 | 4 | 5 | 22 | 0.56% | 23.50% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (910 aa) | ARB_01444 | 98'838.70 | 27 | 34 | 92 | 1.35% | 42.20% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (821 aa) | ARB_05654 | 88'821.60 | 20 | 24 | 78 | 1.14% | 39.60% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (552 aa) | ARB_00083 | 62'360.70 | 13 | 17 | 24 | 0.35% | 32.70% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (255 aa) | ARB_03514 | 27'613.80 | 7 | 9 | 31 | 0.46% | 40.90% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (530 aa) | ARB_06414 | 59'403.00 | 5 | 5 | 10 | 0.15% | 12.90% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (607 aa) | ARB_04499 | 66'514.90 | 15 | 23 | 40 | 0.59% | 44.40% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (354 aa) | ARB_06359 | 37'581.10 | 14 | 16 | 24 | 0.35% | 50.40% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (175 aa) | ARB_01325 | 18'707.50 | 6 | 10 | 41 | 0.60% | 48.30% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (623 aa) | ARB_05535 | 70'472.60 | 10 | 11 | 18 | 0.26% | 21.70% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (246 aa) | ARB_07590 | 26'418.30 | 12 | 19 | 344 | 5.04% | 66.90% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (688 aa) | ARB_06334 | 75'021.90 | 21 | 26 | 57 | 0.84% | 43.80% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (771 aa) | ARB_07629 | 85'022.20 | 8 | 8 | 9 | 0.13% | 15.80% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (762 aa) | ARB_01232 | 83'795.80 | 18 | 27 | 58 | 0.85% | 36.80% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (945 aa) | ARB_06907 | 103'907.50 | 11 | 15 | 16 | 0.24% | 18.40% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (512 aa) | ARB_07056 | 55'227.80 | 11 | 13 | 20 | 0.29% | 40.50% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (593 aa) | ARB_02965 | 63'994.80 | 18 | 25 | 108 | 1.58% | 40.90% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (196 aa) | ARB_07637 | 20'530.80 | 5 | 7 | 10 | 0.15% | 42.10% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (530 aa) | ARB_03264 | 59'162.30 | 10 | 13 | 27 | 0.40% | 24.80% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (172 aa) | ARB_00344 | 18'201.10 | 5 | 7 | 71 | 1.04% | 53.20% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (238 aa) | ARB_07027 | 26'853.40 | 3 | 7 | 39 | 0.57% | 20.30% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (131 aa) | ARB_07026 | 14'085.60 | 5 | 9 | 80 | 1.17% | 73.80% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Abenh-pH7 a | A. benhamiae CBS 112371 null (653 aa) | ARB_04046 | 71'688.10 | 5 | 5 | 5 | 0.07% | 11.00% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (501 aa) | ARB_02372 | 54'522.30 | 9 | 12 | 17 | 0.25% | 28.60% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (565 aa) | ARB_02369 | 61'369.70 | 18 | 24 | 62 | 0.91% | 47.90% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (503 aa) | ARB_05319 | 54'495.60 | 14 | 18 | 24 | 0.35% | 44.40% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (925 aa) | ARB_07867 | 99'074.10 | 12 | 17 | 31 | 0.46% | 21.20% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (206 aa) | ARB_05304 | 21'463.10 | 6 | 8 | 13 | 0.19% | 39.00% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (210 aa) | ARB_00107 | 22'728.10 | 10 | 16 | 203 | 2.98% | 62.20% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (328 aa) | ARB_06052 | 36'392.00 | 14 | 17 | 47 | 0.69% | 63.30% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (1029 aa) | ARB_02327 | 113'054.40 | 16 | 23 | 38 | 0.56% | 26.30% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (398 aa) | ARB_00701 | 40'996.40 | 14 | 21 | 148 | 2.17% | 59.40% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (374 aa) | ARB_03568 | 40'712.70 | 9 | 12 | 19 | 0.28% | 35.70% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (196 aa) | ARB_00595 | 20'913.60 | 11 | 17 | 68 | 1.00% | 81.00% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (405 aa) | ARB_04859 | 43'686.00 | 9 | 12 | 21 | 0.31% | 34.20% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (505 aa) | ARB_06076 | 53'188.50 | 8 | 17 | 147 | 2.16% | 45.80% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (134 aa) | ARB_05536 | 15'101.60 | 5 | 5 | 8 | 0.12% | 37.60% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (354 aa) | ARB_00204 | 38'505.10 | 13 | 15 | 37 | 0.54% | 51.80% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (243 aa) | ARB_07186 | 26'586.30 | 3 | 3 | 4 | 0.06% | 11.20% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (292 aa) | ARB_03620 | 32'033.60 | 4 | 4 | 4 | 0.06% | 17.90% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (491 aa) | ARB_00777 | 52'317.20 | 4 | 4 | 4 | 0.06% | 13.90% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (417 aa) | ARB_03789 | 45'801.80 | 8 | 9 | 10 | 0.15% | 32.00% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (315 aa) | ARB_06966 | 34'730.00 | 5 | 8 | 11 | 0.16% | 22.90% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (149 aa) | ARB_02001 | 16'452.90 | 5 | 7 | 8 | 0.12% | 50.00% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Abenh-pH7 a | A. benhamiae CBS 112371 null (441 aa) | ARB_05372 | 47'817.30 | 4 | 5 | 7 | 0.10% | 11.40% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (360 aa) | ARB_01765 | 41'235.10 | 3 | 3 | 3 | 0.04% | 11.40% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (425 aa) | ARB_01495 | 45'947.10 | 5 | 5 | 12 | 0.18% | 17.00% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (413 aa) | ARB_05307 | 42'717.30 | 6 | 6 | 10 | 0.15% | 26.90% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (566 aa) | ARB_02478 | 61'777.10 | 5 | 5 | 7 | 0.10% | 10.60% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (644 aa) | ARB_00762 | 70'670.60 | 14 | 21 | 96 | 1.41% | 35.30% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (416 aa) | ARB_05309 | 44'871.80 | 4 | 4 | 5 | 0.07% | 18.10% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (187 aa) | ARB_00075 | 19'791.90 | 7 | 9 | 19 | 0.28% | 55.40% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (756 aa) | ARB_05732 | 81'168.90 | 4 | 7 | 18 | 0.26% | 9.80% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (501 aa) | ARB_00532 | 56'090.50 | 6 | 8 | 8 | 0.12% | 25.80% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (179 aa) | ARB_07191 | 19'788.40 | 3 | 3 | 3 | 0.04% | 19.10% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (779 aa) | ARB_06110 | 88'383.60 | 30 | 45 | 118 | 1.73% | 61.20% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (572 aa) | ARB_02107 | 62'387.40 | 3 | 3 | 6 | 0.09% | 9.81% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (414 aa) | ARB_04467 | 45'398.30 | 19 | 29 | 81 | 1.19% | 61.50% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (557 aa) | ARB_01941 | 60'870.20 | 5 | 5 | 6 | 0.09% | 14.20% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (306 aa) | ARB_04618 | 33'949.20 | 4 | 4 | 4 | 0.06% | 15.70% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (342 aa) | ARB_03699 | 36'255.50 | 8 | 10 | 13 | 0.19% | 43.10% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (879 aa) | ARB_02077 | 93'825.10 | 19 | 28 | 70 | 1.03% | 39.60% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (653 aa) | ARB_05859 | 70'106.10 | 9 | 10 | 15 | 0.22% | 18.40% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (354 aa) | ARB_01995 | 38'818.80 | 6 | 6 | 7 | 0.10% | 25.20% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (866 aa) | ARB_02101 | 98'073.30 | 5 | 5 | 5 | 0.07% | 9.60% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (513 aa) | ARB_06983 | 55'982.70 | 8 | 9 | 10 | 0.15% | 25.00% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Abenh-pH7 a | A. benhamiae CBS 112371 null (257 aa) | ARB_03024 | 25'239.30 | 7 | 10 | 26 | 0.38% | 36.70% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (1066 aa) | ARB_06053 | 116'804.20 | 12 | 15 | 15 | 0.22% | 15.80% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (388 aa) | ARB_01627 | 41'241.10 | 12 | 14 | 52 | 0.76% | 51.40% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (585 aa) | ARB_02921 | 63'752.10 | 2 | 2 | 3 | 0.04% | 5.48% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (140 aa) | ARB_06975 | 14'354.00 | 4 | 6 | 9 | 0.13% | 34.50% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (527 aa) | ARB_02407 | 58'544.90 | 5 | 6 | 8 | 0.12% | 12.00% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (401 aa) | ARB_06111 | 41'702.10 | 13 | 15 | 36 | 0.53% | 51.70% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (413 aa) | ARB_01347 | 44'166.80 | 7 | 7 | 10 | 0.15% | 22.60% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (508 aa) | ARB_01886 | 56'227.30 | 2 | 2 | 2 | 0.03% | 6.31% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (403 aa) | ARB_03790 | 42'110.30 | 5 | 7 | 11 | 0.16% | 29.40% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (415 aa) | ARB_02715 | 45'401.10 | 10 | 12 | 13 | 0.19% | 36.50% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (496 aa) | ARB_00494 | 53'225.40 | 30 | 48 | 595 | 8.72% | 66.30% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (246 aa) | ARB_04177 | 27'407.10 | 2 | 2 | 2 | 0.03% | 12.70% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (302 aa) | ARB_02990 | 32'356.50 | 1 | 1 | 1 | 0.01% | 3.99% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (213 aa) | ARB_00773 | 21'751.00 | 5 | 6 | 7 | 0.10% | 40.60% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (250 aa) | ARB_04619 | 27'122.70 | 5 | 6 | 6 | 0.09% | 28.90% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (214 aa) | ARB_05177 | 22'933.00 | 2 | 2 | 2 | 0.03% | 12.20% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (319 aa) | ARB_02306 | 36'287.30 | 3 | 3 | 3 | 0.04% | 11.60% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (426 aa) | ARB_02208 | 46'364.90 | 4 | 4 | 8 | 0.12% | 24.50% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (395 aa) | ARB_00047 | 44'081.90 | 3 | 4 | 5 | 0.07% | 12.70% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (634 aa) | ARB_05085 | 69'232.20 | 8 | 10 | 20 | 0.29% | 24.30% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (583 aa) | ARB_06380 | 64'167.10 | 2 | 2 | 2 | 0.03% | 5.15% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Abenh-pH7 a | A. benhamiae CBS 112371 null (242 aa) | ARB_04889 | 26'743.60 | 5 | 7 | 15 | 0.22% | 29.90% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (864 aa) | ARB_00226 | 95'625.40 | 2 | 2 | 2 | 0.03% | 2.32% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (383 aa) | ARB_08028 | 41'339.10 | 3 | 3 | 4 | 0.06% | 12.60% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (408 aa) | ARB_01032 | 42'987.80 | 6 | 10 | 13 | 0.19% | 29.20% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (923 aa) | ARB_04703 | 104'542.40 | 5 | 5 | 5 | 0.07% | 5.42% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (1102 aa) | ARB_01379 | 121'034.60 | 4 | 6 | 8 | 0.12% | 6.18% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (226 aa) | ARB_01979 | 25'408.90 | 7 | 7 | 13 | 0.19% | 32.00% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (617 aa) | ARB_01353 | 70'350.90 | 24 | 34 | 84 | 1.23% | 55.70% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (168 aa) | ARB_03651 | 18'697.30 | 3 | 4 | 4 | 0.06% | 24.00% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (300 aa) | ARB_00059 | 32'799.20 | 5 | 6 | 11 | 0.16% | 23.10% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (699 aa) | ARB_02220 | 77'510.00 | 45 | 64 | 668 | 9.79% | 64.00% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (453 aa) | ARB_05164 | 50'673.80 | 10 | 12 | 22 | 0.32% | 35.60% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (574 aa) | ARB_01962 | 62'410.60 | 3 | 4 | 4 | 0.06% | 7.85% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (224 aa) | ARB_03853 | 24'951.00 | 3 | 3 | 3 | 0.04% | 13.50% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (207 aa) | ARB_04555 | 21'190.20 | 5 | 6 | 7 | 0.10% | 30.10% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (308 aa) | ARB_06706 | 33'150.80 | 2 | 2 | 2 | 0.03% | 9.77% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (304 aa) | ARB_07886 | 34'727.00 | 5 | 6 | 6 | 0.09% | 17.20% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (728 aa) | ARB_02390 | 79'180.00 | 7 | 8 | 11 | 0.16% | 14.60% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (401 aa) | ARB_02919 | 43'400.10 | 2 | 2 | 2 | 0.03% | 7.50% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (1091 aa) | ARB_00520 | 123'628.70 | 39 | 49 | 110 | 1.61% | 42.20% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (208 aa) | ARB_06407 | 23'067.90 | 5 | 5 | 6 | 0.09% | 37.70% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (187 aa) | ARB_02741 | 18'247.20 | 2 | 4 | 8 | 0.12% | 9.14% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Abenh-pH7 a | A. benhamiae CBS 112371 null (235 aa) | ARB_02963 | 25'210.60 | 2 | 2 | 2 | 0.03% | 19.70% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (193 aa) | ARB_01932 | 20'469.70 | 2 | 2 | 6 | 0.09% | 13.00% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (418 aa) | ARB_05392 | 45'903.20 | 20 | 35 | 336 | 4.93% | 66.90% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (776 aa) | ARB_07441 | 84'044.80 | 2 | 2 | 2 | 0.03% | 4.26% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (513 aa) | ARB_04696 | 55'514.70 | 9 | 9 | 9 | 0.13% | 28.10% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (510 aa) | ARB_00035 | 56'275.40 | 21 | 29 | 64 | 0.94% | 55.20% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (267 aa) | ARB_00862 | 28'612.50 | 9 | 13 | 89 | 1.30% | 38.00% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (336 aa) | ARB_03491 | 37'530.20 | 6 | 6 | 6 | 0.09% | 22.40% |
| Abenh-pH7 a | A. benhamiae CBS 112371 null (309 aa) | ARB_02797 | 32'960.20 | 10 | 17 | 157 | 2.30% | 48.10% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (688 aa) | ARB_06334 | 75'021.90 | 19 | 23 | 37 | 0.49% | 34.60% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (512 aa) | ARB_07056 | 55'227.80 | 12 | 14 | 16 | 0.21% | 41.70% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (328 aa) | ARB_06052 | 36'392.00 | 13 | 16 | 43 | 0.57% | 54.70% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (607 aa) | ARB_04499 | 66'514.90 | 14 | 19 | 39 | 0.52% | 35.50% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (196 aa) | ARB_07637 | 20'530.80 | 4 | 5 | 22 | 0.29% | 30.30% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (623 aa) | ARB_05535 | 70'472.60 | 5 | 5 | 5 | 0.07% | 10.10% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (1029 aa) | ARB_02327 | 113'054.40 | 20 | 29 | 75 | 1.00% | 23.90% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (206 aa) | ARB_05304 | 21'463.10 | 6 | 8 | 37 | 0.49% | 35.60% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (175 aa) | ARB_01325 | 18'707.50 | 4 | 6 | 19 | 0.25% | 35.10% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (238 aa) | ARB_07027 | 26'853.40 | 3 | 7 | 25 | 0.33% | 20.30% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (131 aa) | ARB_07026 | 14'085.60 | 4 | 7 | 50 | 0.67% | 49.20% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (530 aa) | ARB_03264 | 59'162.30 | 12 | 18 | 37 | 0.49% | 35.50% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (210 aa) | ARB_00107 | 22'728.10 | 10 | 17 | 211 | 2.81% | 58.90% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Abenh-pH7 b | A. benhamiae CBS 112371 null (503 aa) | ARB_05319 | 54'495.60 | 15 | 22 | 43 | 0.57% | 48.00% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (593 aa) | ARB_02965 | 63'994.80 | 24 | 36 | 247 | 3.29% | 43.90% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (653 aa) | ARB_04046 | 71'688.10 | 5 | 5 | 7 | 0.09% | 10.90% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (255 aa) | ARB_03514 | 27'613.80 | 6 | 9 | 43 | 0.57% | 37.80% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (552 aa) | ARB_00083 | 62'360.70 | 6 | 7 | 9 | 0.12% | 19.10% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (925 aa) | ARB_07867 | 99'074.10 | 18 | 22 | 32 | 0.43% | 29.80% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (821 aa) | ARB_05654 | 88'821.60 | 25 | 32 | 203 | 2.70% | 36.80% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (762 aa) | ARB_01232 | 83'795.80 | 22 | 31 | 73 | 0.97% | 34.60% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (246 aa) | ARB_07590 | 26'418.30 | 12 | 18 | 254 | 3.38% | 64.10% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (374 aa) | ARB_03568 | 40'712.70 | 8 | 9 | 14 | 0.19% | 33.00% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (910 aa) | ARB_01444 | 98'838.70 | 35 | 50 | 161 | 2.14% | 46.50% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (771 aa) | ARB_07629 | 85'022.20 | 8 | 8 | 9 | 0.12% | 11.20% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (530 aa) | ARB_06414 | 59'403.00 | 5 | 5 | 8 | 0.11% | 12.90% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (398 aa) | ARB_00701 | 40'996.40 | 10 | 15 | 41 | 0.55% | 46.30% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (405 aa) | ARB_04859 | 43'686.00 | 10 | 10 | 19 | 0.25% | 46.30% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (354 aa) | ARB_06359 | 37'581.10 | 20 | 24 | 48 | 0.64% | 52.40% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (172 aa) | ARB_00344 | 18'201.10 | 6 | 10 | 106 | 1.41% | 59.10% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (945 aa) | ARB_06907 | 103'907.50 | 22 | 30 | 48 | 0.64% | 36.90% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (501 aa) | ARB_02372 | 54'522.30 | 11 | 14 | 27 | 0.36% | 31.20% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (565 aa) | ARB_02369 | 61'369.70 | 19 | 25 | 86 | 1.14% | 45.00% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (196 aa) | ARB_00595 | 20'913.60 | 9 | 12 | 52 | 0.69% | 60.00% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (354 aa) | ARB_00204 | 38'505.10 | 12 | 15 | 35 | 0.47% | 47.00% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Abenh-pH7 b | A. benhamiae CBS 112371 null (1102 aa) | ARB_01379 | 121'034.60 | 2 | 2 | 2 | 0.03% | 1.91% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (214 aa) | ARB_05177 | 22'933.00 | 2 | 2 | 5 | 0.07% | 12.20% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (779 aa) | ARB_06110 | 88'383.60 | 27 | 35 | 66 | 0.88% | 46.90% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (923 aa) | ARB_04703 | 104'542.40 | 6 | 6 | 6 | 0.08% | 6.83% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (404 aa) | ARB_05728 | 42'961.40 | 4 | 4 | 5 | 0.07% | 19.60% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (315 aa) | ARB_06966 | 34'730.00 | 6 | 8 | 17 | 0.23% | 26.10% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (513 aa) | ARB_04696 | 55'514.70 | 11 | 13 | 13 | 0.17% | 33.40% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (395 aa) | ARB_00047 | 44'081.90 | 5 | 5 | 5 | 0.07% | 21.60% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (426 aa) | ARB_02208 | 46'364.90 | 3 | 3 | 4 | 0.05% | 9.88% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (226 aa) | ARB_01979 | 25'408.90 | 6 | 6 | 6 | 0.08% | 31.60% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (728 aa) | ARB_02390 | 79'180.00 | 11 | 12 | 15 | 0.20% | 20.60% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (510 aa) | ARB_00035 | 56'275.40 | 24 | 33 | 78 | 1.04% | 62.90% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (134 aa) | ARB_05536 | 15'101.60 | 4 | 4 | 7 | 0.09% | 37.60% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (596 aa) | ARB_00930 | 65'104.30 | 3 | 3 | 3 | 0.04% | 5.55% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (187 aa) | ARB_00075 | 19'791.90 | 5 | 8 | 19 | 0.25% | 38.70% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (193 aa) | ARB_01932 | 20'469.70 | 2 | 2 | 2 | 0.03% | 13.00% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (505 aa) | ARB_06076 | 53'188.50 | 5 | 10 | 39 | 0.52% | 21.80% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (727 aa) | ARB_06651 | 80'067.80 | 20 | 27 | 91 | 1.21% | 45.60% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (401 aa) | ARB_06111 | 41'702.10 | 2 | 2 | 2 | 0.03% | 7.25% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (752 aa) | ARB_07609 | 84'246.10 | 9 | 11 | 11 | 0.15% | 20.10% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (453 aa) | ARB_05164 | 50'673.80 | 11 | 13 | 21 | 0.28% | 30.10% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (557 aa) | ARB_01941 | 60'870.20 | 11 | 11 | 12 | 0.16% | 29.30% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Abenh-pH7 b | A. benhamiae CBS 112371 null (538 aa) | ARB_07085 | 57'758.90 | 6 | 6 | 9 | 0.12% | 16.80% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (342 aa) | ARB_03699 | 36'255.50 | 9 | 14 | 66 | 0.88% | 52.50% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (441 aa) | ARB_05372 | 47'817.30 | 5 | 7 | 8 | 0.11% | 15.20% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (208 aa) | ARB_06407 | 23'067.90 | 5 | 5 | 5 | 0.07% | 31.40% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (657 aa) | ARB_02785 | 71'421.70 | 7 | 8 | 8 | 0.11% | 15.40% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (243 aa) | ARB_07186 | 26'586.30 | 4 | 4 | 4 | 0.05% | 19.40% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (879 aa) | ARB_02077 | 93'825.10 | 16 | 26 | 102 | 1.36% | 37.10% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (417 aa) | ARB_03789 | 45'801.80 | 4 | 5 | 9 | 0.12% | 14.90% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (864 aa) | ARB_00226 | 95'625.40 | 3 | 3 | 3 | 0.04% | 3.36% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (172 aa) | ARB_06080 | 19'252.10 | 5 | 5 | 7 | 0.09% | 32.20% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (566 aa) | ARB_02478 | 61'777.10 | 7 | 10 | 10 | 0.13% | 17.00% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (306 aa) | ARB_04618 | 33'949.20 | 2 | 2 | 2 | 0.03% | 8.85% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (464 aa) | ARB_07628 | 50'817.00 | 2 | 2 | 2 | 0.03% | 4.97% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (414 aa) | ARB_04467 | 45'398.30 | 17 | 24 | 43 | 0.57% | 56.40% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (653 aa) | ARB_05859 | 70'106.10 | 4 | 5 | 6 | 0.08% | 7.52% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (667 aa) | ARB_01864 | 75'175.40 | 1 | 1 | 1 | 0.01% | 2.25% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (418 aa) | ARB_05392 | 45'903.20 | 23 | 40 | 482 | 6.41% | 69.10% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (866 aa) | ARB_02101 | 98'073.30 | 6 | 7 | 8 | 0.11% | 8.79% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (1091 aa) | ARB_00520 | 123'628.70 | 39 | 54 | 176 | 2.34% | 37.70% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (634 aa) | ARB_05085 | 69'232.20 | 6 | 8 | 22 | 0.29% | 19.30% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (292 aa) | ARB_03620 | 32'033.60 | 4 | 4 | 6 | 0.08% | 17.90% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (309 aa) | ARB_02797 | 32'960.20 | 9 | 13 | 34 | 0.45% | 42.20% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Abenh-pH7 b | A. benhamiae CBS 112371 null (213 aa) | ARB_00773 | 21'751.00 | 4 | 4 | 4 | 0.05% | 36.80% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (496 aa) | ARB_00494 | 53'225.40 | 35 | 59 | 777 | 10.30% | 67.90% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (432 aa) | ARB_04981 | 48'517.80 | 1 | 1 | 1 | 0.01% | 2.32% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (179 aa) | ARB_07191 | 19'788.40 | 2 | 2 | 2 | 0.03% | 13.50% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (413 aa) | ARB_05307 | 42'717.30 | 3 | 3 | 6 | 0.08% | 11.90% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (527 aa) | ARB_02407 | 58'544.90 | 8 | 9 | 12 | 0.16% | 16.90% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (305 aa) | ARB_01071 | 33'382.90 | 1 | 1 | 1 | 0.01% | 4.61% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (501 aa) | ARB_00532 | 56'090.50 | 2 | 2 | 2 | 0.03% | 6.80% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (617 aa) | ARB_01353 | 70'350.90 | 27 | 37 | 144 | 1.92% | 54.90% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (267 aa) | ARB_00862 | 28'612.50 | 9 | 15 | 61 | 0.81% | 40.60% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (756 aa) | ARB_05732 | 81'168.90 | 2 | 3 | 7 | 0.09% | 3.84% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (644 aa) | ARB_00762 | 70'670.60 | 17 | 24 | 92 | 1.22% | 37.80% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (574 aa) | ARB_01962 | 62'410.60 | 3 | 3 | 3 | 0.04% | 12.00% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (242 aa) | ARB_04889 | 26'743.60 | 8 | 12 | 22 | 0.29% | 55.60% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (513 aa) | ARB_06983 | 55'982.70 | 13 | 14 | 22 | 0.29% | 44.90% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (597 aa) | ARB_05765 | 64'844.70 | 3 | 3 | 4 | 0.05% | 7.89% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (403 aa) | ARB_03790 | 42'110.30 | 7 | 8 | 9 | 0.12% | 30.80% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (388 aa) | ARB_01627 | 41'241.10 | 8 | 8 | 18 | 0.24% | 26.40% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (300 aa) | ARB_00059 | 32'799.20 | 4 | 4 | 4 | 0.05% | 20.10% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (699 aa) | ARB_02220 | 77'510.00 | 38 | 50 | 358 | 4.76% | 54.90% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (319 aa) | ARB_02306 | 36'287.30 | 3 | 3 | 3 | 0.04% | 11.30% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (257 aa) | ARB_03024 | 25'239.30 | 4 | 5 | 11 | 0.15% | 18.00% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Abenh-pH7 b | A. benhamiae CBS 112371 null (149 aa) | ARB_02001 | 16'452.90 | 4 | 4 | 8 | 0.11% | 27.00% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (415 aa) | ARB_02715 | 45'401.10 | 7 | 10 | 12 | 0.16% | 25.40% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (413 aa) | ARB_01347 | 44'166.80 | 5 | 5 | 6 | 0.08% | 13.30% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (354 aa) | ARB_01995 | 38'818.80 | 2 | 2 | 2 | 0.03% | 7.08% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (1066 aa) | ARB_06053 | 116'804.20 | 6 | 7 | 7 | 0.09% | 9.20% |
| Abenh-pH7 b | A. benhamiae CBS 112371 null (121 aa) | ARB_00658 | 12'788.00 | 1 | 2 | 3 | 0.04% | 13.30% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (910 aa) | ARB_01444 | 98'838.70 | 28 | 32 | 92 | 1.77% | 43.00% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (821 aa) | ARB_05654 | 88'821.60 | 14 | 21 | 78 | 1.50% | 24.30% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (552 aa) | ARB_00083 | 62'360.70 | 12 | 14 | 18 | 0.35% | 32.30% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (210 aa) | ARB_00107 | 22'728.10 | 9 | 16 | 172 | 3.30% | 51.70% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (255 aa) | ARB_03514 | 27'613.80 | 5 | 7 | 21 | 0.40% | 34.30% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (530 aa) | ARB_06414 | 59'403.00 | 3 | 3 | 7 | 0.13% | 6.81% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (945 aa) | ARB_06907 | 103'907.50 | 16 | 21 | 21 | 0.40% | 22.00% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (607 aa) | ARB_04499 | 66'514.90 | 12 | 15 | 27 | 0.52% | 26.20% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (354 aa) | ARB_06359 | 37'581.10 | 11 | 11 | 25 | 0.48% | 34.00% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (175 aa) | ARB_01325 | 18'707.50 | 3 | 4 | 17 | 0.33% | 21.80% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (623 aa) | ARB_05535 | 70'472.60 | 5 | 5 | 7 | 0.13% | 10.60% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (246 aa) | ARB_07590 | 26'418.30 | 11 | 17 | 173 | 3.32% | 62.90% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (688 aa) | ARB_06334 | 75'021.90 | 19 | 25 | 55 | 1.06% | 34.90% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (771 aa) | ARB_07629 | 85'022.20 | 15 | 17 | 18 | 0.35% | 25.70% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (565 aa) | ARB_02369 | 61'369.70 | 16 | 20 | 64 | 1.23% | 36.90% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (762 aa) | ARB_01232 | 83'795.80 | 20 | 28 | 47 | 0.90% | 37.70% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Abenh-pH7 c | A. benhamiae CBS 112371 null (512 aa) | ARB_07056 | 55'227.80 | 16 | 22 | 29 | 0.56% | 50.10% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (593 aa) | ARB_02965 | 63'994.80 | 17 | 22 | 126 | 2.42% | 36.70% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (196 aa) | ARB_07637 | 20'530.80 | 3 | 3 | 5 | 0.10% | 25.10% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (530 aa) | ARB_03264 | 59'162.30 | 7 | 9 | 13 | 0.25% | 17.40% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (172 aa) | ARB_00344 | 18'201.10 | 4 | 7 | 29 | 0.56% | 36.80% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (238 aa) | ARB_07027 | 26'853.40 | 3 | 7 | 18 | 0.35% | 20.30% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (131 aa) | ARB_07026 | 14'085.60 | 5 | 7 | 29 | 0.56% | 53.10% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (653 aa) | ARB_04046 | 71'688.10 | 4 | 4 | 4 | 0.08% | 9.05% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (501 aa) | ARB_02372 | 54'522.30 | 8 | 9 | 15 | 0.29% | 21.40% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (503 aa) | ARB_05319 | 54'495.60 | 13 | 15 | 24 | 0.46% | 40.60% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (925 aa) | ARB_07867 | 99'074.10 | 14 | 17 | 24 | 0.46% | 23.20% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (206 aa) | ARB_05304 | 21'463.10 | 6 | 8 | 20 | 0.38% | 39.00% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (328 aa) | ARB_06052 | 36'392.00 | 5 | 5 | 10 | 0.19% | 20.80% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (1029 aa) | ARB_02327 | 113'054.40 | 18 | 22 | 39 | 0.75% | 20.80% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (398 aa) | ARB_00701 | 40'996.40 | 8 | 13 | 55 | 1.06% | 43.10% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (374 aa) | ARB_03568 | 40'712.70 | 8 | 10 | 15 | 0.29% | 33.00% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (196 aa) | ARB_00595 | 20'913.60 | 5 | 7 | 17 | 0.33% | 28.70% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (405 aa) | ARB_04859 | 43'686.00 | 8 | 8 | 19 | 0.37% | 29.00% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (354 aa) | ARB_01995 | 38'818.80 | 4 | 4 | 5 | 0.10% | 15.90% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (230 aa) | ARB_03731,ARB_05393 | 25'773.70 | 3 | 3 | 3 | 0.06% | 14.80% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (728 aa) | ARB_02390 | 79'180.00 | 8 | 9 | 13 | 0.25% | 17.70% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (342 aa) | ARB_07185 | 37'342.80 | 2 | 2 | 4 | 0.08% | 13.80% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Abenh-pH7 c | A. benhamiae CBS 112371 null (572 aa) | ARB_02107 | 62'387.40 | 2 | 2 | 2 | 0.04% | 4.20% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (923 aa) | ARB_04703 | 104'542.40 | 5 | 5 | 5 | 0.10% | 5.21% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (453 aa) | ARB_05164 | 50'673.80 | 9 | 12 | 22 | 0.42% | 22.80% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (414 aa) | ARB_04467 | 45'398.30 | 14 | 18 | 40 | 0.77% | 45.80% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (243 aa) | ARB_07186 | 26'586.30 | 5 | 6 | 8 | 0.15% | 23.10% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (187 aa) | ARB_00075 | 19'791.90 | 6 | 7 | 23 | 0.44% | 44.60% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (408 aa) | ARB_01032 | 42'987.80 | 5 | 5 | 5 | 0.10% | 26.80% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (121 aa) | ARB_00658 | 12'788.00 | 2 | 2 | 2 | 0.04% | 21.70% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (699 aa) | ARB_02220 | 77'510.00 | 41 | 52 | 424 | 8.14% | 58.60% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (418 aa) | ARB_05392 | 45'903.20 | 18 | 30 | 209 | 4.01% | 60.40% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (491 aa) | ARB_00777 | 52'317.20 | 1 | 1 | 1 | 0.02% | 2.86% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (538 aa) | ARB_07085 | 57'758.90 | 3 | 3 | 3 | 0.06% | 9.31% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (426 aa) | ARB_02208 | 46'364.90 | 5 | 5 | 8 | 0.15% | 22.80% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (305 aa) | ARB_01071 | 33'382.90 | 2 | 2 | 2 | 0.04% | 8.22% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (566 aa) | ARB_02478 | 61'777.10 | 9 | 11 | 15 | 0.29% | 23.40% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (300 aa) | ARB_00059 | 32'799.20 | 3 | 3 | 4 | 0.08% | 13.00% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (172 aa) | ARB_06080 | 19'252.10 | 1 | 1 | 1 | 0.02% | 5.26% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (441 aa) | ARB_05372 | 47'817.30 | 3 | 3 | 8 | 0.15% | 8.86% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (342 aa) | ARB_03699 | 36'255.50 | 6 | 7 | 16 | 0.31% | 28.40% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (727 aa) | ARB_06651 | 80'067.80 | 6 | 6 | 12 | 0.23% | 14.50% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (513 aa) | ARB_06983 | 55'982.70 | 4 | 4 | 4 | 0.08% | 10.40% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (866 aa) | ARB_02101 | 98'073.30 | 5 | 5 | 6 | 0.12% | 7.75% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Abenh-pH7 c | A. benhamiae CBS 112371 null (224 aa) | ARB_03853 | 24'951.00 | 2 | 2 | 2 | 0.04% | 8.52% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (213 aa) | ARB_00773 | 21'751.00 | 4 | 4 | 4 | 0.08% | 36.80% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (653 aa) | ARB_05859 | 70'106.10 | 5 | 5 | 7 | 0.13% | 11.00% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (634 aa) | ARB_05085 | 69'232.20 | 7 | 10 | 18 | 0.35% | 22.10% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (403 aa) | ARB_03790 | 42'110.30 | 5 | 5 | 6 | 0.12% | 15.70% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (417 aa) | ARB_03789 | 45'801.80 | 4 | 4 | 5 | 0.10% | 14.90% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (415 aa) | ARB_02715 | 45'401.10 | 8 | 9 | 12 | 0.23% | 27.80% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (309 aa) | ARB_02797 | 32'960.20 | 10 | 18 | 56 | 1.07% | 48.10% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (864 aa) | ARB_00226 | 95'625.40 | 3 | 3 | 3 | 0.06% | 3.36% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (207 aa) | ARB_04555 | 21'190.20 | 2 | 3 | 4 | 0.08% | 14.10% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (1066 aa) | ARB_06053 | 116'804.20 | 5 | 6 | 8 | 0.15% | 7.98% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (149 aa) | ARB_02001 | 16'452.90 | 4 | 5 | 13 | 0.25% | 23.60% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (574 aa) | ARB_01962 | 62'410.60 | 4 | 4 | 4 | 0.08% | 12.90% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (425 aa) | ARB_01495 | 45'947.10 | 4 | 4 | 8 | 0.15% | 12.70% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (413 aa) | ARB_01347 | 44'166.80 | 3 | 3 | 3 | 0.06% | 8.25% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (140 aa) | ARB_06975 | 14'354.00 | 2 | 3 | 11 | 0.21% | 20.10% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (319 aa) | ARB_02306 | 36'287.30 | 2 | 2 | 2 | 0.04% | 5.35% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (336 aa) | ARB_03491 | 37'530.20 | 2 | 2 | 2 | 0.04% | 6.87% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (505 aa) | ARB_06076 | 53'188.50 | 7 | 13 | 69 | 1.32% | 38.10% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (298 aa) | ARB_07540 | 32'564.70 | 2 | 2 | 2 | 0.04% | 14.10% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (168 aa) | ARB_03651 | 18'697.30 | 3 | 3 | 3 | 0.06% | 23.40% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (257 aa) | ARB_03024 | 25'239.30 | 3 | 4 | 10 | 0.19% | 17.20% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Abenh-pH7 c | A. benhamiae CBS 112371 null (617 aa) | ARB_01353 | 70'350.90 | 22 | 30 | 81 | 1.55% | 41.70% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (214 aa) | ARB_05177 | 22'933.00 | 3 | 3 | 3 | 0.06% | 20.20% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (557 aa) | ARB_01941 | 60'870.20 | 6 | 6 | 7 | 0.13% | 14.60% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (226 aa) | ARB_01979 | 25'408.90 | 7 | 8 | 11 | 0.21% | 32.00% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (395 aa) | ARB_00047 | 44'081.90 | 2 | 3 | 3 | 0.06% | 7.36% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (413 aa) | ARB_05307 | 42'717.30 | 2 | 2 | 3 | 0.06% | 7.77% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (510 aa) | ARB_00035 | 56'275.40 | 20 | 25 | 56 | 1.07% | 51.70% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (779 aa) | ARB_06110 | 88'383.60 | 24 | 34 | 70 | 1.34% | 41.80% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (496 aa) | ARB_00494 | 53'225.40 | 27 | 46 | 536 | 10.30% | 61.20% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (1091 aa) | ARB_00520 | 123'628.70 | 31 | 36 | 81 | 1.55% | 33.20% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (513 aa) | ARB_04696 | 55'514.70 | 6 | 6 | 6 | 0.12% | 20.50% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (459 aa) | ARB_05157 | 49'026.40 | 1 | 1 | 1 | 0.02% | 1.75% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (250 aa) | ARB_04619 | 27'122.70 | 2 | 3 | 5 | 0.10% | 14.10% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (267 aa) | ARB_00862 | 28'612.50 | 9 | 14 | 112 | 2.15% | 39.80% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (292 aa) | ARB_03620 | 32'033.60 | 3 | 3 | 3 | 0.06% | 14.40% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (354 aa) | ARB_00204 | 38'505.10 | 8 | 10 | 22 | 0.42% | 21.20% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (630 aa) | ARB_06361 | 69'690.80 | 3 | 3 | 3 | 0.06% | 6.36% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (527 aa) | ARB_02407 | 58'544.90 | 10 | 10 | 10 | 0.19% | 22.60% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (383 aa) | ARB_08028 | 41'339.10 | 1 | 1 | 1 | 0.02% | 3.40% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (306 aa) | ARB_04618 | 33'949.20 | 2 | 2 | 2 | 0.04% | 8.85% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (388 aa) | ARB_01751 | 42'248.00 | 2 | 2 | 2 | 0.04% | 7.75% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (134 aa) | ARB_05536 | 15'101.60 | 3 | 5 | 7 | 0.13% | 29.30% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Abenh-pH7 c | A. benhamiae CBS 112371 null (179 aa) | ARB_07191 | 19'788.40 | 3 | 3 | 3 | 0.06% | 19.10% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (388 aa) | ARB_01627 | 41'241.10 | 7 | 8 | 29 | 0.56% | 28.70% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (644 aa) | ARB_00762 | 70'670.60 | 15 | 18 | 70 | 1.34% | 35.50% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (756 aa) | ARB_05732 | 81'168.90 | 4 | 5 | 8 | 0.15% | 9.80% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (242 aa) | ARB_04889 | 26'743.60 | 4 | 5 | 7 | 0.13% | 19.90% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (208 aa) | ARB_06407 | 23'067.90 | 3 | 3 | 3 | 0.06% | 20.30% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (401 aa) | ARB_06111 | 41'702.10 | 7 | 8 | 19 | 0.37% | 24.00% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (879 aa) | ARB_02077 | 93'825.10 | 13 | 20 | 51 | 0.98% | 25.70% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (596 aa) | ARB_00930 | 65'104.30 | 2 | 2 | 2 | 0.04% | 4.54% |
| Abenh-pH7 c | A. benhamiae CBS 112371 null (315 aa) | ARB_06966 | 34'730.00 | 4 | 6 | 9 | 0.17% | 17.80% |

**Supplementary Table 2a: Shotgun MS analysis of *M. canis* grown in SP medium at pH 4.0 and pH 7.0.**

| Identified Proteins (196) | Accession Number | Molecular Weight | Mcanis-pH4-a | Mcanis-pH4-b | Mcanis-pH4-c | Mcanis-pH7-a | Mcanis-pH7-b | Mcanis-pH7-c |
|---|---|---|---|---|---|---|---|---|
| Tripeptidyl-peptidase SedB | SED2_NANOT | 65 kDa | 157 | 221 | 247 | 2 | 0 | 19 |
| Probable neutral protease 2 homolog MCYG_07155 | NPIID_NANOT | 38 kDa | 201 | 61 | 152 | 12 | 12 | 9 |
| Putative uncharacterized protein MCYG_07328 | C5FYB1_NANOT | 21 kDa | 168 | 1 | 148 | 1 | 12 | 5 |
| Tripeptidyl-peptidase SedD | SED4_NANOT | 66 kDa | 153 | 128 | 113 | 4 | 7 | 15 |
| Subtilisin-like protease 3 Sub3 | SUB3_MICCA | 41 kDa | 59 | 6 | 100 | 169 | 59 | 202 |
| Aspartic endopeptidase Pep1 | PEPF_NANOT | 41 kDa | 206 | 19 | 97 | 6 | 0 | 0 |
| Peptidase S41 MCYG_03610 | C5FM69_NANOT | 77 kDa | 44 | 53 | 94 | 5 | 13 | 26 |
| Beta-glucosidase 4 MCYG_01527 | C5FHG8_NANOT | 33 kDa | 99 | 92 | 90 | 29 | 40 | 93 |
| Metallocarboxypeptidase A-like protein MCYG_01475 | MCPAL_NANOT | 46 kDa | 117 | 6 | 84 | 33 | 4 | 22 |
| Sulphydryl oxidase Sox MCYG_02499 | C5FFZ5_NANOT | 44 kDa | 28 | 19 | 81 | 4 | 5 | 13 |
| Polysaccharide deacetylase family protein MCYG_07538 | C5FYX1_NANOT | 33 kDa | 41 | 3 | 73 | 78 | 18 | 142 |
| FAD binding domain-containing protein MCYG_04968 | C5FQJ6_NANOT | 55 kDa | 42 | 12 | 68 | 75 | 50 | 95 |
| Putative uncharacterized protein MCYG_05599 | C5FSC7_NANOT | 21 kDa | 55 | 71 | 63 | 9 | 1 | 7 |
| Dipeptidyl-peptidase 5 DPPV | DPP5_NANOT | 80 kDa | 52 | 0 | 61 | 0 | 11 | 1 |
| Putative uncharacterized protein MCYG_03713 | C5FJN3_NANOT | 26 kDa | 43 | 59 | 60 | 0 | 8 | 4 |
| Carboxypeptidase cpdS MCYG_07753 | C5FX94_NANOT | 58 kDa | 36 | 36 | 56 | 0 | 2 | 1 |
| Laccase-1 MCYG_08571 | C5G0U9_NANOT | 65 kDa | 44 | 31 | 56 | 0 | 0 | 0 |
| Putative uncharacterized protein MCYG_02207 | C5FJ58_NANOT | 61 kDa | 28 | 0 | 55 | 0 | 0 | 0 |
| 1,2-alpha-D-mannosidase MCYG_00423 | C5FCK1_NANOT | 56 kDa | 38 | 13 | 54 | 15 | 7 | 50 |
| Glucoamylase MCYG_06367 | C5FUG4_NANOT | 68 kDa | 35 | 0 | 53 | 388 | 174 | 662 |
| Extracellular metalloproteinase 3 Mep3 | MEP3_MICCA | 69 kDa | 22 | 2 | 50 | 38 | 33 | 120 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Carboxypeptidase S1 homolog A SCPA | SPCA_NANOT | 72 kDa | 33 | 27 | 47 | 0 | 4 | 1 |
| Peptidase M28 MCYG_04217 | C5FP82_NANOT | 56 kDa | 19 | 16 | 44 | 0 | 0 | 5 |
| Extracellular cell wall glucanase Crf1/allergen Asp F9 MCYG_04874 | C5FQA2_NANOT | 45 kDa | 56 | 18 | 40 | 0 | 0 | 0 |
| Metallocarboxypeptidase A MCPA | MCPA_NANOT | 47 kDa | 44 | 13 | 39 | 8 | 1 | 10 |
| Extracellular metalloproteinase 5 Mep5 | MEP5_MICCA | 70 kDa | 21 | 0 | 34 | 0 | 0 | 1 |
| Ecm33 MCYG_01225 | C5FEL3_NANOT | 41 kDa | 35 | 8 | 32 | 13 | 7 | 11 |
| Ser/Thr protein phosphatase MCYG_06525 | C5FUX2_NANOT | 72 kDa | 41 | 14 | 32 | 0 | 0 | 0 |
| Carbohydrate-binding protein MCYG_01090 | C5FEG8_NANOT | 42 kDa | 10 | 6 | 30 | 5 | 0 | 66 |
| IgE-binding protein MCYG_03081 | C5FKP0_NANOT | 20 kDa | 43 | 44 | 29 | 11 | 8 | 7 |
| Oxalate decarboxylase oxdD MCYG_04379 | C5FNE6_NANOT | 44 kDa | 17 | 0 | 29 | 19 | 16 | 21 |
| Putative uncharacterized protein MCYG_08572 | C5G0V0_NANOT | 33 kDa | 18 | 2 | 27 | 0 | 0 | 1 |
| Gamma-glutamyltranspeptidase MCYG_05524 | C5FS52_NANOT | 63 kDa | 5 | 0 | 25 | 3 | 18 | 4 |
| FAD binding domain-containing protein MCYG_06815 | C5FVR2_NANOT | 63 kDa | 11 | 12 | 22 | 1 | 0 | 6 |
| Aspartic protease MCYG_05372 | C5FRQ0_NANOT | 50 kDa | 12 | 5 | 21 | 0 | 0 | 0 |
| 6-hydroxy-D-nicotine oxidase MCYG_04909 | C5FQD7_NANOT | 56 kDa | 10 | 15 | 20 | 0 | 0 | 0 |
| 6-hydroxy-D-nicotine oxidase MCYG_04127 | C5FN72_NANOT | 63 kDa | 11 | 0 | 19 | 43 | 33 | 82 |
| Acid phosphatase MCYG_07729 | C5FX70_NANOT | 31 kDa | 12 | 4 | 19 | 0 | 0 | 0 |
| Beta-1,3-glucanosyltransferase 3 MCYG_03246 | C5FL55_NANOT | 57 kDa | 29 | 0 | 18 | 6 | 2 | 4 |
| DUF1237 domain-containing protein MCYG_03876 | C5FMF4_NANOT | 61 kDa | 4 | 3 | 18 | 9 | 1 | 13 |
| Phytase MCYG_02670 | C5FGG6_NANOT | 51 kDa | 19 | 0 | 17 | 0 | 0 | 0 |
| Mucin MCYG_07854 | C5FXJ5_NANOT | 77 kDa | 5 | 0 | 16 | 78 | 62 | 150 |
| Putative uncharacterized protein MCYG_00366 | C5FC55_NANOT | 62 kDa | 11 | 0 | 16 | 5 | 20 | 27 |
| Phosphoglycerate mutase family protein MCYG_08379 | C5G0A7_NANOT | 37 kDa | 7 | 0 | 15 | 9 | 8 | 11 |
| Carboxypeptidase Y MCYG_05486 | C5FS14_NANOT | 66 kDa | 16 | 13 | 15 | 1 | 0 | 0 |
| Cell wall protein PhiA MCYG_00375 | C5FC64_NANOT | 20 kDa | 9 | 19 | 14 | 0 | 0 | 9 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Exo-beta-1,3-glucanase Exg0 MCYG_06600 | C5FV47_NANOT | 34 kDa | 8 | 7 | 14 | 10 | 1 | 17 |
| Beta-glucosidase 1 MCYG_00928 | C5FE06_NANOT | 94 kDa | 0 | 0 | 13 | 40 | 25 | 75 |
| Lysophospholipase MCYG_02652 | C5FGE8_NANOT | 68 kDa | 9 | 4 | 13 | 3 | 7 | 3 |
| N,O-diacetylmuramidase MCYG_02645 | C5FGE1_NANOT | 25 kDa | 9 | 4 | 13 | 0 | 0 | 0 |
| Amidase MCYG_00475 | C5FCQ3_NANOT | 62 kDa | 0 | 0 | 12 | 81 | 79 | 211 |
| Putative uncharacterized protein MCYG_04152 | C5FN97_NANOT | 21 kDa | 12 | 0 | 12 | 9 | 4 | 3 |
| Carboxylesterase family protein MCYG_00869 | C5FDU7_NANOT | 62 kDa | 22 | 0 | 12 | 0 | 0 | 2 |
| Glycosyl hydrolase MCYG_03093 | C5FKQ2_NANOT | 88 kDa | 7 | 8 | 12 | 0 | 4 | 2 |
| Carboxypeptidase S1 homolog B SCPB | SCPB_NANOT | 73 kDa | 15 | 2 | 12 | 0 | 0 | 0 |
| Putative uncharacterized protein MCYG_00562 | C5FCZ0_NANOT | 26 kDa | 13 | 0 | 12 | 0 | 0 | 0 |
| Carboxylesterase MCYG_01781 | C5FHY2_NANOT | 55 kDa | 8 | 0 | 12 | 0 | 0 | 0 |
| Carboxy-cis,cis-muconate cyclase MCYG_00595 | C5FD23_NANOT | 42 kDa | 0 | 0 | 12 | 0 | 0 | 0 |
| Glutaminase GtaA MCYG_00814 | C5FDF2_NANOT | 95 kDa | 0 | 0 | 11 | 9 | 4 | 23 |
| 1,3(4)-beta-glucanase MCYG_05925 | C5FTA3_NANOT | 49 kDa | 18 | 5 | 11 | 0 | 0 | 0 |
| Ribonuclease T2-like protein MCYG_02204 | C5FJ55_NANOT | 42 kDa | 5 | 6 | 10 | 0 | 1 | 3 |
| Putative uncharacterized protein MCYG_06727 | C5FVH4_NANOT | 37 kDa | 1 | 0 | 9 | 0 | 0 | 2 |
| Extracellular metalloproteinase 1 Mep1 | MEP1_MICCA | 70 kDa | 4 | 0 | 8 | 0 | 0 | 3 |
| Glycosyl hydrolase family 3 N terminal domain-containing protein MCYG_07820 | C5FXG1_NANOT | 40 kDa | 1 | 4 | 7 | 2 | 0 | 1 |
| Acid trehalase MCYG_02758 | C5FGQ4_NANOT | 115 kDa | 3 | 0 | 7 | 0 | 0 | 0 |
| GDSL Lipase/Acylhydrolase family protein MCYG_04422 | C5FNI9_NANOT | 28 kDa | 4 | 0 | 7 | 0 | 0 | 0 |
| Beta-hexosaminidase MCYG_02200 | C5FJ51_NANOT | 68 kDa | 0 | 0 | 6 | 16 | 9 | 17 |
| Putative uncharacterized protein MCYG_03189 | C5FKZ8_NANOT | 47 kDa | 7 | 0 | 6 | 0 | 0 | 8 |
| Lcc2 MCYG_02578 | C5FG74_NANOT | 74 kDa | 8 | 3 | 6 | 0 | 0 | 0 |
| Alkaline proteinase MCYG_01476 | C5FH27_NANOT | 42 kDa | 1 | 0 | 6 | 0 | 0 | 7 |
| Putative uncharacterized protein MCYG_03822 | C5FMA0_NANOT | 56 kDa | 2 | 0 | 6 | 0 | 0 | 0 |
| Dipeptidyl peptidase 4 DPPIV | A0S5V9_MICCA | 88 kDa | 0 | 0 | 5 | 54 | 39 | 90 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Phosphatidylglycerol/phosphatidylinositol transfer protein MCYG_02226 | C5FFG0_NANOT | 19 kDa | 6 | 13 | 5 | 46 | 27 | 31 |
| Putative uncharacterized protein MCYG_04951 | C5FQH9_NANOT | 15 kDa | 0 | 24 | 5 | 0 | 3 | 11 |
| Putative uncharacterized protein MCYG_03515 | C5FLX4_NANOT | 23 kDa | 0 | 2 | 5 | 3 | 5 | 14 |
| Predicted protein MCYG_01508 | C5FHE9_NANOT | 19 kDa | 4 | 2 | 5 | 0 | 0 | 2 |
| Probable leucine aminopeptidase MCYG_04170 | LAP5_NANOT | 38 kDa | 8 | 0 | 5 | 0 | 0 | 0 |
| Putative uncharacterized protein MCYG_04944 | C5FQH2_NANOT | 19 kDa | 2 | 4 | 5 | 0 | 0 | 0 |
| Tripeptidyl-peptidase SedC | SED3_NANOT | 65 kDa | 0 | 0 | 5 | 0 | 0 | 0 |
| ThiJ/PfpI family protein MCYG_02762 | C5FGQ8_NANOT | 25 kDa | 0 | 0 | 5 | 0 | 0 | 0 |
| Alpha-glucosidase MCYG_00092 | C5FBM0_NANOT | 101 kDa | 4 | 0 | 4 | 9 | 14 | 17 |
| Probable neutral protease 2 homolog MCYG_05201 | NPIIA_NANOT | 40 kDa | 12 | 3 | 4 | 6 | 8 | 2 |
| Glycerophosphoryl diester phosphodiesterase family protein MCYG_05364 | C5FRP2_NANOT | 45 kDa | 1 | 0 | 4 | 3 | 3 | 11 |
| FAD binding domain-containing protein MCYG_08167 | C5FZP5_NANOT | 54 kDa | 1 | 0 | 4 | 0 | 0 | 3 |
| Feruloyl esterase B MCYG_06844 | C5FVU1_NANOT | 58 kDa | 3 | 0 | 4 | 0 | 0 | 0 |
| 2,6-dihydropseudooxynicotine hydrolase MCYG_02065 | C5FIH7_NANOT | 49 kDa | 2 | 0 | 4 | 0 | 0 | 0 |
| Copper radical oxidase MCYG_08682 | C5G160_NANOT | 96 kDa | 4 | 1 | 3 | 56 | 32 | 92 |
| Proteinase inhibitor I4 MCYG_03085 | C5FKP4_NANOT | 40 kDa | 0 | 0 | 3 | 1 | 3 | 13 |
| Alkaline serine protease MCYG_02748 | C5FGP4_NANOT | 41 kDa | 2 | 0 | 3 | 0 | 8 | 2 |
| FAD binding domain-containing protein MCYG_07459 | C5FYP2_NANOT | 54 kDa | 0 | 0 | 3 | 1 | 0 | 2 |
| Putative uncharacterized protein MCYG_01908 | C5FIA9_NANOT | 97 kDa | 0 | 0 | 3 | 0 | 0 | 2 |
| Endo-beta-1,3-glucanase MCYG_06694 | C5FVE1_NANOT | 74 kDa | 1 | 0 | 3 | 0 | 0 | 0 |
| Putative uncharacterized protein MCYG_05202 | C5FR80_NANOT | 19 kDa | 1 | 0 | 3 | 0 | 0 | 0 |
| Aminopeptidase Y Lap1 | LAP1_NANOT | 53 kDa | 0 | 0 | 2 | 156 | 301 | 798 |
| Leucine aminopeptidase 2 Lap2 | LAP2_NANOT | 41 kDa | 1 | 1 | 2 | 10 | 24 | 26 |
| Putative uncharacterized protein MCYG_00150 | C5FBS8_NANOT | 19 kDa | 4 | 0 | 2 | 20 | 11 | 18 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Putative uncharacterized protein MCYG_06096 | C5FTS4_NANOT | 57 kDa | 0 | 0 | 2 | 15 | 6 | 24 |
| Subtilisin-like protease 7 Sub7 | SUB7_NANOT | 41 kDa | 2 | 0 | 2 | 21 | 6 | 19 |
| Putative uncharacterized protein MCYG_03637 | C5FM96_NANOT | 25 kDa | 9 | 2 | 2 | 4 | 0 | 0 |
| Oxalate decarboxylase MCYG_02705 | C5FGK1_NANOT | 44 kDa | 2 | 0 | 2 | 3 | 5 | 5 |
| ATP-dependent permease MCYG_02170 | C5FJ21_NANOT | 107 kDa | 0 | 0 | 2 | 3 | 1 | 8 |
| LysM domain-containing protein MCYG_08445 | C5G0H3_NANOT | 42 kDa | 6 | 0 | 2 | 3 | 0 | 1 |
| Extracellular matrix protein MCYG_07613 | C5FWV4_NANOT | 23 kDa | 6 | 2 | 2 | 0 | 0 | 0 |
| Glucan 1,3-beta-glucosidase MCYG_05874 | C5FT52_NANOT | 46 kDa | 0 | 0 | 2 | 0 | 7 | 1 |
| Aldose 1-epimerase MCYG_00654 | C5FD82_NANOT | 46 kDa | 0 | 1 | 2 | 0 | 2 | 2 |
| LysM domain-containing protein MCYG_04103 | C5FN48_NANOT | 48 kDa | 4 | 0 | 2 | 0 | 0 | 0 |
| Predicted protein MCYG_04621 | C5FNU9_NANOT | 36 kDa | 0 | 0 | 2 | 0 | 0 | 0 |
| Endochitinase 1 MCYG_00676 | C5FDA4_NANOT | 44 kDa | 0 | 0 | 1 | 34 | 14 | 75 |
| Predicted protein MCYG_04553 | C5FNN1_NANOT | 28 kDa | 0 | 0 | 1 | 17 | 16 | 52 |
| Glutamate carboxypeptidase 2 MCYG_07431 | C5FYL4_NANOT | 78 kDa | 0 | 0 | 1 | 22 | 12 | 39 |
| Putative uncharacterized protein MCYG_08685 | C5G163_NANOT | 79 kDa | 1 | 0 | 1 | 2 | 3 | 5 |
| Putative uncharacterized protein MCYG_04701 | C5FP29_NANOT | 27 kDa | 6 | 0 | 1 | 0 | 0 | 0 |
| Secreted protein MCYG_06024 | C5FTK2_NANOT | 22 kDa | 0 | 0 | 1 | 1 | 0 | 2 |
| HMF1 MCYG_06048 | C5FTM6_NANOT | 13 kDa | 0 | 2 | 1 | 0 | 0 | 0 |
| Endonuclease/exonuclease/phosphatase family protein MCYG_03887 | C5FMG5_NANOT | 33 kDa | 0 | 0 | 1 | 0 | 0 | 0 |
| Alkaline phosphatase MCYG_04625 | C5FNV3_NANOT | 56 kDa | 0 | 0 | 0 | 358 | 64 | 377 |
| Carboxypeptidase 2 MCPB | MCPB_NANOT | 60 kDa | 0 | 0 | 0 | 64 | 51 | 127 |
| Extracellular metalloproteinase 4 Mep4 | MEP4_MICCA | 71 kDa | 0 | 0 | 0 | 37 | 45 | 148 |
| Alpha-mannosidase MCYG_06176 | C5FTX3_NANOT | 123 kDa | 0 | 0 | 0 | 72 | 13 | 102 |
| Hydrophobic surface binding protein B MCYG_01969 | C5FIQ9_NANOT | 19 kDa | 0 | 2 | 0 | 17 | 27 | 64 |
| Neutral ceramidase MCYG_06637 | C5FV84_NANOT | 83 kDa | 0 | 0 | 0 | 25 | 18 | 67 |
| Fumarylacetoacetase MCYG_00527 | C5FCV5_NANOT | 47 kDa | 0 | 0 | 0 | 27 | 21 | 39 |
| Dipeptidase 1 MCYG_02918 | C5FK77_NANOT | 47 kDa | 2 | 0 | 0 | 15 | 21 | 38 |
| LysM domain-containing protein MCYG_04646 | C5FNX4_NANOT | 28 kDa | 0 | 0 | 0 | 29 | 15 | 20 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Nucleoside diphosphate kinase MCYG_01839 | C5FI40_NANOT | 17 kDa | 0 | 0 | 0 | 14 | 22 | 15 |
| Secreted protein MCYG_00341 | C5FC30_NANOT | 23 kDa | 0 | 0 | 0 | 27 | 6 | 15 |
| Putative uncharacterized protein MCYG_07164 | C5FXU7_NANOT | 49 kDa | 0 | 0 | 0 | 10 | 2 | 26 |
| Multicopper oxidase MCYG_01191 | C5FF09_NANOT | 75 kDa | 0 | 0 | 0 | 7 | 7 | 25 |
| Putative uncharacterized protein MCYG_01574 | C5FHL5_NANOT | 28 kDa | 0 | 0 | 0 | 20 | 5 | 13 |
| Arabinan endo-1,5-alpha-L-arabinosidase A MCYG_02777 | C5FGS3_NANOT | 37 kDa | 0 | 0 | 0 | 8 | 9 | 15 |
| Aspartylglucosaminidase family protein MCYG_04957 | C5FQI5_NANOT | 45 kDa | 0 | 0 | 0 | 6 | 7 | 15 |
| Aconitase MCYG_03299 | C5FLA8_NANOT | 84 kDa | 0 | 0 | 0 | 4 | 16 | 3 |
| Cutinase MCYG_05629 | C5FSF7_NANOT | 28 kDa | 0 | 0 | 0 | 7 | 4 | 12 |
| Putative uncharacterized protein MCYG_04556 | C5FNN4_NANOT | 50 kDa | 0 | 0 | 0 | 4 | 7 | 11 |
| Putative uncharacterized protein MCYG_07025 | C5FWC2_NANOT | 32 kDa | 0 | 0 | 0 | 3 | 2 | 18 |
| 4-hydroxyphenylpyruvate dioxygenase MCYG_00524 | C5FCV2_NANOT | 45 kDa | 0 | 0 | 0 | 8 | 7 | 7 |
| Beta-mannosidase MCYG_06102 | C5FTT0_NANOT | 98 kDa | 0 | 0 | 0 | 3 | 5 | 14 |
| G-protein complex beta subunit CpcB MCYG_01069 | C5FEE7_NANOT | 35 kDa | 0 | 0 | 0 | 9 | 5 | 10 |
| Exochitinase 1 MCYG_05745 | C5FSS3_NANOT | 53 kDa | 0 | 0 | 0 | 3 | 4 | 13 |
| Putative uncharacterized protein MCYG_07611 | C5FWV2_NANOT | 29 kDa | 0 | 0 | 0 | 1 | 8 | 12 |
| Superoxide dismutase [Cu-Zn] MCYG_02077 | C5FII9_NANOT | 16 kDa | 0 | 0 | 0 | 18 | 1 | 3 |
| Polysaccharide deacetylase family protein MCYG_04253 | C5FPB8_NANOT | 30 kDa | 0 | 0 | 0 | 1 | 4 | 14 |
| Putative uncharacterized protein MCYG_02350 | C5FJB0_NANOT | 16 kDa | 0 | 0 | 0 | 0 | 4 | 10 |
| HypA MCYG_01872 | C5FI73_NANOT | 51 kDa | 0 | 0 | 0 | 6 | 4 | 7 |
| Superoxide dismutase MCYG_06348 | C5FUE5_NANOT | 25 kDa | 0 | 0 | 0 | 9 | 2 | 7 |
| Fumarate reductase flavoprotein subunit MCYG_02604 | C5FGA0_NANOT | 68 kDa | 0 | 0 | 0 | 6 | 5 | 5 |
| Class II aldolase/adducin domain-containing protein MCYG_00391 | C5FC80_NANOT | 32 kDa | 0 | 0 | 0 | 3 | 5 | 7 |
| Uricase MCYG_00197 | C5FBX5_NANOT | 34 kDa | 0 | 0 | 0 | 2 | 7 | 3 |
| Putative uncharacterized protein MCYG_07766 | C5FXA7_NANOT | 24 kDa | 0 | 0 | 0 | 5 | 3 | 7 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Putative uncharacterized protein MCYG_03989 | C5FMR7_NANOT | 46 kDa | 0 | 0 | 0 | 5 | 3 | 7 |
| Glutathione S-transferase Gst3 MCYG_07941 | C5FXT2_NANOT | 41 kDa | 0 | 0 | 0 | 5 | 1 | 5 |
| Aminopeptidase P MCYG_01718 | C5FHR9_NANOT | 69 kDa | 0 | 0 | 0 | 4 | 4 | 4 |
| GH17932p MCYG_04430 | C5FNJ7_NANOT | 23 kDa | 0 | 0 | 0 | 5 | 4 | 3 |
| Amidohydrolase MCYG_03185 | C5FKZ4_NANOT | 33 kDa | 0 | 0 | 0 | 1 | 4 | 7 |
| Proline iminopeptidase MCYG_04539 | C5FNL7_NANOT | 51 kDa | 0 | 0 | 0 | 1 | 2 | 9 |
| Dienelactone hydrolase family protein MCYG_02976 | C5FKD5_NANOT | 32 kDa | 0 | 0 | 0 | 3 | 3 | 1 |
| Cyanate hydratase MCYG_03856 | C5FMD4_NANOT | 18 kDa | 0 | 0 | 0 | 4 | 1 | 5 |
| Dihydroorotase MCYG_02534 | C5FG30_NANOT | 39 kDa | 0 | 0 | 0 | 1 | 1 | 8 |
| Ser/Thr protein phosphatase MCYG_02775 | C5FGS1_NANOT | 68 kDa | 0 | 0 | 0 | 1 | 1 | 7 |
| Alkaline phosphatase MCYG_05903 | C5FT81_NANOT | 66 kDa | 0 | 0 | 0 | 1 | 0 | 7 |
| PBSP domain-containing protein MCYG_01682 | C5FHE4_NANOT | 33 kDa | 0 | 0 | 0 | 2 | 3 | 2 |
| Dihydrolipoamide dehydrogenase MCYG_08327 | C5G055_NANOT | 46 kDa | 0 | 0 | 0 | 4 | 4 | 0 |
| Beta-glucosidase MCYG_00545 | C5FCX3_NANOT | 135 kDa | 0 | 0 | 0 | 0 | 3 | 6 |
| Chitinase MCYG_04644 | C5FNX2_NANOT | 159 kDa | 0 | 0 | 0 | 0 | 4 | 6 |
| Citrate synthase MCYG_03094 | C5FKQ3_NANOT | 44 kDa | 0 | 0 | 0 | 4 | 0 | 3 |
| Predicted protein MCYG_08251 | C5FZX9_NANOT | 18 kDa | 0 | 0 | 0 | 2 | 2 | 3 |
| Secretory phospholipase A2 MCYG_00583 | C5FD11_NANOT | 17 kDa | 0 | 0 | 0 | 3 | 2 | 4 |
| LysM domain-containing protein MCYG_02074 | C5FII6_NANOT | 22 kDa | 4 | 0 | 0 | 5 | 0 | 0 |
| Peptidase S8 and S53 MCYG_01699 | C5FHQ0_NANOT | 75 kDa | 0 | 0 | 0 | 1 | 0 | 7 |
| Alanine-glyoxylate aminotransferase MCYG_08492 | C5G0M0_NANOT | 41 kDa | 0 | 0 | 0 | 2 | 1 | 3 |
| Thimet oligopeptidase MCYG_02264 | C5FFJ8_NANOT | 82 kDa | 0 | 0 | 0 | 2 | 0 | 3 |
| Chitinase 4 MCYG_08403 | C5G0D1_NANOT | 39 kDa | 0 | 0 | 0 | 3 | 0 | 4 |
| Chitinase 3 MCYG_07133 | C5FWN0_NANOT | 24 kDa | 2 | 1 | 0 | 1 | 0 | 2 |
| Aspartyl aminopeptidase MCYG_06746 | C5FVJ3_NANOT | 74 kDa | 0 | 0 | 0 | 3 | 0 | 4 |
| Putative uncharacterized protein MCYG_08076 | C5FZF4_NANOT | 34 kDa | 0 | 0 | 0 | 1 | 0 | 3 |
| Glyoxalase family protein MCYG_00690 | C5FDK6_NANOT | 17 kDa | 0 | 0 | 0 | 0 | 0 | 6 |
| Serine proteinase MCYG_02070 | C5FII2_NANOT | 52 kDa | 0 | 0 | 0 | 0 | 1 | 3 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Actin MCYG_05457 | C5FRY5_NANOT | 42 kDa | 4 | 0 | 0 | 0 | 0 | 0 |
| Subtilisin-like protease 4 Sub4 | SUB4_NANOT | 42 kDa | 0 | 0 | 0 | 0 | 2 | 1 |
| Phosphoserine aminotransferase MCYG_05388 | C5FRR6_NANOT | 47 kDa | 0 | 0 | 0 | 0 | 0 | 4 |
| Phosphoribosylglycinamide formyltransferase MCYG_05959 | C5FTD7_NANOT | 24 kDa | 0 | 0 | 0 | 1 | 1 | 2 |
| Glycoprotein X MCYG_08739 | C5G1B7_NANOT | 98 kDa | 0 | 0 | 0 | 3 | 0 | 1 |
| Putative uncharacterized protein MCYG_06389 | C5FUI6_NANOT | 38 kDa | 0 | 0 | 0 | 0 | 2 | 1 |
| Stress responsive A/B barrel domain-containing protein MCYG_02249 | C5FFI3_NANOT | 12 kDa | 0 | 0 | 0 | 0 | 2 | 1 |
| Extracellular serine-threonine rich protein MCYG_04810 | C5FQ38_NANOT | 93 kDa | 0 | 0 | 0 | 4 | 0 | 0 |
| Esterase MCYG_01130 | C5FEU8_NANOT | 60 kDa | 0 | 0 | 0 | 0 | 4 | 0 |
| N-carbamoyl-L-amino acid hydrolase MCYG_03207 | C5FL16_NANOT | 50 kDa | 0 | 0 | 0 | 2 | 0 | 1 |
| Anthranilate synthase multifunctional enzyme MCYG_01660 | C5FHC2_NANOT | 82 kDa | 0 | 0 | 0 | 0 | 1 | 1 |
| Chitinase MCYG_04741 | C5FPW9_NANOT | 130 kDa | 0 | 0 | 0 | 0 | 3 | 0 |
| Cysteinyl-tRNA synthetase MCYG_01772 | C5FHX3_NANOT | 90 kDa | 0 | 0 | 0 | 0 | 0 | 2 |
| Peptidyl-prolyl cis-trans isomerase D MCYG_08316 | C5G044_NANOT | 38 kDa | 0 | 0 | 0 | 0 | 1 | 0 |
| Lactoylglutathione lyase MCYG_08655 | C5G133_NANOT | 36 kDa | 0 | 0 | 0 | 1 | 0 | 0 |
| Peroxisomal matrix protein MCYG_00580 | C5FD08_NANOT | 18 kDa | 0 | 0 | 0 | 0 | 1 | 0 |
| Putative uncharacterized protein MCYG_07934 | C5FXS5_NANOT | 34 kDa | 0 | 0 | 0 | 0 | 0 | 1 |
| Calnexin MCYG_01115 | C5FEJ3_NANOT | 62 kDa | 0 | 0 | 0 | 0 | 1 | 0 |
| Putative uncharacterized protein MCYG_01528 | C5FHG9_NANOT | 251 kDa | 0 | 0 | 0 | 1 | 0 | 0 |

**Supplementary Table 2b: Shotgun MS analysis of *A. benhamiae* grown in SP medium at pH 4.0 and pH 7.0.**

| Identified Proteins (175) | Accession Number | Molecular Weight | pH4 Abenh-a | pH4 Abenh-b | pH4 Abenh-c | pH7 Abenh-a | pH7 Abenh-b | pH7 Abenh-c | Predicted fonction |
|---|---|---|---|---|---|---|---|---|---|
| A. benhamiae CBS 112371 null (727 aa) | ARB_06651 | 80 kDa | 277 | 645 | 410 | 0 | 91 | 12 | DPP V |
| A. benhamiae CBS 112371 null (172 aa) | ARB_00344 | 18 kDa | 263 | 218 | 193 | 71 | 106 | 29 | GPI anchored cell wall protein |
| A. benhamiae CBS 112371 null (404 aa) | ARB_05728 | 43 kDa | 184 | 273 | 94 | 0 | 5 | 0 | Pep1 |
| A. benhamiae CBS 112371 null (699 aa) | ARB_02220 | 78 kDa | 177 | 384 | 108 | 668 | 358 | 424 | peptidase S41 protein family |
| A. benhamiae CBS 112371 null (398 aa) | ARB_00701 | 41 kDa | 119 | 178 | 33 | 148 | 41 | 55 | subtilisin-like protease SUB3 |
| A. benhamiae CBS 112371 null (309 aa) | ARB_02797 | 33 kDa | 101 | 169 | 89 | 157 | 34 | 56 | GPI-anchored cell wall beta-1,3-endoglucanase EglC |
| A. benhamiae CBS 112371 null (634 aa) | ARB_05085 | 69 kDa | 96 | 142 | 61 | 20 | 22 | 18 | metalloprotease |
| A. benhamiae CBS 112371 null (187 aa) | ARB_00075 | 20 kDa | 92 | 112 | 180 | 19 | 19 | 23 | cell wall protein PhiA |
| A. benhamiae CBS 112371 null (653 aa) | ARB_04046 | 72 kDa | 83 | 54 | 46 | 5 | 7 | 4 | carboxypeptidase S1 |
| A. benhamiae CBS 112371 null (717 aa) | ARB_01345 | 79 kDa | 74 | 114 | 53 | 0 | 0 | 0 | extracelular serine carboxypeptidase |
| A. benhamiae CBS 112371 null (597 aa) | ARB_05765 | 65 kDa | 73 | 153 | 81 | 0 | 4 | 0 | tripeptidyl-peptidase (TppA) |
| A. benhamiae CBS 112371 null (418 aa) | ARB_05392 | 46 kDa | 52 | 52 | 37 | 336 | 482 | 209 | endochitinase |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| A. benhamiae CBS 112371 null (440 aa) | ARB_05253 | 45 kDa | 52 | 56 | 44 | 0 | 0 | 0 | glycosyl hydrolase or cell wall glucanase |
| A. benhamiae CBS 112371 null (667 aa) | ARB_01864 | 75 kDa | 50 | 113 | 73 | 0 | 1 | 1 | Ser/Thr protein phosphatase family protein |
| A. benhamiae CBS 112371 null (342 aa) | ARB_03699 | 36 kDa | 49 | 135 | 58 | 13 | 66 | 16 | polysaccharide deacetylase family protein |
| A. benhamiae CBS 112371 null (236 aa) | ARB_05911 | 25 kDa | 49 | 93 | 51 | 0 | 1 | 0 | glycosyl hydrolase, putative |
| A. benhamiae CBS 112371 null (510 aa) | ARB_00035 | 56 kDa | 48 | 46 | 30 | 64 | 78 | 56 | mannosyl-oligosaccharide 1,2-alpha-mannosidase |
| A. benhamiae CBS 112371 null (414 aa) | ARB_04467 | 45 kDa | 48 | 66 | 35 | 81 | 43 | 40 | glucanase |
| A. benhamiae CBS 112371 null (879 aa) | ARB_02077 | 94 kDa | 46 | 51 | 39 | 70 | 102 | 51 | exo-beta-1,3-glucanase |
| A. benhamiae CBS 112371 null (503 aa) | ARB_05319 | 55 kDa | 46 | 64 | 37 | 24 | 43 | 24 | FAD-dependent oxygenase |
| A. benhamiae CBS 112371 null (530 aa) | ARB_06414 | 59 kDa | 45 | 70 | 32 | 10 | 8 | 7 | carboxypeptidase |
| A. benhamiae CBS 112371 null (779 aa) | ARB_06110 | 88 kDa | 41 | 42 | 25 | 118 | 66 | 70 | DPP IV |
| A. benhamiae CBS 112371 null (945 aa) | ARB_06907 | 104 kDa | 41 | 41 | 31 | 16 | 48 | 21 | hypothetical protein |
| A. benhamiae CBS 112371 null (374 aa) | ARB_03568 | 41 kDa | 38 | 39 | 41 | 19 | 14 | 15 | leucine aminopeptidase 2 |
| A. benhamiae CBS 112371 null (552 aa) | ARB_00083 | 62 kDa | 35 | 69 | 35 | 24 | 9 | 18 | serine peptidase, family S28, putative |
| A. benhamiae CBS 112371 null (131 aa) | ARB_07026 | 14 kDa | 33 | 33 | 7 | 80 | 50 | 29 | carboxypeptidase A1 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| A. benhamiae CBS 112371 null (206 aa) | ARB_05304 | 21 kDa | 33 | 51 | 32 | 13 | 37 | 20 | allergenic cerato-platanin Asp F13 |
| A. benhamiae CBS 112371 null (623 aa) | ARB_05535 | 70 kDa | 31 | 37 | 19 | 18 | 5 | 7 | glutaminase, putative |
| A. benhamiae CBS 112371 null (223 aa) | ARB_03106 | 25 kDa | 31 | 26 | 18 | 0 | 1 | 0 | conserved hypothetical protein |
| A. benhamiae CBS 112371 null (354 aa) | ARB_06359 | 38 kDa | 28 | 59 | 29 | 24 | 48 | 25 | glycosyl hydrolase |
| A. benhamiae CBS 112371 null (196 aa) | ARB_07637 | 21 kDa | 26 | 22 | 21 | 10 | 22 | 5 | IgE-binding protein |
| A. benhamiae CBS 112371 null (388 aa) | ARB_01627 | 41 kDa | 25 | 75 | 51 | 52 | 18 | 29 | GPI-anchored cell wall protein Pst1, putative |
| A. benhamiae CBS 112371 null (481 aa) | ARB_05360 | 53 kDa | 25 | 33 | 18 | 0 | 1 | 0 | extracellular lipase, putative |
| A. benhamiae CBS 112371 null (196 aa) | ARB_00595 | 21 kDa | 23 | 11 | 8 | 68 | 52 | 17 | conserved hypothetical protein |
| A. benhamiae CBS 112371 null (530 aa) | ARB_03264 | 59 kDa | 23 | 36 | 20 | 27 | 37 | 13 | conserved hypothetical protein |
| A. benhamiae CBS 112371 null (771 aa) | ARB_07629 | 85 kDa | 22 | 28 | 22 | 9 | 9 | 18 | alpha-1,2-mannosidase, putative subfamily |
| A. benhamiae CBS 112371 null (910 aa) | ARB_01444 | 99 kDa | 20 | 23 | 17 | 92 | 161 | 92 | endo-1,3-beta-glucanase |
| A. benhamiae CBS 112371 null (925 aa) | ARB_07867 | 99 kDa | 18 | 21 | 24 | 31 | 32 | 24 | copper radical oxidase |
| A. benhamiae CBS 112371 null (134 aa) | ARB_05536 | 15 kDa | 18 | 21 | 18 | 8 | 7 | 7 | glutaminase |
| A. benhamiae CBS 112371 null (403 aa) | ARB_03790 | 42 kDa | 18 | 11 | 14 | 11 | 9 | 6 | alkaline serine protease |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| A. benhamiae CBS 112371 null (198 aa) | ARB_03673 | 21 kDa | 18 | 12 | 12 | 0 | 0 | 0 | predicted protein |
| A. benhamiae CBS 112371 null (417 aa) | ARB_03789 | 46 kDa | 17 | 16 | 7 | 10 | 9 | 5 | secreted carboxypeptidase McpA |
| A. benhamiae CBS 112371 null (491 aa) | ARB_00777 | 52 kDa | 16 | 2 | 2 | 4 | 1 | 1 | serine protease |
| A. benhamiae CBS 112371 null (505 aa) | ARB_06076 | 53 kDa | 15 | 6 | 1 | 147 | 39 | 69 | subtilisin protease SUB7 |
| A. benhamiae CBS 112371 null (1029 aa) | ARB_02327 | 113 kDa | 15 | 36 | 22 | 38 | 75 | 39 | zinc-binding oxidoreductase, putative |
| A. benhamiae CBS 112371 null (242 aa) | ARB_04889 | 27 kDa | 15 | 17 | 9 | 15 | 22 | 7 | GDSL Lipase/Acylhydrolase family protein |
| A. benhamiae CBS 112371 null (226 aa) | ARB_01979 | 25 kDa | 15 | 22 | 13 | 13 | 6 | 11 | conserved hypothetical protein |
| A. benhamiae CBS 112371 null (238 aa) | ARB_07027 | 27 kDa | 14 | 21 | 13 | 39 | 25 | 18 | secreted carboxypeptidase McpA (maybe A1) |
| A. benhamiae CBS 112371 null (405 aa) | ARB_04859 | 44 kDa | 14 | 11 | 9 | 21 | 19 | 19 | oxalate decarboxylase, putative |
| A. benhamiae CBS 112371 null (441 aa) | ARB_05372 | 48 kDa | 13 | 13 | 8 | 7 | 8 | 8 | aldose 1-epimerase family protein, putative |
| A. benhamiae CBS 112371 null (821 aa) | ARB_05654 | 89 kDa | 12 | 17 | 15 | 78 | 203 | 78 | beta-glucosidase, putative |
| A. benhamiae CBS 112371 null (512 aa) | ARB_07056 | 55 kDa | 12 | 16 | 18 | 20 | 16 | 29 | FAD/FMN-containing isoamyl alcohol oxidase MreA-like |
| A. benhamiae CBS 112371 null (315 aa) | ARB_06966 | 35 kDa | 12 | 10 | 7 | 11 | 17 | 9 | conserved hypothetical protein |
| A. benhamiae CBS 112371 null (197 aa) | ARB_00926 | 21 kDa | 12 | 16 | 22 | 0 | 0 | 0 | amidohydrolase (très putative) |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| A. benhamiae CBS 112371 null (883 aa) | ARB_07888 | 100 kDa | 12 | 13 | 18 | 0 | 0 | 0 | glycosyl hydrolase |
| A. benhamiae CBS 112371 null (175 aa) | ARB_01325 | 19 kDa | 11 | 7 | 6 | 41 | 19 | 17 | phosphatidylglycerol/phosphatidylinositol transfer protein |
| A. benhamiae CBS 112371 null (336 aa) | ARB_03491 | 38 kDa | 11 | 9 | 7 | 6 | 0 | 2 | GPI anchored protein, putative |
| A. benhamiae CBS 112371 null (634 aa) | ARB_06472 | 70 kDa | 11 | 16 | 8 | 0 | 0 | 0 | metalloprotease MEP5, putative |
| A. benhamiae CBS 112371 null (644 aa) | ARB_05919 | 69 kDa | 11 | 7 | 12 | 0 | 0 | 0 | lysophospholipase Plb2 |
| A. benhamiae CBS 112371 null (395 aa) | ARB_02015 | 43 kDa | 10 | 15 | 7 | 0 | 0 | 0 | phosphogluconolactonase |
| A. benhamiae CBS 112371 null (187 aa) | ARB_02741 | 18 kDa | 10 | 0 | 3 | 8 | 0 | 1 | GPI anchored CFEM domain protein |
| A. benhamiae CBS 112371 null (255 aa) | ARB_03514 | 28 kDa | 9 | 2 | 6 | 31 | 43 | 21 | class III chitinase ChiA2 |
| A. benhamiae CBS 112371 null (426 aa) | ARB_02208 | 46 kDa | 9 | 4 | 3 | 8 | 4 | 8 | oxalate decarboxylase |
| A. benhamiae CBS 112371 null (224 aa) | ARB_03853 | 25 kDa | 9 | 11 | 9 | 3 | 1 | 2 | conserved hypothetical protein (DUF1349) |
| A. benhamiae CBS 112371 null (585 aa) | ARB_02921 | 64 kDa | 9 | 13 | 5 | 3 | 0 | 0 | gamma-glutamyltranspeptidase |
| A. benhamiae CBS 112371 null (213 aa) | ARB_06108 | 24 kDa | 9 | 0 | 0 | 0 | 0 | 0 | predicted protein |
| A. benhamiae CBS 112371 null (593 aa) | ARB_02965 | 64 kDa | 8 | 24 | 7 | 108 | 247 | 126 | amidase family protein |
| A. benhamiae CBS 112371 null (413 aa) | ARB_01347 | 44 kDa | 8 | 19 | 16 | 10 | 6 | 3 | ribonuclease T2 family, putative |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| A. benhamiae CBS 112371 null (250 aa) | ARB_04619 | 27 kDa | 8 | 6 | 7 | 6 | 1 | 5 | secreted protein |
| A. benhamiae CBS 112371 null (501 aa) | ARB_02372 | 55 kDa | 7 | 27 | 10 | 17 | 27 | 15 | FAD dependent oxidoreductase, putative |
| A. benhamiae CBS 112371 null (657 aa) | ARB_06019 | 72 kDa | 7 | 5 | 4 | 0 | 0 | 0 | carboxypeptidase S1, putative |
| A. benhamiae CBS 112371 null (243 aa) | ARB_07186 | 27 kDa | 6 | 6 | 6 | 4 | 4 | 8 | extracellular lipase, putative |
| A. benhamiae CBS 112371 null (496 aa) | ARB_04170 | 52 kDa | 6 | 16 | 6 | 0 | 0 | 0 | aspartic-type endopeptidase (OpsB), putative |
| A. benhamiae CBS 112371 null (351 aa) | ARB_03276 | 39 kDa | 6 | 11 | 9 | 0 | 0 | 0 | endonuclease/exonuclease/phosphatase |
| A. benhamiae CBS 112371 null (509 aa) | ARB_05721 | 57 kDa | 6 | 14 | 4 | 0 | 0 | 0 | carboxypeptidase Y, putative |
| A. benhamiae CBS 112371 null (910 aa) | ARB_06590 | 102 kDa | 6 | 2 | 1 | 0 | 0 | 0 | dipeptidyl aminopeptidase |
| A. benhamiae CBS 112371 null (354 aa) | ARB_00204 | 39 kDa | 5 | 2 | 1 | 37 | 35 | 22 | chitinase |
| A. benhamiae CBS 112371 null (566 aa) | ARB_02478 | 62 kDa | 5 | 12 | 9 | 7 | 10 | 15 | FAD binding domain containing protein |
| A. benhamiae CBS 112371 null (191 aa) | ARB_05566 | 21 kDa | 5 | 19 | 15 | 0 | 0 | 0 | predicted protein |
| A. benhamiae CBS 112371 null (508 aa) | ARB_01886 | 56 kDa | 5 | 4 | 1 | 2 | 0 | 0 | xaa-Pro aminopeptidase |
| A. benhamiae CBS 112371 null (257 aa) | ARB_03024 | 25 kDa | 4 | 2 | 4 | 26 | 11 | 10 | extracellular serine-rich protein |
| A. benhamiae CBS 112371 null (866 aa) | ARB_02101 | 98 kDa | 4 | 15 | 7 | 5 | 8 | 6 | alpha-glucosidase AgdA, putative |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| A. benhamiae CBS 112371 null (408 aa) | ARB_01032 | 43 kDa | 4 | 0 | 0 | 13 | 0 | 5 | subtilisin-like protease SUB4 |
| A. benhamiae CBS 112371 null (546 aa) | ARB_07487 | 59 kDa | 4 | 5 | 2 | 0 | 0 | 0 | 1,3-beta-glucanosyltransferase |
| A. benhamiae CBS 112371 null (607 aa) | ARB_04499 | 67 kDa | 3 | 1 | 0 | 40 | 39 | 27 | alkaline phosphatase |
| A. benhamiae CBS 112371 null (401 aa) | ARB_06111 | 42 kDa | 3 | 3 | 1 | 36 | 2 | 19 | alkaline serine protease (oryzin) |
| A. benhamiae CBS 112371 null (395 aa) | ARB_00047 | 44 kDa | 3 | 3 | 4 | 5 | 5 | 3 | conserved hypothetical protein |
| A. benhamiae CBS 112371 null (342 aa) | ARB_07185 | 37 kDa | 3 | 3 | 2 | 3 | 1 | 4 | carboxylesterase |
| A. benhamiae CBS 112371 null (214 aa) | ARB_05177 | 23 kDa | 3 | 0 | 1 | 2 | 5 | 3 | predicted protein |
| A. benhamiae CBS 112371 null (1055 aa) | ARB_03719 | 115 kDa | 3 | 9 | 3 | 0 | 0 | 0 | glycosyl hydrolase |
| A. benhamiae CBS 112371 null (231 aa) | ARB_01545 | 21 kDa | 3 | 0 | 0 | 0 | 0 | 0 | extracelular proline-glycine rich protein |
| A. benhamiae CBS 112371 null (211 aa) | ARB_05178 | 22 kDa | 3 | 0 | 0 | 0 | 0 | 0 | prp 6 CRoW domain containing protein |
| A. benhamiae CBS 112371 null (267 aa) | ARB_00862 | 29 kDa | 2 | 2 | 1 | 89 | 61 | 112 | hydrophobic surface binding protein B |
| A. benhamiae CBS 112371 null (756 aa) | ARB_05732 | 81 kDa | 2 | 1 | 0 | 18 | 7 | 8 | cysteine desulfurase (en partie…) |
| A. benhamiae CBS 112371 null (292 aa) | ARB_03620 | 32 kDa | 2 | 5 | 1 | 4 | 6 | 3 | conserved hypothetical protein |
| A. benhamiae CBS 112371 null (409 aa) | ARB_03431 | 44 kDa | 2 | 8 | 0 | 0 | 0 | 0 | extracellular sialidase/neuraminidase |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| A. benhamiae CBS 112371 null (157 aa) | ARB_03576 | 16 kDa | 2 | 0 | 0 | 1 | 0 | 0 | conserved hypothetical protein |
| A. benhamiae CBS 112371 null (246 aa) | ARB_07590 | 26 kDa | 1 | 0 | 0 | 344 | 254 | 173 | secreted protein |
| A. benhamiae CBS 112371 null (140 aa) | ARB_06975 | 14 kDa | 1 | 0 | 0 | 9 | 3 | 11 | hydrophobin, putative |
| A. benhamiae CBS 112371 null (574 aa) | ARB_01962 | 62 kDa | 1 | 0 | 0 | 4 | 3 | 4 | beta-xylosidase, putative |
| A. benhamiae CBS 112371 null (193 aa) | ARB_01932 | 20 kDa | 1 | 1 | 0 | 6 | 2 | 1 | GPI anchored cell wall protein, putative |
| A. benhamiae CBS 112371 null (207 aa) | ARB_04555 | 21 kDa | 1 | 1 | 1 | 7 | 3 | 4 | deoxyuridine 5'-triphosphate nucleotidohydrolase |
| A. benhamiae CBS 112371 null (457 aa) | ARB_05933 | 50 kDa | 1 | 7 | 2 | 0 | 0 | 0 | phytase |
| A. benhamiae CBS 112371 null (246 aa) | ARB_07466 | 26 kDa | 1 | 2 | 2 | 0 | 0 | 0 | hypothetical protein |
| A. benhamiae CBS 112371 null (496 aa) | ARB_00494 | 53 kDa | 0 | 16 | 2 | 595 | 777 | 536 | Leucine aminopeptidase 1 |
| A. benhamiae CBS 112371 null (210 aa) | ARB_00107 | 23 kDa | 0 | 0 | 0 | 203 | 211 | 172 | secreted protein |
| A. benhamiae CBS 112371 null (1091 aa) | ARB_00520 | 124 kDa | 0 | 0 | 0 | 110 | 176 | 81 | alpha-mannosidase |
| A. benhamiae CBS 112371 null (617 aa) | ARB_01353 | 70 kDa | 0 | 1 | 1 | 84 | 144 | 81 | chitobiase , putative |
| A. benhamiae CBS 112371 null (644 aa) | ARB_00762 | 71 kDa | 0 | 0 | 0 | 96 | 92 | 70 | metalloprotease (maybe Mep4) |
| A. benhamiae CBS 112371 null (565 aa) | ARB_02369 | 61 kDa | 0 | 0 | 0 | 62 | 86 | 64 | lipase (or carboxylesterase, putative) |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| A. benhamiae CBS 112371 null (762 aa) | ARB_01232 | 84 kDa | 0 | 0 | 0 | 58 | 73 | 47 | neutral/alkaline non-lysosomal ceramidase |
| A. benhamiae CBS 112371 null (688 aa) | ARB_06334 | 75 kDa | 0 | 0 | 0 | 57 | 37 | 55 | mucin |
| A. benhamiae CBS 112371 null (328 aa) | ARB_06052 | 36 kDa | 0 | 0 | 0 | 47 | 43 | 10 | xylanase D (or xylosidase, putative) |
| A. benhamiae CBS 112371 null (453 aa) | ARB_05164 | 51 kDa | 0 | 0 | 0 | 22 | 21 | 22 | conserved hypothetical protein |
| A. benhamiae CBS 112371 null (527 aa) | ARB_02407 | 59 kDa | 0 | 8 | 2 | 8 | 12 | 10 | carboxypeptidase (2?) |
| A. benhamiae CBS 112371 null (415 aa) | ARB_02715 | 45 kDa | 0 | 0 | 0 | 13 | 12 | 12 | microsomal dipeptidase or dipeptidyl aminopeptidase |
| A. benhamiae CBS 112371 null (728 aa) | ARB_02390 | 79 kDa | 0 | 0 | 0 | 11 | 15 | 13 | glutamate carboxypeptidase |
| A. benhamiae CBS 112371 null (513 aa) | ARB_06983 | 56 kDa | 0 | 0 | 0 | 10 | 22 | 4 | aminopeptidase I zinc metalloprotease M18 |
| A. benhamiae CBS 112371 null (149 aa) | ARB_02001 | 16 kDa | 0 | 0 | 0 | 8 | 8 | 13 | secretory phospholipase A2 |
| A. benhamiae CBS 112371 null (1066 aa) | ARB_06053 | 117 kDa | 0 | 0 | 0 | 15 | 7 | 8 | ser/Thr protein phosphatase |
| A. benhamiae CBS 112371 null (653 aa) | ARB_05859 | 70 kDa | 0 | 0 | 0 | 15 | 6 | 7 | FAD dependent oxidoreductase, putative |
| A. benhamiae CBS 112371 null (513 aa) | ARB_04696 | 56 kDa | 0 | 0 | 0 | 9 | 13 | 6 | conserved hypothetical protein |
| A. benhamiae CBS 112371 null (557 aa) | ARB_01941 | 61 kDa | 0 | 0 | 0 | 6 | 12 | 7 | fumarylacetoacetate hydrolase family protein |
| A. benhamiae CBS 112371 null (300 aa) | ARB_00059 | 33 kDa | 0 | 0 | 0 | 11 | 4 | 4 | class II aldolase/adducin domain-containing protein |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| A. benhamiae CBS 112371 null (213 aa) | ARB_00773 | 22 kDa | 0 | 0 | 0 | 7 | 4 | 4 | Cu,Zn superoxide dismutase |
| A. benhamiae CBS 112371 null (413 aa) | ARB_05307 | 43 kDa | 0 | 0 | 0 | 10 | 6 | 3 | subtilisin-like protease SUB6 |
| A. benhamiae CBS 112371 null (425 aa) | ARB_01495 | 46 kDa | 0 | 0 | 0 | 12 | 0 | 8 | subtilisin-like protease SUB2 |
| A. benhamiae CBS 112371 null (923 aa) | ARB_04703 | 105 kDa | 0 | 0 | 0 | 5 | 6 | 5 | beta-mannosidase, putative |
| A. benhamiae CBS 112371 null (538 aa) | ARB_07085 | 58 kDa | 0 | 2 | 0 | 0 | 9 | 3 | tannase, putative |
| A. benhamiae CBS 112371 null (208 aa) | ARB_06407 | 23 kDa | 0 | 0 | 0 | 6 | 5 | 3 | conserved hypothetical protein |
| A. benhamiae CBS 112371 null (354 aa) | ARB_01995 | 39 kDa | 0 | 0 | 0 | 7 | 2 | 5 | FAD dependent oxidoreductase protein |
| A. benhamiae CBS 112371 null (388 aa) | ARB_01751 | 42 kDa | 0 | 6 | 4 | 1 | 1 | 2 | PAF acetylhydrolase family protein |
| A. benhamiae CBS 112371 null (572 aa) | ARB_02107 | 62 kDa | 0 | 1 | 2 | 6 | 0 | 2 | glutathione reductase |
| A. benhamiae CBS 112371 null (606 aa) | ARB_07893 | 69 kDa | 0 | 9 | 3 | 0 | 0 | 0 | hypothetical protein |
| A. benhamiae CBS 112371 null (752 aa) | ARB_07609 | 84 kDa | 0 | 0 | 0 | 0 | 11 | 0 | mycelial catalase Cat1 |
| A. benhamiae CBS 112371 null (501 aa) | ARB_00532 | 56 kDa | 0 | 0 | 0 | 8 | 2 | 1 | tyrosinase |
| A. benhamiae CBS 112371 null (172 aa) | ARB_06080 | 19 kDa | 0 | 0 | 0 | 0 | 7 | 1 | ssDNA binding protein, putative |
| A. benhamiae CBS 112371 null (319 aa) | ARB_02306 | 36 kDa | 0 | 0 | 0 | 3 | 3 | 2 | N-acetyltransferase family protein, putative |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| A. benhamiae CBS 112371 null (1102 aa) | ARB_01379 | 121 kDa | 0 | 0 | 0 | 8 | 2 | 0 | ABC transporter |
| A. benhamiae CBS 112371 null (306 aa) | ARB_04618 | 34 kDa | 0 | 0 | 0 | 4 | 2 | 2 | conserved hypothetical protein |
| A. benhamiae CBS 112371 null (383 aa) | ARB_08028 | 41 kDa | 0 | 0 | 0 | 4 | 0 | 1 | aminotransferase |
| A. benhamiae CBS 112371 null (179 aa) | ARB_07191 | 20 kDa | 0 | 0 | 0 | 3 | 2 | 3 | conserved hypothetical protein |
| A. benhamiae CBS 112371 null (864 aa) | ARB_00226 | 96 kDa | 0 | 0 | 0 | 2 | 3 | 3 | raffinose synthase protein Sip1, putative |
| A. benhamiae CBS 112371 null (168 aa) | ARB_03651 | 19 kDa | 0 | 0 | 0 | 4 | 1 | 3 | 3-demethylubiquinone-9 3-methyltransferase, putative |
| A. benhamiae CBS 112371 null (657 aa) | ARB_02785 | 71 kDa | 0 | 0 | 0 | 0 | 8 | 0 | 1-pyrroline-5-carboxylate dehydrogenase |
| A. benhamiae CBS 112371 null (121 aa) | ARB_00658 | 13 kDa | 0 | 0 | 0 | 0 | 3 | 2 | hypothetical protein |
| A. benhamiae CBS 112371 null (312 aa) | ARB_02251 | 34 kDa | 0 | 3 | 2 | 0 | 0 | 0 | hypothetical protein |
| A. benhamiae CBS 112371 null (418 aa) | ARB_07536 | 47 kDa | 0 | 5 | 2 | 0 | 0 | 0 | hypothetical protein |
| A. benhamiae CBS 112371 null (304 aa) | ARB_07886 | 35 kDa | 0 | 0 | 0 | 6 | 1 | 0 | lactoylglutathione lyase (Glo1), putative |
| A. benhamiae CBS 112371 null (596 aa) | ARB_00930 | 65 kDa | 0 | 0 | 1 | 0 | 3 | 2 | beta-lactamase, putative |
| A. benhamiae CBS 112371 null (630 aa) | ARB_06361 | 70 kDa | 0 | 0 | 2 | 1 | 0 | 3 | carboxypeptidase S1 |
| A. benhamiae CBS 112371 null (416 aa) | ARB_05309 | 45 kDa | 0 | 0 | 0 | 5 | 0 | 1 | asparaginase |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| A. benhamiae CBS 112371 null (246 aa) | ARB_04177 | 27 kDa | 0 | 0 | 0 | 2 | 0 | 0 | glycerophosphoryl diester phosphodiesterase |
| A. benhamiae CBS 112371 null (366 aa) | ARB_05022 | 40 kDa | 0 | 1 | 3 | 0 | 0 | 0 | fucose-specific lectin FleA |
| A. benhamiae CBS 112371 null (601 aa) | ARB_04101 | 65 kDa | 0 | 4 | 0 | 0 | 0 | 0 | serine protease, putative |
| A. benhamiae CBS 112371 null (305 aa) | ARB_01071 | 33 kDa | 0 | 0 | 0 | 0 | 1 | 2 | peptidyl-prolyl cis-trans isomerase, putative |
| A. benhamiae CBS 112371 null (459 aa) | ARB_05157 | 49 kDa | 0 | 0 | 0 | 1 | 0 | 1 | LysM domain protein, putative |
| A. benhamiae CBS 112371 null (636 aa) | ARB_02406 | 70 kDa | 0 | 3 | 1 | 0 | 0 | 0 | metalloprotease |
| A. benhamiae CBS 112371 null (230 aa) | ARB_03731 | 26 kDa | 0 | 0 | 1 | 0 | 0 | 3 | polyubiquitin UbiD/Ubi4, putative |
| A. benhamiae CBS 112371 null (308 aa) | ARB_06706 | 33 kDa | 0 | 0 | 0 | 2 | 1 | 0 | PBSP domain-containing protein |
| A. benhamiae CBS 112371 null (464 aa) | ARB_07628 | 51 kDa | 0 | 0 | 0 | 0 | 2 | 1 | methylcitrate synthase |
| A. benhamiae CBS 112371 null (401 aa) | ARB_02919 | 43 kDa | 0 | 0 | 0 | 2 | 0 | 0 | aspartyl proteinase (vacuolar protease A) |
| A. benhamiae CBS 112371 null (508 aa) | ARB_01498 | 56 kDa | 0 | 2 | 0 | 0 | 0 | 0 | carboxylesterase, putative |
| A. benhamiae CBS 112371 null (583 aa) | ARB_06380 | 64 kDa | 0 | 0 | 0 | 2 | 0 | 1 | general amidase GmdA, putative |
| A. benhamiae CBS 112371 null (298 aa) | ARB_07540 | 33 kDa | 0 | 0 | 0 | 1 | 0 | 2 | hydrolase, carbon-nitrogen family, putative |
| A. benhamiae CBS 112371 null (405 aa) | ARB_06224 | 45 kDa | 0 | 1 | 1 | 0 | 0 | 0 | thioredoxin reductase |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| A. benhamiae CBS 112371 null (634 aa) | ARB_05828 | 72 kDa | 0 | 1 | 1 | 0 | 0 | 0 | diphenol oxidase |
| A. benhamiae CBS 112371 null (432 aa) | ARB_04981 | 49 kDa | 0 | 0 | 0 | 0 | 1 | 0 | prolyl peptidase |
| A. benhamiae CBS 112371 null (360 aa) | ARB_01765 | 41 kDa | 0 | 0 | 0 | 3 | 0 | 0 | spermidine synthase |
| A. benhamiae CBS 112371 null (669 aa) | ARB_01230 | 71 kDa | 0 | 2 | 0 | 0 | 0 | 0 | kelch repeat protein |
| A. benhamiae CBS 112371 null (235 aa) | ARB_02963 | 25 kDa | 0 | 0 | 0 | 2 | 0 | 0 | ThiJ/PfpI family protein |
| A. benhamiae CBS 112371 null (149 aa) | ARB_04828 | 16 kDa | 0 | 2 | 0 | 0 | 0 | 0 | cytochrome c |
| A. benhamiae CBS 112371 null (776 aa) | ARB_07441 | 84 kDa | 0 | 0 | 0 | 2 | 0 | 0 | aconitase |
| A. benhamiae CBS 112371 null (214 aa) | ARB_02516 | 24 kDa | 0 | 0 | 1 | 0 | 0 | 0 | D-tyrosyl-tRNA(Tyr) deacylase |
| A. benhamiae CBS 112371 null (302 aa) | ARB_02990 | 32 kDa | 0 | 0 | 0 | 1 | 0 | 0 | ubiquinol-cytochrome c reductase iron-sulfur subunit |

**Supplementary Table 3a: *M. canis* hydrolases identified by MS in SP medium at pH 4.0 and pH 7.0.**

## Glycosidases

| Identified Proteins | Accession Number | Molecular Weight | Mcanis-pH4-a | Mcanis-pH4-b | Mcanis-pH4-c | Mcanis-pH7-a | Mcanis-pH7-b | Mcanis-pH7-c |
|---|---|---|---|---|---|---|---|---|
| Beta-glucosidase 4 MCYG_01527 | C5FHG8_NANOT | 33 kDa | 99 | 92 | 90 | 29 | 40 | 93 |
| 1,2-alpha-D-mannosidase MCYG_00423 | C5FCK1_NANOT | 56 kDa | 38 | 13 | 54 | 15 | 7 | 50 |
| Glucoamylase MCYG_06367 | C5FUG4_NANOT | 68 kDa | 35 | 0 | 53 | 388 | 174 | 662 |
| Extracellular cell wall glucanase Crf1/allergen Asp F9 MCYG_04874 | C5FQA2_NANOT | 45 kDa | 56 | 18 | 40 | 0 | 0 | 0 |
| Exo-beta-1,3-glucanase Exg0 MCYG_06600 | C5FV47_NANOT | 34 kDa | 8 | 7 | 14 | 10 | 1 | 17 |
| Beta-glucosidase 1 MCYG_00928 | C5FE06_NANOT | 94 kDa | 0 | 0 | 13 | 40 | 25 | 75 |
| N,O-diacetylmuramidase MCYG_02645 | C5FGE1_NANOT | 25 kDa | 9 | 4 | 13 | 0 | 0 | 0 |
| Amidase MCYG_00475 | C5FCQ3_NANOT | 62 kDa | 0 | 0 | 12 | 81 | 79 | 211 |
| Glycosyl hydrolase MCYG_03093 | C5FKQ2_NANOT | 88 kDa | 7 | 8 | 12 | 0 | 4 | 2 |
| 1,3(4)-beta-glucanase MCYG_05925 | C5FTA3_NANOT | 49 kDa | 18 | 5 | 11 | 0 | 0 | 0 |
| Glycosyl hydrolase family 3 N terminal domain-containing protein MCYG_07820 | C5FXG1_NANOT | 40 kDa | 1 | 4 | 7 | 2 | 0 | 1 |
| Acid trehalase MCYG_02758 | C5FGQ4_NANOT | 115 kDa | 3 | 0 | 7 | 0 | 0 | 0 |
| Beta-hexosaminidase MCYG_02200 | C5FJ51_NANOT | 68 kDa | 0 | 0 | 6 | 16 | 9 | 17 |
| Alpha-glucosidase MCYG_00092 | C5FBM0_NANOT | 101 kDa | 4 | 0 | 4 | 9 | 14 | 17 |
| Endo-beta-1,3-glucanase MCYG_06694 | C5FVE1_NANOT | 74 kDa | 1 | 0 | 3 | 0 | 0 | 0 |
| Glucan 1,3-beta-glucosidase MCYG_05874 | C5FT52_NANOT | 46 kDa | 0 | 0 | 2 | 0 | 7 | 1 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Endochitinase 1 MCYG_00676 | C5FDA4_NANOT | 44 kDa | 0 | 0 | 1 | 34 | 14 | 75 |
| Alpha-mannosidase MCYG_06176 | C5FTX3_NANOT | 123 kDa | 0 | 0 | 0 | 72 | 13 | 102 |
| Arabinan endo-1,5-alpha-L-arabinosidase A MCYG_02777 | C5FGS3_NANOT | 37 kDa | 0 | 0 | 0 | 8 | 9 | 15 |
| Aspartylglucosaminidase family protein MCYG_04957 | C5FQI5_NANOT | 45 kDa | 0 | 0 | 0 | 6 | 7 | 15 |
| Beta-mannosidase MCYG_06102 | C5FTT0_NANOT | 98 kDa | 0 | 0 | 0 | 3 | 5 | 14 |
| Exochitinase 1 MCYG_05745 | C5FSS3_NANOT | 53 kDa | 0 | 0 | 0 | 3 | 4 | 13 |
| Beta-glucosidase MCYG_00545 | C5FCX3_NANOT | 135 kDa | 0 | 0 | 0 | 0 | 3 | 6 |
| Chitinase MCYG_04644 | C5FNX2_NANOT | 159 kDa | 0 | 0 | 0 | 0 | 4 | 6 |
| Chitinase 4 MCYG_08403 | C5G0D1_NANOT | 39 kDa | 0 | 0 | 0 | 3 | 0 | 4 |
| Chitinase 3 MCYG_07133 | C5FWN0_NANOT | 24 kDa | 2 | 1 | 0 | 1 | 0 | 2 |
| Chitinase MCYG_04741 | C5FPW9_NANOT | 130 kDa | 0 | 0 | 0 | 0 | 3 | 0 |

## Lipases

| Identified Proteins | Accession Number | Molecular Weight | Mcanis-pH4-a | Mcanis-pH4-b | Mcanis-pH4-c | Mcanis-pH7-a | Mcanis-pH7-b | Mcanis-pH7-c |
|---|---|---|---|---|---|---|---|---|
| Lysophospholipase MCYG_02652 | C5FGE8_NANOT | 68 kDa | 9 | 4 | 13 | 3 | 7 | 3 |
| GDSL Lipase/Acylhydrolase family protein MCYG_04422 | C5FNI9_NANOT | 28 kDa | 4 | 0 | 7 | 0 | 0 | 0 |
| Neutral ceramidase MCYG_06637 | C5FV84_NANOT | 83 kDa | 0 | 0 | 0 | 25 | 18 | 67 |
| Secretory phospholipase A2 MCYG_00583 | C5FD11_NANOT | 17 kDa | 0 | 0 | 0 | 3 | 2 | 4 |

## Phosphatases

| Identified Proteins | Accession Number | Molecular Weight | Mcanis-pH4-a | Mcanis-pH4-b | Mcanis-pH4-c | Mcanis-pH7-a | Mcanis-pH7-b | Mcanis-pH7-c |
|---|---|---|---|---|---|---|---|---|
| Ser/Thr protein phosphatase MCYG_06525 | C5FUX2_NANOT | 72 kDa | 41 | 14 | 32 | 0 | 0 | 0 |
| Acid phosphatase MCYG_07729 | C5FX70_NANOT | 31 kDa | 12 | 4 | 19 | 0 | 0 | 0 |
| Phytase MCYG_02670 | C5FGG6_NANOT | 51 kDa | 19 | 0 | 17 | 0 | 0 | 0 |
| HMF1 MCYG_06048 | C5FTM6_NANOT | 13 kDa | 0 | 2 | 1 | 0 | 0 | 0 |
| Endonuclease/exonuclease/phosphatase family protein MCYG_03887 | C5FMG5_NANOT | 33 kDa | 0 | 0 | 1 | 0 | 0 | 0 |
| Alkaline phosphatase MCYG_04625 | C5FNV3_NANOT | 56 kDa | 0 | 0 | 0 | 358 | 64 | 377 |
| Nucleoside diphosphate kinase MCYG_01839 | C5FI40_NANOT | 17 kDa | 0 | 0 | 0 | 14 | 22 | 15 |
| Ser/Thr protein phosphatase MCYG_02775 | C5FGS1_NANOT | 68 kDa | 0 | 0 | 0 | 1 | 1 | 7 |
| Alkaline phosphatase MCYG_05903 | C5FT81_NANOT | 66 kDa | 0 | 0 | 0 | 1 | 0 | 7 |

## Other hydrolases

| Identified Proteins | Accession Number | Molecular Weight | Mcanis-pH4-a | Mcanis-pH4-b | Mcanis-pH4-c | Mcanis-pH7-a | Mcanis-pH7-b | Mcanis-pH7-c |
|---|---|---|---|---|---|---|---|---|
| Gamma-glutamyltranspeptidase MCYG_05524 | C5FS52_NANOT | 63 kDa | 5 | 0 | 25 | 3 | 18 | 4 |
| Carboxylesterase family protein MCYG_00869 | C5FDU7_NANOT | 62 kDa | 22 | 0 | 12 | 0 | 0 | 2 |
| Carboxylesterase MCYG_01781 | C5FHY2_NANOT | 55 kDa | 8 | 0 | 12 | 0 | 0 | 0 |
| Glutaminase GtaA MCYG_00814 | C5FDF2_NANOT | 95 kDa | 0 | 0 | 11 | 9 | 4 | 23 |
| Ribonuclease T2-like protein MCYG_02204 | C5FJ55_NANOT | 42 kDa | 5 | 6 | 10 | 0 | 1 | 3 |
| Glycerophosphoryl diester phosphodiesterase family protein MCYG_05364 | C5FRP2_NANOT | 45 kDa | 1 | 0 | 4 | 3 | 3 | 11 |
| Feruloyl esterase B MCYG_06844 | C5FVU1_NANOT | 58 kDa | 3 | 0 | 4 | 0 | 0 | 0 |
| 2,6-dihydropseudooxynicotine hydrolase MCYG_02065 | C5FIH7_NANOT | 49 kDa | 2 | 0 | 4 | 0 | 0 | 0 |
| Fumarylacetoacetase MCYG_00527 | C5FCV5_NANOT | 47 kDa | 0 | 0 | 0 | 27 | 21 | 39 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Cutinase MCYG_05629 | C5FSF7_NANOT | 28 kDa | 0 | 0 | 0 | 7 | 4 | 12 |
| Amidohydrolase MCYG_03185 | C5FKZ4_NANOT | 33 kDa | 0 | 0 | 0 | 1 | 4 | 7 |
| Dienelactone hydrolase family protein MCYG_02976 | C5FKD5_NANOT | 32 kDa | 0 | 0 | 0 | 3 | 3 | 1 |
| Thimet oligopeptidase MCYG_02264 | C5FFJ8_NANOT | 82 kDa | 0 | 0 | 0 | 2 | 0 | 3 |
| Glyoxalase family protein MCYG_00690 | C5FDK6_NANOT | 17 kDa | 0 | 0 | 0 | 0 | 0 | 6 |
| Esterase MCYG_01130 | C5FEU8_NANOT | 60 kDa | 0 | 0 | 0 | 0 | 4 | 0 |
| N-carbamoyl-L-amino acid hydrolase MCYG_03207 | C5FL16_NANOT | 50 kDa | 0 | 0 | 0 | 2 | 0 | 1 |

**Supplementary Table 3b: *A. benhamiae* hydrolases identified by MS in SP medium at pH 4.0 and pH 7.0.**

## Glucosidases

| Identified Proteins (from CBS 112371) | Accession Number | Molecular Weight | pH4 Abenh-a | pH4 Abenh-b | pH4 Abenh-c | pH7 Abenh-a | pH7 Abenh-b | pH7 Abenh-c | Predicted fonction |
|---|---|---|---|---|---|---|---|---|---|
| A. benh (309 aa) | ARB_02797 | 33 kDa | 101 | 169 | 89 | 157 | 34 | 56 | GPI-anchored cell wall beta-1,3-endoglucanase EglC |
| A. benh (418 aa) | ARB_05392 | 46 kDa | 52 | 52 | 37 | 336 | 482 | 209 | endochitinase |
| A. benh (440 aa) | ARB_05253 | 45 kDa | 52 | 56 | 44 | 0 | 0 | 0 | glycosyl hydrolase or cell wall glucanase |
| A. benh (236 aa) | ARB_05911 | 25 kDa | 49 | 93 | 51 | 0 | 1 | 0 | glycosyl hydrolase, putative |
| A. benh (510 aa) | ARB_00035 | 56 kDa | 48 | 46 | 30 | 64 | 78 | 56 | mannosyl-oligosaccharide 1,2-alpha-mannosidase |
| A. benh (414 aa) | ARB_04467 | 45 kDa | 48 | 66 | 35 | 81 | 43 | 40 | glucanase |
| A. benh (879 aa) | ARB_02077 | 94 kDa | 46 | 51 | 39 | 70 | 102 | 51 | exo-beta-1,3-glucanase |
| A. benh (623 aa) | ARB_05535 | 70 kDa | 31 | 37 | 19 | 18 | 5 | 7 | glutaminase, putative |
| A. benh (354 aa) | ARB_06359 | 38 kDa | 28 | 59 | 29 | 24 | 48 | 25 | glycosyl hydrolase |
| A. benh (771 aa) | ARB_07629 | 85 kDa | 22 | 28 | 22 | 9 | 9 | 18 | alpha-1,2-mannosidase, putative subfamily |
| A. benh (910 aa) | ARB_01444 | 99 kDa | 20 | 23 | 17 | 92 | 161 | 92 | endo-1,3-beta-glucanase |
| A. benh (821 aa) | ARB_05654 | 89 kDa | 12 | 17 | 15 | 78 | 203 | 78 | beta-glucosidase, putative |
| A. benh (883 aa) | ARB_07888 | 100 kDa | 12 | 13 | 18 | 0 | 0 | 0 | glycosyl hydrolase |
| A. benh (395 aa) | ARB_02015 | 43 kDa | 10 | 15 | 7 | 0 | 0 | 0 | phosphogluconolactonase |
| A. benh (593 aa) | ARB_02965 | 64 kDa | 8 | 24 | 7 | 108 | 247 | 126 | amidase family protein |
| A. benh (354 aa) | ARB_00204 | 39 kDa | 5 | 2 | 1 | 37 | 35 | 22 | chitinase |
| A. benh (866 aa) | ARB_02101 | 98 kDa | 4 | 15 | 7 | 5 | 8 | 6 | alpha-glucosidase AgdA, putative |
| A. benh (1055 aa) | ARB_03719 | 115 kDa | 3 | 9 | 3 | 0 | 0 | 0 | glycosyl hydrolase |
| A. benh (1091 aa) | ARB_00520 | 124 kDa | 0 | 0 | 0 | 110 | 176 | 81 | alpha-mannosidase |
| A. benh (923 aa) | ARB_04703 | 105 kDa | 0 | 0 | 0 | 5 | 6 | 5 | beta-mannosidase, putative |
| A. benh (864 aa) | ARB_00226 | 96 kDa | 0 | 0 | 0 | 2 | 3 | 3 | raffinose synthase protein Sip1, putative |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| A. benh (596 aa) | ARB_00930 | 65 kDa | 0 | 0 | 1 | 0 | 3 | 2 | beta-lactamase, putative |
| A. benh (583 aa) | ARB_06380 | 64 kDa | 0 | 0 | 0 | 2 | 0 | 1 | general amidase GmdA, putative |
| A. benh (255 aa) | ARB_03514 | 28 kDa | 9 | 2 | 6 | 31 | 43 | 21 | class III chitinase ChiA2 |
| A. benh (409 aa) | ARB_03431 | 44 kDa | 2 | 8 | 0 | 0 | 0 | 0 | extracellular sialidase/neuraminidase |
| A. benh (574 aa) | ARB_01962 | 62 kDa | 1 | 0 | 0 | 4 | 3 | 4 | beta-xylosidase, putative |
| A. benh (617 aa) | ARB_01353 | 70 kDa | 0 | 1 | 1 | 84 | 144 | 81 | chitobiase , putative |
| A. benh (328 aa) | ARB_06052 | 36 kDa | 0 | 0 | 0 | 47 | 43 | 10 | xylanase D (or xylosidase, putative) |
| A. benh (762 aa) | ARB_01232 | 84 kDa | 0 | 0 | 0 | 58 | 73 | 47 | neutral/alkaline non-lysosomal ceramidase |

## Lipases

| Identified Proteins | Accession Number | Molecular Weight | pH4 Abenh-a | pH4 Abenh-b | pH4 Abenh-c | pH7 Abenh-a | pH7 Abenh-b | pH7 Abenh-c | Predicted fonction |
|---|---|---|---|---|---|---|---|---|---|
| A. benh (481 aa) | ARB_05360 | 53 kDa | 25 | 33 | 18 | 0 | 1 | 0 | extracellular lipase, putative |
| A. benh (242 aa) | ARB_04889 | 27 kDa | 15 | 17 | 9 | 15 | 22 | 7 | GDSL Lipase/Acylhydrolase family protein |
| A. benh (644 aa) | ARB_05919 | 69 kDa | 11 | 7 | 12 | 0 | 0 | 0 | lysophospholipase Plb2 |
| A. benh (243 aa) | ARB_07186 | 27 kDa | 6 | 6 | 6 | 4 | 4 | 8 | extracellular lipase, putative |
| A. benh (565 aa) | ARB_02369 | 61 kDa | 0 | 0 | 0 | 62 | 86 | 64 | lipase, putative |
| A. benh (149 aa) | ARB_02001 | 16 kDa | 0 | 0 | 0 | 8 | 8 | 13 | secretory phospholipase A2 |

## Phosphatases

| Identified Proteins | Accession Number | Molecular Weight | pH4 Abenh-a | pH4 Abenh-b | pH4 Abenh-c | pH7 Abenh-a | pH7 Abenh-b | pH7 Abenh-c | Predicted fonction |
|---|---|---|---|---|---|---|---|---|---|

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| A. benh (351 aa) | ARB_03276 | 39 kDa | 6 | 11 | 9 | 0 | 0 | 0 | endonuclease/exonuclease/phosphatase |
| A. benh (607 aa) | ARB_04499 | 67 kDa | 3 | 1 | 0 | 40 | 39 | 27 | alkaline phosphatase |
| A. benh (457 aa) | ARB_05933 | 50 kDa | 1 | 7 | 2 | 0 | 0 | 0 | phytase |
| A. benh (1066 aa) | ARB_06053 | 117 kDa | 0 | 0 | 0 | 15 | 7 | 8 | ser/Thr protein phosphatase |
| A. benh (667 aa) | ARB_01864 | 75 kDa | 50 | 113 | 73 | 0 | 1 | 1 | Ser/Thr protein phosphatase family protein |

## Other Hydrolases

| Identified Proteins | Accession Number | Molecular Weight | pH4 Abenh-a | pH4 Abenh-b | pH4 Abenh-c | pH7 Abenh-a | pH7 Abenh-b | pH7 Abenh-c | Predicted fonction |
|---|---|---|---|---|---|---|---|---|---|
| A. benh (134 aa) | ARB_05536 | 15 kDa | 18 | 21 | 18 | 8 | 7 | 7 | glutaminase |
| A. benh (197 aa) | ARB_00926 | 21 kDa | 12 | 16 | 22 | 0 | 0 | 0 | amidohydrolase, putative |
| A. benh (585 aa) | ARB_02921 | 64 kDa | 9 | 13 | 5 | 3 | 0 | 0 | gamma-glutamyltranspeptidase |
| A. benh (342 aa) | ARB_07185 | 37 kDa | 3 | 3 | 2 | 3 | 1 | 4 | carboxylesterase |
| A. benh (207 aa) | ARB_04555 | 21 kDa | 1 | 1 | 1 | 7 | 3 | 4 | deoxyuridine 5'-triphosphate nucleotidohydrolase |
| A. benh (557 aa) | ARB_01941 | 61 kDa | 0 | 0 | 0 | 6 | 12 | 7 | fumarylacetoacetate hydrolase family protein |
| A. benh (538 aa) | ARB_07085 | 58 kDa | 0 | 2 | 0 | 0 | 9 | 3 | tannase, putative |
| A. benh (501 aa) | ARB_00532 | 56 kDa | 0 | 0 | 0 | 8 | 2 | 1 | tyrosinase |
| A. benh (416 aa) | ARB_05309 | 45 kDa | 0 | 0 | 0 | 5 | 0 | 1 | asparaginase |
| A. benh (246 aa) | ARB_04177 | 27 kDa | 0 | 0 | 0 | 2 | 0 | 0 | glycerophosphoryl diester phosphodiesterase |
| A. benh (508 aa) | ARB_01498 | 56 kDa | 0 | 2 | 0 | 0 | 0 | 0 | carboxylesterase, putative |
| A. benh (298 aa) | ARB_07540 | 33 kDa | 0 | 0 | 0 | 1 | 0 | 2 | hydrolase, carbon-nitrogen family, putative |
| A. benh (214 aa) | ARB_02516 | 24 kDa | 0 | 0 | 1 | 0 | 0 | 0 | D-tyrosyl-tRNA(Tyr) deacylase |
| A. benh (388 aa) | ARB_01751 | 42 kDa | 0 | 6 | 4 | 1 | 1 | 2 | PAF acetylhydrolase family protein |

**Supplementary Table 4a: Ratio average of all secreted proteases from *M. canis* based on the Scaffold scoring. Data associated to the figure 3A.**

| Identified Proteases | Accession Number | Molecular Weight | Mcanis-a pH4 | Mcanis-b pH4 | Mcanis-c pH4 | Average | Mcanis-a pH7 | Mcanis-b pH7 | Mcanis-c pH7 | Average |
|---|---|---|---|---|---|---|---|---|---|---|
| SedB (S53) | SED2_NANOT | 65 kDa | 6.47 | 19.13 | 8.61 | **11.41** | 0.08 | 0.00 | 0.38 | **0.15** |
| NPIIA (M35) | NPIID_NANOT | 38 kDa | 8.28 | 5.28 | 5.30 | **6.29** | 0.45 | 0.66 | 0.18 | **0.43** |
| SedD (S53) | SED4_NANOT | 66 kDa | 6.30 | 11.08 | 3.94 | **7.11** | 0.15 | 0.39 | 0.30 | **0.28** |
| Sub3 (S8A) | SUB3_MICCA | 41 kDa | 2.43 | 0.52 | 3.49 | **2.15** | 6.39 | 3.26 | 3.99 | **4.55** |
| Pep1 (A1) | PEPF_NANOT | 41 kDa | 8.49 | 1.65 | 3.38 | **4.51** | 0.23 | 0.00 | 0.00 | **0.08** |
| S41 | C5FM69_NANOT | 77 kDa | 1.81 | 4.59 | 3.28 | **3.23** | 0.19 | 0.72 | 0.51 | **0.47** |
| McpAl (M14) | MCPAL_NANOT | 46 kDa | 4.82 | 0.52 | 2.93 | **2.76** | 1.25 | 0.22 | 0.43 | **0.63** |
| DppV (S9) | DPP5_NANOT | 80 kDa | 2.14 | 0.00 | 2.13 | **1.42** | 0.00 | 0.61 | 0.02 | **0.21** |
| CpS5 (S10) | C5FX94_NANOT | 58 kDa | 1.48 | 3.12 | 1.95 | **2.18** | 0.00 | 0.11 | 0.02 | **0.04** |
| Mep3 (M36) | MEP3_MICCA | 69 kDa | 0.91 | 0.17 | 1.74 | **0.94** | 1.44 | 1.83 | 2.37 | **1.88** |
| CpS1 (S10) | SPCA_NANOT | 72 kDa | 1.36 | 2.34 | 1.64 | **1.78** | 0.00 | 0.22 | 0.02 | **0.08** |
| M28 | C5FP82_NANOT | 56 kDa | 0.78 | 1.39 | 1.53 | **1.23** | 0.00 | 0.00 | 0.10 | **0.03** |
| McpA (M14) | MCPA_NANOT | 47 kDa | 1.81 | 1.13 | 1.36 | **1.43** | 0.30 | 0.06 | 0.20 | **0.19** |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| S28 B | C5FJ58_NANOT | 61 kDa | 1.15 | 0.00 | 1.92 | **1.02** | 0 | 0 | 0 | **0.00** |
| Mep5 (M36) | MEP5_MICCA | 70 kDa | 0.87 | 0.00 | 1.19 | **0.68** | 0.00 | 0.00 | 0.02 | **0.01** |
| OpsB (A1) | C5FRQ0_NANOT | 50 kDa | 0.49 | 0.43 | 0.73 | **0.55** | 0.00 | 0.00 | 0.00 | **0.00** |
| CpS7 (S10) | C5FS14_NANOT | 66 kDa | 0.66 | 1.13 | 0.52 | **0.77** | 0.04 | 0.00 | 0.00 | **0.01** |
| CpS3 (S10) | SCPB_NANOT | 73 kDa | 0.62 | 0.17 | 0.42 | **0.40** | 0.00 | 0.00 | 0.00 | **0.00** |
| Mep1 (M36) | MEP1_MICCA | 70 kDa | 0.16 | 0.00 | 0.28 | **0.15** | 0.00 | 0.00 | 0.06 | **0.02** |
| Sub9 (S8A) | C5FH27_NANOT | 42 kDa | 0.04 | 0.00 | 0.21 | **0.08** | 0.00 | 0.00 | 0.14 | **0.05** |
| DppIV (S9B) | A0S5V9_MICCA | 88 kDa | 0.00 | 0.00 | 0.17 | **0.06** | 2.04 | 2.16 | 1.78 | **1.99** |
| Lap3 (M28E) | LAP5_NANOT | 38 kDa | 0.33 | 0.00 | 0.17 | **0.17** | 0.00 | 0.00 | 0.00 | **0.00** |
| SedC (S53) | SED3_NANOT | 65 kDa | 0.00 | 0.00 | 0.17 | **0.06** | 0.00 | 0.00 | 0.00 | **0.00** |
| NPIIB (M35) | NPIIA_NANOT | 40 kDa | 0.49 | 0.26 | 0.14 | **0.30** | 0.23 | 0.44 | 0.04 | **0.24** |
| Sub10 (S8A) | C5FGP4_NANOT | 41 kDa | 0.08 | 0.00 | 0.10 | **0.06** | 0.00 | 0.44 | 0.04 | **0.16** |
| Lap1 (M28E) | LAP1_NANOT | 53 kDa | 0.00 | 0.00 | 0.07 | **0.02** | 5.90 | 16.65 | 15.76 | **12.77** |
| Lap2 (M28A) | LAP2_NANOT | 41 kDa | 0.04 | 0.09 | 0.07 | **0.07** | 0.38 | 1.33 | 0.51 | **0.74** |
| Sub7 (S8A) | SUB7_NANOT | 41 kDa | 0.08 | 0.00 | 0.07 | **0.05** | 0.79 | 0.33 | 0.38 | **0.50** |
| Gltcp (M28) | C5FYL4_NANOT | 78 kDa | 0.00 | 0.00 | 0.03 | **0.01** | 0.83 | 0.66 | 0.77 | **0.76** |
| McpB (M14) | MCPB_NANOT | 60 kDa | 0.00 | 0.00 | 0.00 | **0.00** | 2.42 | 2.82 | 2.51 | **2.58** |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Mep4 (M36) | MEP4_MICCA | 71 kDa | 0.00 | 0.00 | 0.00 | **0.00** | 1.40 | 2.49 | 2.92 | **2.27** |
| DppI (M19) | C5FK77_NANOT | 47 kDa | 0.08 | 0.00 | 0.00 | **0.03** | 0.57 | 1.16 | 0.75 | **0.83** |
| ApP (M28*) | C5FHR9_NANOT | 69 kDa | 0.00 | 0.00 | 0.00 | **0.00** | 0.15 | 0.22 | 0.08 | **0.15** |
| Pap (S33) | C5FNL7_NANOT | 51 kDa | 0.00 | 0.00 | 0.00 | **0.00** | 0.04 | 0.11 | 0.18 | **0.11** |
| Subl (S8*) | C5FHQ0_NANOT | 75 kDa | 0.00 | 0.00 | 0.00 | **0.00** | 0.04 | 0.00 | 0.14 | **0.06** |
| Aap (M18*) | C5FVJ3_NANOT | 74 kDa | 0.00 | 0.00 | 0.00 | **0.00** | 0.11 | 0.00 | 0.08 | **0.06** |
| Sub8 (S8A) | C5FII2_NANOT | 52 kDa | 0.00 | 0.00 | 0.00 | **0.00** | 0.00 | 0.06 | 0.06 | **0.04** |
| Sub4 (S8A) | SUB4_NANOT | 42 kDa | 0.00 | 0.00 | 0.00 | **0.00** | 0.00 | 0.11 | 0.02 | **0.04** |
| | | **Sum** | 52.20 | 52.99 | 47.56 | **50.92** | 25.43 | 37.06 | 34.73 | **32.40** |

**Supplementary Table 4b: Ratio average of all secreted proteases from *A. benhamiae* based on the Scaffold scoring. Data associated to the figure 3B.**

| Identified Proteases (predictive fonction) | Accession Number | Molecular Weight | Abenh-a pH4 | Abenh-b pH4 | Abenh-c pH4 | Average | Abenh-a pH7 | Abenh-b pH7 | Abenh-c pH7 | Average |
|---|---|---|---|---|---|---|---|---|---|---|
| DppV (727 aa) | ARB_06651 | 80 kDa | 9.18 | 14.40 | 15.58 | **13.05** | 0.00 | 1.74 | 0.33 | **0.69** |
| Pep1 (404 aa) | ARB_05728 | 43 kDa | 6.09 | 6.09 | 3.57 | **5.25** | 0.00 | 0.10 | 0.00 | **0.03** |
| Peptidase S41 (699 aa) | ARB_02220 | 78 kDa | 5.86 | 8.57 | 4.10 | **6.18** | 12.92 | 6.83 | 11.58 | **10.44** |
| Sub3 (398 aa) | ARB_00701 | 41 kDa | 3.94 | 3.97 | 1.25 | **3.06** | 2.86 | 0.78 | 1.50 | **1.72** |
| Mep3 (634 aa) | ARB_05085 | 69 kDa | 3.18 | 3.17 | 2.32 | **2.89** | 0.39 | 0.42 | 0.49 | **0.43** |
| CpS1 (653 aa) | ARB_04046 | 72 kDa | 2.75 | 1.21 | 1.75 | **1.90** | 0.10 | 0.13 | 0.11 | **0.11** |
| S28 B (717 aa) | ARB_01345 | 79 kDa | 2.45 | 2.54 | 2.01 | **2.34** | 0.00 | 0.00 | 0.00 | **0.00** |
| SedB (597 aa) | ARB_05765 | 65 kDa | 2.42 | 3.42 | 3.08 | **2.97** | 0.00 | 0.08 | 0.00 | **0.03** |
| CpS4 (530 aa) | ARB_06414 | 59 kDa | 1.49 | 1.56 | 1.22 | **1.42** | 0.19 | 0.15 | 0.19 | **0.18** |
| DppIV (779 aa) | ARB_06110 | 88 kDa | 1.36 | 0.94 | 0.95 | **1.08** | 2.28 | 1.26 | 1.91 | **1.82** |
| Lap1 (374 aa) | ARB_03568 | 41 kDa | 1.26 | 0.87 | 1.56 | **1.23** | 0.37 | 0.27 | 0.41 | **0.35** |
| S28 A (552 aa) | ARB_00083 | 62 kDa | 1.16 | 1.54 | 1.33 | **1.34** | 0.46 | 0.17 | 0.49 | **0.38** |
| McpA (131 aa) | ARB_07026 | 14 kDa | 1.09 | 0.74 | 0.27 | **0.70** | 1.55 | 0.95 | 0.79 | **1.10** |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Sub9 (403 aa) | ARB_03790 | 42 kDa | 0.60 | 0.25 | 0.53 | **0.46** | 0.21 | 0.17 | 0.16 | **0.18** |
| McpAl (417 aa) | ARB_03789 | 46 kDa | 0.56 | 0.36 | 0.27 | **0.40** | 0.19 | 0.17 | 0.14 | **0.17** |
| Sub8 (491 aa) | ARB_00777 | 52 kDa | 0.53 | 0.04 | 0.08 | **0.22** | 0.08 | 0.02 | 0.03 | **0.04** |
| Sub7 (505 aa) | ARB_06076 | 53 kDa | 0.50 | 0.13 | 0.04 | **0.22** | 2.84 | 0.74 | 1.88 | **1.82** |
| McpA' (238 aa) | ARB_07027 | 27 kDa | 0.46 | 0.47 | 0.49 | **0.48** | 0.75 | 0.48 | 0.49 | **0.57** |
| Mep5 (634 aa) | ARB_06472 | 70 kDa | 0.36 | 0.36 | 0.30 | **0.34** | 0.00 | 0.00 | 0.00 | **0.00** |
| CpS2 (657 aa) | ARB_06019 | 72 kDa | 0.23 | 0.11 | 0.15 | **0.17** | 0.00 | 0.00 | 0.00 | **0.00** |
| OpsB (496 aa) | ARB_04170 | 52 kDa | 0.20 | 0.36 | 0.23 | **0.26** | 0.00 | 0.00 | 0.00 | **0.00** |
| CpS6 (509 aa) | ARB_05721 | 57 kDa | 0.20 | 0.31 | 0.15 | **0.22** | 0.00 | 0.00 | 0.00 | **0.00** |
| Dpp (910 aa) | ARB_06590 | 102 kDa | 0.20 | 0.04 | 0.04 | **0.09** | 0.00 | 0.00 | 0.00 | **0.00** |
| Peptidase D (508 aa) | ARB_01886 | 56 kDa | 0.17 | 0.09 | 0.04 | **0.10** | 0.04 | 0.00 | 0.00 | **0.01** |
| Sub4 (408 aa) | ARB_01032 | 43 kDa | 0.13 | 0.00 | 0.00 | **0.04** | 0.25 | 0.00 | 0.14 | **0.13** |
| Sub11 (401 aa) | ARB_06111 | 42 kDa | 0.10 | 0.07 | 0.04 | **0.07** | 0.70 | 0.04 | 0.52 | **0.42** |
| Lap2 (496 aa) | ARB_00494 | 53 kDa | 0.00 | 0.36 | 0.08 | **0.14** | 11.50 | 14.83 | 14.63 | **13.65** |
| Mep4 (644 aa) | ARB_00762 | 71 kDa | 0.00 | 0.00 | 0.00 | **0.00** | 1.86 | 1.76 | 1.91 | **1.84** |
| McpB (527 aa) | ARB_02407 | 59 kDa | 0.00 | 0.18 | 0.08 | **0.08** | 0.15 | 0.23 | 0.27 | **0.22** |
| Dppl (415 aa) | ARB_02715 | 45 kDa | 0.00 | 0.00 | 0.00 | **0.00** | 0.25 | 0.23 | 0.33 | **0.27** |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Gltcp (728 aa) | ARB_02390 | 79 kDa | 0.00 | 0.00 | 0.00 | **0.00** | 0.21 | 0.29 | 0.35 | **0.28** |
| Aap (513 aa) | ARB_06983 | 56 kDa | 0.00 | 0.00 | 0.00 | **0.00** | 0.19 | 0.42 | 0.11 | **0.24** |
| Sub6 (413 aa) | ARB_05307 | 43 kDa | 0.00 | 0.00 | 0.00 | **0.00** | 0.19 | 0.11 | 0.08 | **0.13** |
| Sub2 (425 aa) | ARB_01495 | 46 kDa | 0.00 | 0.00 | 0.00 | **0.00** | 0.23 | 0.00 | 0.22 | **0.15** |
| CpS3 (630 aa) | ARB_06361 | 70 kDa | 0.00 | 0.00 | 0.08 | **0.03** | 0.02 | 0.00 | 0.08 | **0.03** |
| SedD (601 aa) | ARB_04101 | 65 kDa | 0.00 | 0.09 | 0.00 | **0.03** | 0.00 | 0.00 | 0.00 | **0.00** |
| Mep1 (636 aa) | ARB_02406 | 70 kDa | 0.00 | 0.07 | 0.04 | **0.03** | 0.00 | 0.00 | 0.00 | **0.00** |
| Pep2 (401 aa) | ARB_02919 | 43 kDa | 0.00 | 0.00 | 0.00 | **0.00** | 0.04 | 0.00 | 0.00 | **0.01** |
| Pap (432 aa) | ARB_04981 | 49 kDa | 0.00 | 0.00 | 0.00 | **0.00** | 0.00 | 0.02 | 0.00 | **0.01** |
| | | **sum** | 46.47 | 52.30 | 41.60 | **46.79** | 40.84 | 32.38 | 39.15 | **37.45** |

**Supplementary Table 5: Homologous proteases with a signal sequence in *Aspergillus fumigatus, Arthroderma. benhamiae* and *Microsporum canis*.**

| Family/ Sub-family | Protein | *A. fumigatus*[1] | *M. canis*[2] | *A. benhamiae*[3] |
|---|---|---|---|---|
| | | | | |
| A1 | Aspartic endopeptidase PEP2 (Vacuolar protease A) | AFUA_3G11400 O42630 | MCYG_05527 C5FS55 | ARB_02919 D4B385 |
| A1 | Aspartic endopeptidase PEP1 (Aspergillopepsin I) | AFUA_5G13300 P41748 | MCYG_00144 C5FBS2 | ARB_05728 D4ANC3 |
| A1 | Aspartic-type endopeptidase ctsD | AFUA_4G07040 Q4WNV0 | MCYG_01216 C5FEK4 | ARB_01619 D4AZK1 |
| A1 | Putative aspergillopepsin A-like aspartic endopeptidase | AFUA_2G15950 Q4WZS3 | MCYG_07979 C5FZ57 | ARB_07403 D4AT39 |
| A1 | Probable aspartic-type endopeptidase (opsB) | AFUA_6G05350 Q4WDN4 | MCYG_05372 C5FRQ0 | ARB_04170 D4AIS3 |
| A1 | Aspartic-type endopeptidase, putative | - | MCYG_06955 C5FW52 | ARB_04018 D4AIC4 |
| A1 | Aspartic-type endopeptidase, putative | AFUA_3G01220 Q4WFS2 | - | - |
| A1 | Aspartic-type endopeptidase, putative | AFUA_6G03260 Q4WD26 | - | - |
| G1 | *Aspergillopepsin, putative* | AFUA_3G02970 Q4WF96 | - | - |
| G1 | *Aspergillopepsin, putative* | AFUA_7G01200 Q4WAG2 | - | - |

| | | | | |
|---|---|---|---|---|
| M12 | *ADAM family of metalloprotease ADM-A* | *AFUA_6G14420* Q58I96 | - | - |
| M12 | *ADAM family of metalloprotease ADM-B* | *AFUA_4G11150* Q4WQ08 | *MCYG_06406* C5FUK3 | *ARB_02289* D4B1G0 |
| M14 | Metallocarboxypeptidase, putative | AFUA_2G08790 Q4X1U0 | MCYG_04493 C5FPR9 | ARB_04942 D4AKU7 |
| M14 | Metallocarboxypeptidase A (MCPA) | - | MCYG_06789 C5FVN6 | ARB_07026 / ARB_07027 D4AS12 |
| M14 | Metallocarboxypeptidase A-like protein (MCPAL) | - | MCYG_01475 C5FH26 | ARB_03789 D4B5N0 |
| M14 | Carboxypeptidase 2 Metallocarboxypeptidase B (MCPB) | - | MCYG_07414 C5FYJ7 | ARB_02407 D4B1S6 |
| M19 | Dipeptidase 1 | - | MCYG_02918 C5FK77 | ARB_02715 D4B2N2 |
| M20A | Probable peptidase | AFUA_6G06800 Q4WNC9 | MCYG_07204 C5FXY7 | ARB_01041 D4AXX2 |
| M24 | Aminopeptidase P (AMPP) | AFUA_5G08050 Q4WUD3 | MCYG_01718 C5FHR9 | ARB_06742 D4ARJ9 |
| M24 | Methionine aminopeptidase | AFUA_4G06930 Q4WNT9 | MCYG_01318 C5FF46 | ARB_02691 D4B2G3 |
| M24 | Peptidase D | AFUA_1G14920 Q4WRV9 | MCYG_06503 C5FUV0 | ARB_01886 D4B0B2 |
| M28A | Aminopeptidase Y (Leucine aminopeptidase 2) | AFUA_3G00650 Q4WFX9 | MCYG_06199 C5FTZ6 | ARB_00494 D4AWC9 |
| M28A | Aminopeptidase, putative | AFUA_2G00220 Q4WIY5 | - | - |
| M28E | Leucine aminopeptidase 1 | AFUA_4G04210 Q4W9P4 | MCYG_02286 C5FFM0 | ARB_03568 D4B528 |

| | | | | |
|---|---|---|---|---|
| M28E | Probable leucine aminopeptidase | - | MCYG_03459 C5FLR8 | ARB_01443 D4AZ23 |
| M28E | Probable leucine aminopeptidase | - | MCYG_08380 C5G0A8 | ARB_03492 D4B4V2 |
| M28E | Probable leucine aminopeptidase | - | MCYG_04170 C5FNB5 | ARB_00576 D4AWL0 |
| M28 | Peptidase family M28 protein | - | MCYG_04217 C5FP82 | ARB_04732 D4AM42 |
| M28 | Peptidase family M28 protein | AFUA_1G05960 Q4WJH4 | MCYG_00832 C5FDH0 | ARB_05554 D4AMV1 |
| M28 | Putative glutamate carboxypeptidase | - | MCYG_07431 C5FYL4 | ARB_02390 D4B1R0 |
| M28 | Glutaminyl-peptide cyclotransferase | AFUA_4G08280 Q4WP74 | MCYG_05716 C5FSP4 | ARB_00230 D4AVL6 |
| M35 | Probable neutral protease 2 homolog | AFUA_4G13750 Q4WQR6 | MCYG_01991 C5FIT1 | ARB_00849 D4AXC1 |
| M35 | Probable neutral protease 2 homolog | - | MCYG_07155 C5FWQ2 | ARB_03949 D4B639 |
| M35 | Probable neutral protease 2 homolog (Deuterolysin B) | - | MCYG_00239 C5FCA7 | ARB_05817 D4ANL2 |
| M35 | Probable neutral protease 2 homolog (Deuterolysin A) | AFUA_4G02700 Q4WA45 | MCYG_04257 C5FPC2 | ARB_04769 D4AM79 |
| M35 | Probable neutral protease 2 homolog | - | MCYG_05201 C5FR79 | ARB_04336 D4AJ87 |
| M36 | Extracellular metalloproteinase 1 (Fungalysin MEP1) | - | MCYG_07415 C5FYJ8 | ARB_02406 D4B1S5 |
| M36 | Extracellular metalloproteinase 2 (Fungalysin MEP2) | - | MCYG_02168 C5FJ19 | ARB_01382 D4AYW3 |

| | | | | |
|---|---|---|---|---|
| M36 | Extracellular metalloproteinase 3 (Fungalysin MEP3) | - | MCYG_04620 C5FNU8 | ARB_05085 D4AL88 |
| M36 | Extracellular metalloproteinase 4 (Fungalysin MEP4) | - | MCYG_02088 C5FIK0 | ARB_00762 D4AX35 |
| M36 | Extracellular metalloproteinase 5 (Fungalysin MEP5) | AFUA_8G07080 P46075 | MCYG_07688 C5FX29 | ARB_06472 D4AQG5 |
| M43 | Probable metalloproteinase | AFUA_1G07730 Q4WJ01 | MCYG_04966 C5FQJ4 | ARB_05317 D4ALW9 |
| M43 | Probable metalloproteinase | - | MCYG_03238 C5FL47 | ARB_07495 D4ATD1 |
| S8A | Subtilisin-like protease 1 (SUB1) | - | MCYG_04495 C5FPS1 | ARB_04944 D4AKU9 |
| S8A | Subtilisin-like protease 2 (SUB2) | AFUA_4G11800 P28296 | MCYG_08690 C5G168 | ARB_01495 D4AZ75 |
| S8A | Subtilisin-like protease 3 (SUB3) | - | MCYG_04040 C5FMY5 | ARB_00701 D4AWY5 |
| S8A | Subtilisin-like protease 4 (SUB4) | - | MCYG_07213 C5FXZ6 | ARB_01032 D4AXW3 |
| S8A | Subtilisin-like protease 5 (SUB5) | - | MCYG_08753 C5G1D1 | ARB_02223 D4B194 |
| S8A | Subtilisin-like protease 6 (SUB6) | - | MCYG_04955 C5FQI3 | ARB_05307 D4ALV9 |
| S8A | Subtilisin-like protease 7 (SUB7) | - | MCYG_02345 C5FJA5 | ARB_06076 D4APA9 |
| S8A | Subtilisin-like protease 8 (SUB8) | AFUA_5G09210 P87184 | MCYG_02070 C5FII2 | ARB_00777 D4AX50 |
| S8A | Subtilisin-like protease 9 (SUB9) | - | MCYG_01476 C5FH27 | ARB_03790 D4B5N1 |

| | | | | |
|---|---|---|---|---|
| S8A | Probable alkaline serine protease | - | MCYG_02748 C5FGP4 | - |
| S8A | Subtilisin-like protease 10 (SUB10) | - | MCYG_07696 C5FX37 | ARB_06467 D4AQG0 |
| S8A | Subtilisin-like protease 11 (SUB11) | - | MCYG_07961 C5FZ39 | ARB_06111 D4APE3 |
| S8A | Subtilisin-like protease 12 (SUB12) | - | - | ARB_06416 D4AQA9 |
| S8B | Pheromone processing endoprotease (KexB) | AFUA_4G12970 Q4WQI8 | MCYG_01100 C5FEH8 | ARB_00131 D4AVC2 |
| S8B | Pheromone processing endoprotease (KexB-like) | - | MCYG_08580 (Fragment) C5G0V8 | ARB_07953 D4AUN6 |
| S8 | Alkaline serine protease (PR1)/allergen F18-like | AFUA_7G04930 Q4WGG2 | - | - |
| S9B | Dipeptidyl peptidase 4 | AFUA_4G09320 Q4WPH9 | MCYG_02383 C5FJE3 | ARB_06110 D4APE2 |
| S9B | Dipeptidyl aminopeptidase | AFUA_3G07850 Q4WX13 | MCYG_07560 C5FYZ3 | ARB_06590 D4AQT0 |
| S9 | Dipeptidyl-peptidase 5 | AFUA_2G09030 P0C959 | MCYG_01626 C5FH88 | ARB_06651 D4ARB1 |
| S9 | Oligopeptidase family protein | AFUA_8G04730 Q4WCE3 | MCYG_07650 C5FWZ1 | ARB_06511 D4AQK4 |
| S10 | Carboxypeptidase S1 homolog A | AFUA_5G07330 Q5VJK9 | MCYG_06933 C5FW30 | ARB_04046 D4AIF1 |
| S10 | Carboxypeptidase S1 homolog B | AFUA_8G04120 Q4WCK3 | MCYG_02825 C5FGX1 | ARB_06019 D4AP52 |
| S10 | Carboxypeptidase S1 homolog | AFUA_1G00420 Q4WL26 | - | - |

| | | | | |
|---|---|---|---|---|
| S10 | Carboxypeptidase S1 homolog | - | MCYG_07817<br>C5FXF8 | ARB_06361<br>D4AQ54 |
| S10 | Carboxypeptidase S1 homolog | - | MCYG_04298<br>C5FPG3 | ARB_04807<br>D4AKG4 |
| S10 | Carboxypeptidase Y homolog | AFUA_6G13540<br>Q5VJG9 | MCYG_07094<br>C5FWJ1 | ARB_01491<br>D4AZ71 |
| S10 | Carboxypeptidase Y homolog | AFUA_5G14610<br>Q4WW68 | MCYG_00135<br>C5FBR3 | ARB_05721<br>D4ANB6 |
| S10 | Carboxypeptidase Y homolog | - | MCYG_05486<br>C5FS14 | ARB_07161<br>D4ASE6 |
| S10 | Carboxypeptidase Y homolog | AFUA_5G01200<br>Q4WDZ3 | - | - |
| S10 | Carboxypeptidase Y homolog | AFUA_3G12210<br>Q4WY91 | - | - |
| S10 | Carboxypeptidase cpdS homolog | AFUA_6G00310<br>Q4W8Y5 | MCYG_00613<br>C5FD41 | ARB_02032<br>D4B0Q6 |
| S10 | Carboxypeptidase cpdS homolog | AFUA_2G03510<br>Q5VJG7 | MCYG_07753<br>C5FX94 | ARB_06414<br>D4AQA7 |
| S10 | Carboxypeptidase cpdS homolog | AFUA_4G07270<br>Q4WNX3 | MCYG_01181<br>C5FEZ9 | ARB_01587<br>D4AZG9 |
| S10 | Pheromone processing carboxypeptidase Kex1 | AFUA_1G08940<br>Q4WTK9 | MCYG_06129<br>C5FTV7 | ARB_03758<br>D4B5L8 |
| S26 | Microsomal signal peptidase subunit | AFUA_3G12840<br>Q4WYF4 | MCYG_04817<br>C5FQ45 | ARB_05208<br>D4ALL0 |
| S28 | Serine peptidase, family S28, putative | AFUA_2G01250<br>Q4WIN2 | - | - |
| S28 | Serine peptidase, family S28, putative | AFUA_4G03790<br>Q4W9T5 | MCYG_00366<br>C5FC55 | ARB_00083<br>D4AV74 |

| | | | | |
|---|---|---|---|---|
| S28 | Serine peptidase, family S28, putative | AFUA_2G17330 Q4WZD5 | MCYG_02207 C5FJ58 | ARB_01345 D4AYS6 |
| S41 | Peptidase S41 family protein | - | MCYG_03610 C5FM69 | ARB_02220 D4B191 |
| S41 | Peptidase S41 family protein | - | MCYG_08247 C5FZX5 | ARB_02997 D4B3G0 |
| S41 | Peptidase S41 family protein | - | - | ARB_07195 D4ASI0 |
| S41 | Peptidase S41 family protein | AFUA_1G17400 Q4WR63 | - | - |
| S53 | Tripeptidyl-peptidase SED1 (Sedolisin-A) | AFUA_6G10250 Q70DX9 | MCYG_01559 C5FHK0 | - |
| S53 | Tripeptidyl-peptidase SED2 (Sedolisin-B) | AFUA_4G03490 Q70J59 | MCYG_00184 C5FBW2 | ARB_05765 D4ANG0 |
| S53 | Tripeptidyl-peptidase SED3 (Sedolisin-C) | AFUA_3G08930 Q70GH4 | MCYG_06077 C5FTQ5 | ARB_04677 D4AK75 |
| S53 | Tripeptidyl-peptidase SED4 (Sedolisin-D) | AFUA_4G14000 Q4WQU0 | MCYG_05446 C5FRX4 | ARB_04101 D4AIK6 |
| S53 | Peptidase S53 family protein | AFUA_7G06220 Q4WGU1 | - | - |
| S53 | Peptidase S53 family protein | AFUA_1G11400 Q6MYZ3 | - | - |

[1] *A. fumigatus* complete genome of strain Af293/CBS 101355/ FGSCA1100 (Nierman *et al*., 2005)

[2] *M. canis* complete genome sequenced strain CBS 113480 (Cuomo *et al*., Submitted to GenBank/EMBL in, 2008)

[3] *A. benhamiae* complete genome sequenced strain CBS 112371 (Burmester *et al*., 2011)

[1,2,3] Proteins are from UniprotKB database (http://www.uniprot.org/). UniProtKB accession numbers are indicated below the gene name. Closest homologs were identified by Blast search using the *A. fumigatus* and/or *M. canis* proteins as query.

Colour code: White - found in all species; Yellow - dermatophyte-specific proteases (found in dermatophytes, but not in *Aspergillus*); Purple - *Aspergillus*-specific;

## Side project on *Pichia pastoris*

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, July 2010, p. 4269–4276 Vol. 76, No. 13
0099-2240/10/$12.00 doi:10.1128/AEM.00412-10


# Secretion of an Endogenous Subtilisin by *Pichia pastoris* Strains GS115 and KM71[▽]

Karine Salamin,[1] Dev Sriranganadane,[1] Barbara Léchenne,[1] Olivier Jousson,[2] and Michel Monod[1]*

*Service de Dermatologie et Vénéréologie, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland,[1] and Centre for Integrative Biology (CIBIO), University of Trento, Trento, Italy[2]*

Received 15 February 2010/Accepted 6 May 2010

**The methylotrophic yeast *Pichia pastoris* is widely used for the expression of heterologous enzymes. While the purity of the desired expression product is of major importance for many applications, we found that recombinant enzymes produced in methanol medium were contaminated by a 37-kDa endogenous yeast protease. This enzyme was completely inhibited by phenylmethanesulfonyl fluoride (PMSF) but not by 1,10-phenanthroline, EDTA, and pepstatin A, suggesting the nature of a serine protease. Its secretion was abolished in *P. pastoris* strains GS115 and KM71 by specific mutagenesis of a subtilisin gene (*SUB2*) but not by inactivation of the gene encoding vacuolar proteinase B (*PRB*). Bioinformatic comparisons of Sub2 protein with subtilisins from other fungal genomes and phylogenetic analyses indicated that this enzyme is not an orthologue of the vacuolar protease cerevisin generally present in yeasts but is more closely related to another putative subtilisin found in a small number of yeast genomes. During growth of *P. pastoris*, Sub2 was produced as a secreted enzyme at a concentration of 10 μg/ml of culture supernatant after overexpression of the full-length *SUB2* gene. During fermentative production of recombinant enzymes in methanol medium, 1 ml of *P. pastoris* culture supernatant was found to contain approximately 3 ng of Sub2, while the enzyme was not detected during growth in a medium containing glycerol as a carbon source. The mutant strain GS115-*sub2* was subsequently used as a host for the production of recombinant proteases without endogenous subtilisin contamination.**

The methylotrophic yeast *Pichia pastoris* has been used successfully to express a wide range of heterologous proteins. *Pichia pastoris* has two genes that encode alcohol oxidase, *AOX1* and *AOX2*. The transcription of the former gene is tightly regulated by methanol, while the latter is expressed in small quantities (7, 11). Alcohol oxidase is not found in the cell when *P. pastoris* grows in the presence of glycerol, glucose, or ethanol, but in the presence of methanol, alcohol oxidase enzyme 1 (Aox1) amounts to 5% of total cellular proteins in shake-flask cultures and over 30% of total cellular proteins in fermenter cultures (6). The procedure for producing a secreted recombinant protein by using *P. pastoris* consists of cloning the cDNA encoding the protein of interest downstream of a signal sequence under the control of the *AOX1* promoter in a *P. pastoris* expression vector. In general, the *P. pastoris* acid phosphatase gene (*PHO1*) signal sequence or the α-factor signal peptide sequence is used for entry of the secretory pathway of the yeast (19). The construct, which carries in addition to the cloned coding sequence of interest a gene for selection after transformation of *P. pastoris*, is inserted into the *P. pastoris* genome at the *AOX1* locus via homologous recombination. Selected transformants are screened for recombinant protein production after induction in a medium containing methanol.

A fundamental objective of our research on fungi (*Aspergillus* spp. and dermatophytes) is to gain a comprehensive view of the enzymes that allow the digestion of an insoluble protein structure, such as keratinized tissues, into oligopeptides and free amino acids (32, 33). Highly purified proteases are needed to analyze the different steps in protein and peptide digestions. However, purification of an individual protease from fungal culture supernatant and cell extract is a laborious process, especially when the protease of interest is produced in small amounts and simultaneously with other proteases. Therefore, *P. pastoris* is a powerful tool for individual production of numerous secreted proteases as recombinant proteins in substantial amounts for further characterization and applications (1, 2, 4, 24, 31, 34, 41).

During assays of sequential digestion of large peptides by exoproteases, we realized that purified fractions of recombinant leucine aminopeptidases, dipeptidyl peptidases, and tripeptidyl peptidases of the sedolisin family after gel filtration and ion-exchange chromatography had additional endoproteolytic activities that were similar in all trials. In the present study, we identified and characterized a contaminating protease of the subtilisin family which is secreted in minor amounts by *P. pastoris* during growth in methanol medium. A strain deficient in this endogenous secreted subtilisin activity was constructed for further heterologous gene expression experiments.

## MATERIALS AND METHODS

**Strains and plasmids.** *Pichia pastoris* GS115 and KM71 (Invitrogen, Carlsbad, CA), pPICZA, and previously constructed plasmids pKJ113-*LAP2* and pKJ113-*DPPIV* for expression of the genes encoding *Aspergillus fumigatus* DppIV (AfuDppIV) and *A. fumigatus* Lap2 (AfuLap2), respectively, were used (2, 31). *Escherichia coli* LE392 was used for the propagation of the bacteriophage λEMBL3 (Promega, Madison, WI). Direct cloning of PCR products in the pDrive vector was performed using a PCR cloning kit (Qiagen, Hombrechtikon,

* Corresponding author. Mailing address: Service de Dermatologie et Vénéréologie, Centre Hospitalier Universitaire Vaudois, BT422, 1011 Lausanne, Switzerland. Phone: 41 21 3140376. Fax: 41 21 3140378. E-mail: Michel.Monod@chuv.ch.

▽ Published ahead of print on 14 May 2010.

Switzerland). All plasmid subcloning experiments were performed with *E. coli* XL1-Blue with plasmids pUC19 and pPICZA (Invitrogen).

**Cloning of the *P. pastoris* gene encoding PrB.** A *P. pastoris* genomic λEMBL3 library was constructed as previously described for *Candida parapsilosis* (10). *Saccharomyces cerevisiae* DNA encoding vacuolar proteinase B (PrB) was used as a probe for cloning of the orthologous gene in *P. pastoris*. Recombinant plaques ($2 \times 10^4$) of the genomic library were immobilized on GeneScreen nylon membranes (NEN Life Science Products). The filters were hybridized with $^{32}$P-labeled probe under low-stringency conditions (30). All positive plaques were purified, and the associated bacteriophage DNAs were isolated as described previously (16). Agarose gel electrophoresis of restricted recombinant bacteriophage λEMBL3 DNA, Southern blotting, and subcloning of hybridizing fragments from bacteriophages into pUC19 were performed using standard protocols (36). DNA was sequenced by Microsynth (Balgach, Switzerland).

***Pichia pastoris* transformation.** *Pichia pastoris* strains were transformed by electroporation with 5 to 10 μg of linearized plasmid DNA. Using pPICZA construct DNA, transformants were selected on YPDS medium containing 100 μg ml$^{-1}$ Zeocin (Invitrogen). His$^+$ GS115 and GS115-*sub2* transformants were selected on histidine-deficient medium (1 M sorbitol, 1% [wt/vol] dextrose, 1.34% [wt/vol] yeast nitrogen base [YNB] without amino acids, $4 \times 10^{-5}$% [wt/vol] biotin, $5 \times 10^{-3}$% amino acids [i.e., $5 \times 10^{-3}$% {wt/vol} each L-glutamic acid, L-methionine, L-lysine, L-leucine, and L-isoleucine], 2% [wt/vol] agarose). Subsequently, the transformants were screened for insertion of the construct at the *AOX1* site on minimal-methanol plates (1.34% [wt/vol] YNB without amino acids, $4 \times 10^{-5}$% [wt/vol] biotin, 0.5% [vol/vol] methanol, 2% [wt/vol] agarose). Transformants unable to grow on media containing only methanol as a carbon source were retained for further investigations. They were assumed to contain the construct at the correct yeast genomic location by integration events in the *AOX1* locus displacing the *AOX1* coding region.

**Native secreted subtilisin and heterologous enzyme production in *P. pastoris*.** *Pichia pastoris* GS115 and KM71 as well as *P. pastoris* transformants were grown to near saturation (optical density [OD] of 20 at 600 nm) at 30°C in 10 ml of glycerol-based yeast media (0.1 M potassium phosphate buffer at pH 6.0, containing 1% [wt/vol] yeast extract, 2% [wt/vol] peptone, 1.34% [wt/vol] YNB without amino acids, 1% [vol/vol] glycerol, and $4 \times 10^{-5}$% [wt/vol] biotin). Cells were harvested by centrifugation and resuspended in 2 ml of the same medium with 0.5% (vol/vol) methanol instead of glycerol and incubated for 2 days. Thereafter, the culture supernatants were separated from the yeast cells by centrifugation and retained for protein analysis. Salts and low-molecular-weight solutes were removed from 2.5 ml of *P. pastoris* culture supernatant by passing through a PD10 column (Amersham Pharmacia, Buckinghamshire, United Kingdom) by using 20 mM Tris-HCl buffer (pH 7.5) before testing for proteolytic activity. Protein concentrations were measured by the Bradford method with a commercial reagent (Bio-Rad, Hercules, CA) and by densitometry on SDS-PAGE gels with the aid of Adobe Photoshop software (Adobe Systems Inc., San Jose, CA) with different amounts of bovine serum albumin as standards.

**Protein gel electrophoresis.** Extracts were analyzed by SDS-PAGE (25) with a separation gel of 12% polyacrylamide. To detect proteolytic activity of culture supernatant, gelatin at a concentration of 0.1% was added to the gel. In this case, 10 μl of *P. pastoris* culture supernatant was simply added to an equal volume of SDS-PAGE sample buffer without β-mercaptoethanol (125 mM Tris, pH 6.8, 4% SDS, 20% glycerol, 0.002% bromophenol blue) and the mixture was loaded without heating onto SDS-PAGE gels. Samples were pretreated with phenylmethanesulfonyl fluoride (PMSF) or EDTA at a concentration of 10 or 200 mM, respectively, during 10 min at room temperature before the samples were loaded for the characterization of proteolytic activities. After electrophoresis, the gels were incubated twice for 15 min at room temperature in 10 volumes of 2.5% Triton X-100 under constant agitation. The gels were then incubated overnight (16 h) with 10 mM sodium phosphate buffer, pH 7.0 or pH 4.0, in the presence of or in the absence of PMSF or EDTA when the samples had been treated with these proteolytic inhibitors before loading. The gels were stained for 30 min with 0.1% Coomassie brilliant blue R-250 (Bio-Rad) in ethanol-acetic acid-water (40:10:50) and destained with the same solvent.

**Proteolytic activity.** Proteolytic activity was assayed using resorufin-labeled casein as previously described (5) and the chromogenic substrate *N*-succinyl-Ala-Ala-Pro-Phe-*p*-nitrounilide (*N*-Suc-Ala-Ala-Pro-Phe-pNA), (Sigma) (8, 28). Sodium acetate buffer (pH 3 to 6.5), Tris-HCl buffer (pH 7 to 9), and glycine-NaOH buffer (pH 9 to 11) were used. With *N*-Suc-Ala-Ala-Pro-Phe-pNA, a substrate stock solution was prepared at a 20 mM concentration (12 mg/ml) in dimethyl sulfoxide (DMSO) and stored at −20°C. The reaction mixture contained a concentration of 1.0 mM substrate and enzyme preparation (between 0.1 to 1.0 μg per assay) in 100 μl of 50 mM buffer, at different pH values. After incubation at 37°C for 60 min, the reaction was terminated by the addition of 5 μl of glacial acetic acid followed by the addition of 0.9 ml of water to the mixture. The released pNA was measured by spectrometry as the change in $A_{405}$. A control with a blank substrate and blank culture broth was carried out in parallel. The enzyme activities were expressed in mU (nmol of released pNA/min at pH 7.0) with *N*-Suc-Ala-Ala-Pro-Phe-pNA as a substrate.

Inhibitors were from a protease inhibitor set (Roche Diagnostics, Rotkreuz, Switzerland), and stock solutions were prepared as recommended by the supplier. Sub2 (0.1 μg) in 0.2 ml of 20 mM Tris-HCl buffer (pH 7.0) was incubated for 10 min at 37°C either with PMSF (10 μg/ml) or with antipain (50 μg/ml), bestatin (40 μg/ml), chymostatin (6 μg/ml), E64 (10 μg/ml), leupeptin (5 μg/ml), pepstatin (0.7 μg/ml), phosphoramidon (330 μg/ml), EDTA (0.2 mg/ml), or aprotinin (2 μg/ml), following the recommendations of the supplier. The mixture was incubated for 10 min at 37°C, and assays were subsequently performed after the addition of the substrate to the mixture. An appropriate control without inhibitor was assayed simultaneously.

**Protein identification by liquid chromatography-tandem mass spectrometry (LC-MS-MS).** Coomassie blue-stained bands were excised from the SDS-PAGE gels and in-gel digested with sequencing-grade trypsin (Promega) as previously described (37, 40). Extracted peptides were analyzed on a hybrid linear trap LTQ Orbitrap mass spectrometer (Thermo Fisher, Bremen, Germany) interfaced via a TriVersa Nanomate (Advion Biosciences, Norwich, United Kingdom) to an Agilent 1100 nano-high-performance-liquid-chromatography (nano-HPLC) system (Agilent Technologies, Waldbronn, Germany). Solvents used for the mobile phase were 95:5 $H_2O$-acetonitrile (vol/vol) with 0.1% formic acid (solvent A) and 5:95 $H_2O$-acetonitrile (vol/vol) with 0.1% formic acid (solvent B).

Peptides were loaded onto a ZORBAX 300SB $C_{18}$ trapping microcolumn (5 mm by 300 μm, with an inner diameter of 5 μm; Agilent) in $H_2O$-acetonitrile at 97:3 (vol/vol) plus 0.1% formic acid at a flow rate of 10 μl/min. After 5 min, they were back-flush eluted and separated on a reversed-phase nanocolumn, a ZORBAX 300SB $C_{18}$ column (75 μm inner diameter by 15 cm, 3.5 μm; Agilent), at a flow rate of 300 nl/min with a gradient from 5 to 85% acetonitrile in 0.1% formic acid as follows: 5 min at 0% solvent B, from 0 to 25% solvent B in 35 min, 25 to 50% solvent B in 15 min, 50 to 90% solvent B in 5 min, 90% solvent B during 10 min, 90 to 0% solvent B in 5 min, and 0% for 15 min (total time, 90 min).

For spraying, a 400-nozzle ESI (electrospray ionization) chip (Advion Biosciences) was used with a voltage of 1.65 kV, and the mass spectrometer capillary transfer temperature was set at 200°C. In data-dependent acquisition controlled by Xcalibur 2.0.7 software (Thermo Fisher), the four most intense precursor ions detected in the full MS survey performed in the Orbitrap (range, 350 to 1,500 *m/z*; resolution, 60,000 at *m/z* 400) were selected and fragmented. MS-MS was triggered by a minimum signal threshold of 10,000 counts and carried out at a relative collision energy of 35%, with isolation width of 4.0 atomic mass units (amu). Only precursors with a charge higher than 1 were selected for collision-induced dissociation (CID) fragmentation and fragment ions were analyzed in the LTQ linear trap. The *m/z* of fragmented precursors was then dynamically excluded, with a tolerance of 0.01 amu, from any selection during 120 s.

From raw files, MS-MS spectra were exported as mgf (Mascot generic file, text format) files by using the extract_msn.exe script (Thermo Fisher) with the following settings: peptide mass range, 500 to 5,000; minimum total ion intensity threshold, 500; minimum number of fragment ions, 15; minimum signal-to-noise ratio needed for a peak to be written, 3.

Samples were analyzed using Mascot 2.2 (Matrix Science, London, United Kingdom). Mascot was set up to search a custom-built database containing the sequences of the vacuolar proteinase B (YscB) and of contaminants (enzymes and keratins). Semispecific cleavage at K and R (not before P) was used as the enzyme definition, with a maximum of one missed cleavage allowed. Mascot was searched with a fragment ion mass tolerance of 0.50 Da and a parent ion tolerance of 10 ppm. The iodoacetamide derivative of cysteine was specified in Mascot as a fixed modification. Deamidation of asparagine and glutamine, oxidation of methionine, and protein N acetylation were specified as variable modifications.

**Phylogenetic analysis.** Amino acid sequences of *P. pastoris* PrB and Sub2 were used as a query to seek orthologous sequences in 54 available fungal genomes by using the blastp algorithm. A total of 27 subtilisins from selected species and showing a blast score of >200 were submitted to phylogenetic analyses. The subset included sequences from yeasts (from the subphylum *Saccharomycotina*, *Saccharomyces cerevisiae*, *P. pastoris*, *Pichia guilliermondii*, *Candida albicans*, *Candida tropicalis*, and *Yarrowia lipolytica*), filamentous ascomycetes (from the subphylum *Pezizomycotina*, *Aspergillus fumigatus*, *Aspergillus clavatus*, and *Trichophyton rubrum*), and basidiomycetes (*Ustilago maydis*, *Laccaria bicolor*, and *Cryptococcus neoformans*). Proteinase K from *Tritirachium album* was also included, and the type sequence of S8 Merops peptidase family, subtilisin Carlsberg from *Bacillus licheniformis*, was used as a potential outgroup.

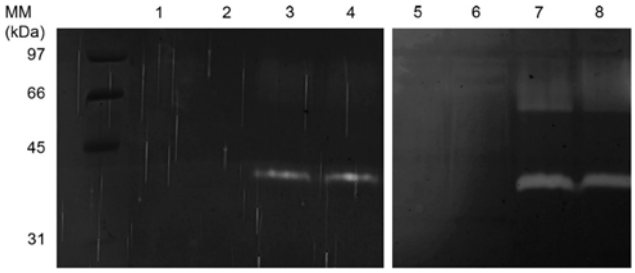



FIG. 1. Native secreted subtilisin produced by *P. pastoris* GS115, KM71, and derivative strains in methanol medium. (Left panel) Ten microliters of *P. pastoris* culture supernatant was loaded onto the gel (lane 1, GS115-*sub2*; lane 2, KM71-*sub2*; lane 3, GS115; lane 4, KM71). (Right panel) *P. pastoris* GS115 and KM71 were grown to near saturation (OD of 20 at 600 nm) at 30°C in 10 ml of glycerol-based yeast medium for 2 days. Cells were harvested by centrifugation and resuspended in 2 ml of the same medium with 0.5% (vol/vol) methanol instead of glycerol and incubated for 2 days. For lanes 5 (GS115) and 6 (KM71), 10 μl of 50×-concentrated culture supernatant in glycerol-based yeast medium was loaded onto the gel. For lanes 7 (GS115) and 8 (KM71), 10 μl of 10×-concentrated culture supernatant in methanol medium as appropriate controls was loaded onto the gel. MM, molecular mass.

Amino acid sequences were aligned using ClustalW as implemented in BioEdit Sequence Alignment Editor software (18). Phylogenetic analyses were performed using the following reconstruction methods and parameters: PhyML (17) with Shimodaira-Hasegawa (SH)-like approximate likelihood ratio test, four substitution rate categories, and estimation of gamma distribution parameter and proportion of invariable sites; BIONJ (12), using a Dayhoff point accepted mutation (PAM) substitution matrix; TNT (15) with sectorial search and tree fusing options; MrBayes (35) with general time-reversible (GTR) likelihood model and 100,000 Markov chain Monte Carlo generations. Phylogenetic trees were edited by using Dendroscope (20).

**Nucleotide sequence accession number.** The *SUB2* nucleotide sequence has been deposited in the GenBank database with the accession number GU46877.

## RESULTS

**Endogenous proteolytic activity of *P. pastoris* GS115 and KM71.** By use of SDS-PAGE gels containing gelatin as a substrate, two different proteolytic activities were detected in the supernatant of *P. pastoris* cultures in methanol medium. One protease showed a distinct band with an apparent molecular mass of 37 kDa (Fig. 1). A second activity was represented by a large proteolytic zone with an apparent molecular mass between 55 and 80 kDa. The 37-kDa protease was completely inhibited when the gel was incubated in the presence of 1 mM PMSF but not in the presence of 1,10-phenanthroline or EDTA, suggesting that it was a serine protease (data not shown). The 55- to 80-kDa protease was not inhibited by PMSF or the aforementioned chelating agents. Gelatinolytic activities of both strains were stronger at pH 7.0 than they were at pH 4.0 (data not shown).

**Cloning of a gene conferring native *P. pastoris* proteolytic activity.** Cloning experiments were performed before genome sequences were available (9, 27). In a first attempt, we tried to disrupt the gene encoding vacuolar protease B (PrB) in *P. pastoris* strain GS115. Protease B is a serine protease of the subtilisin family and was postulated to be secreted by *P. pastoris* (38). A gene encoding the putative *P. pastoris* PrB (*PRB*) was cloned from a *P. pastoris* genomic library as described in Materials and Methods. A central part of *PRB* was subsequently amplified using P1 and P2 as primers (Table 1) and using genomic DNA of *P. pastoris* GS115 as a template. The PCR product (785 bp) was digested by BamHI and XhoI restriction enzymes and ligated to the large fragment of *P. pastoris* expression vector pPICZA cut with BglII and XhoI. The resulting plasmid (pBL1263) corresponded to plasmid pPICZA in which the *AOX1* promoter was replaced by part of *PRB*. Plasmid pBL1263 was cut by a unique BglII restriction site in the cloned fragment, and linearized DNA was used to transform *P. pastoris* GS115. Transformants resistant to Zeocin were screened for gelatinolytic activities on SDS-PAGE and verified for targeted integration of pBL1263 in GS115 by Southern blotting (3; data not shown). As a result, *PRB*-targeted disruption did not abolish the gelatinolytic activity in the *P. pastoris* cell culture supernatant. Therefore, we attempted to clone another gene encoding a subtilisin for subsequent disruption.

Two primers, P3 and P4 (Table 1), were designed from an alignment of genes encoding known fungal subtilisins. PCR amplification of *P. pastoris* genomic DNA produced two 400-bp products which could be distinguished by BglII digestion. The two fragments were separately cloned into the multiple cloning site of the pDrive vector by using a PCR fragment cloning kit and sequenced. One fragment encoded part of PrB, and the second fragment apparently encoded an amino acid sequence from another subtilisin, which was called Sub2. The latter fragment was recovered from the pDrive construct after BamHI-XhoI digestion and ligated to the large fragment of *P. pastoris* expression vector pPICZA digested with restriction enzymes BglII and XhoI. The resulting plasmid (pKS1) cut by Bsu96I (SauI) at a unique restriction site was used to transform *P. pastoris* GS115 and KM71 (Fig. 2). Transformants resistant to Zeocin were screened on SDS-PAGE gels to which gelatin

TABLE 1. Primers used in this study

| Primer[a] | Oligonucleotide sequence (5′–3′)[b] | Location | PCR product size (bp) (cloning site) |
|---|---|---|---|
| P1 | GTT*GGATCC*ATCCAGCAGTTGCTTTCATTG | *P. pastoris PRB* | 785 (BamHI-XhoI) |
| P2 | CTT*CTCGAG*AGTACCAGACAAGGTAGCAG | Complement of *PRB* | |
| P3 | GGYCACGGXACXCACGTXGCXGGXAC | Subtilisin genes | 495 |
| P4 | GTGRGGXGTXGCCATXGAXGTXCC | Complement of subtilisin genes | |
| P5 | GTT*TTCGAA*GAAATGAAAATTATCAGATTAGCATCA | *SUB2* | 1,455 (SfuI-XhoI) |
| P6 | CTT*CTCGAG*TTAATAATCCCAAAATTCAGT | Complement of *SUB2* | |

[a] P3 and P4 were designed on the basis of alignments of nucleotide sequences encoding *A. fumigatus* Alp1 (GenBank CAA77666), *Y. lipolytica* secreted alkaline protease (MEROPS000340), *S. cerevisiae* PrB, *C. albicans* PrB (MEROPS137808), and *C. albicans* putative secreted subtilisin (MEROPS16558).
[b] Restriction sites are indicated in italics.

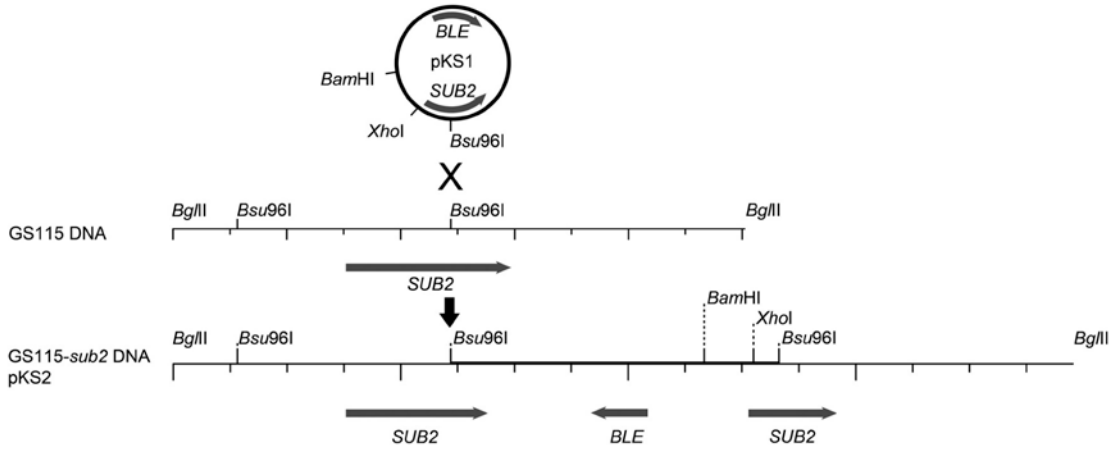



FIG. 2. Chromosomal integration of pKS1 in GS115 and KM71 transformants. *SUB2*, *P. pastoris SUB2* gene coding for the secreted subtilisin; *BLE*, *Streptoalloteichus hindustanus BLE* gene for resistance to Zeocin. The sequence of the 9-kb BglII-BglII fragment in GS115 and KM71 transformants is identical to the sequence of pKS2.

was added. One GS115 transformant out of 10 and 1 KM71 transformant out of 8 did not show any 37-kDa proteolytic component in the supernatant after growth in methanol medium (Fig. 1). These clones were designated GS115-*sub2* and KM71-*sub2*, respectively.

In order to clone the full-length *SUB2* gene, 1-μg samples of GS115-*sub2* genomic DNA were separately digested by different enzymes (DraI, BglII, ClaI, PstI, and EcoRI) and religated, and the products were then used to transform *E. coli*. Five hygromycin-resistant clones obtained from DNA digested by BglII were shown to harbor a 9-kb plasmid (pKS2; Fig. 2). Plasmid sequencing revealed the beginning and the end of *SUB2* upstream and downstream of the pPICZA sequence, respectively (Fig. 2). The *SUB2* sequence was identical to that encoding the protein with the GenBank accession number CAY68404 ["Vacuolar proteinase B (yscB), a serine protease of the subtilisin family"] in the *Pichia pastoris* chromosome 1 complete sequence (GenBank accession number FN3922319, locus tag PAS_chr1-4_548) (9).

Sub2 was predicted to be encoded by 1,431 nucleotides starting from the ATG codon and appeared to be synthesized as a precursor in a preprotein form of 477 amino acids. Inspection of the N-terminal amino acid sequence of the precursor beginning from the Met1 residue suggested the existence of a signal peptide in the protease precursor polypeptide with a hydrophobic core. Two putative signal peptidase cleavage sites in accordance with the −3 −1 von Heijne's rule (39) were found after the Ala and Ser residues at positions 16 and 18, respectively (Fig. 3). Based on alignment with different fungal secreted subtilisins (28), the N-terminal amino acid sequence of the mature Sub2 was apparently preceded by a precursor protein peptide (prepropeptide) of 168 amino acids, and the mature protein is 309 amino acids long, with a calculated molecular mass of 34 kDa and an isoelectric point at pH 5.69. In addition to Ser 402 (position relative to the Met1 residue of the prepropeptide), the alignment of Sub2 with other subtilisins revealed Asp and His residues of the catalytic triad in position 212 and 244, respectively.

**Biochemical characterization of Sub2 obtained from a Sub2-overproducing strain of *P. pastoris*.** DNA encoding Sub2


FIG. 3. Prepropeptide Sub2 amino acid sequence. Vertical arrows indicate putative signal sequence cleavage sites. The Asp, His, and Ser residues of the catalytic triad are indicated by asterisks. Detected MS peptides generated by tryptic digestion of Sub2 secreted by *P. pastoris* are underlined.

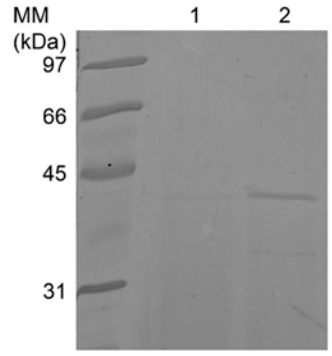



FIG. 4. Overexpression of *P. pastoris SUB2* in *P. pastoris* KM71 (lane 2). Lane 1 shows the culture supernatant of KM71 as a negative control. Ten microliters of *P. pastoris* culture supernatant was loaded onto the gel.

was amplified using primers P5 and P6 (Table 1) and *P. pastoris* genomic DNA as a target. The PCR product was digested with restriction enzymes SfuI and XhoI, for which a recognition site was previously designed at the 5′ extremity of the primers, and ligated to pPICZA cut with the same restriction enzymes. Linearized DNA of the generated plasmid was used to transform *Pichia pastoris* KM71, and Zeocin-resistant transformants were screened for Sub2 overproduction in the culture supernatant. One clone out of 10 was found to secrete Sub2 at a rate of 10 μg/ml. This clone was called KM71-*SUB2* (Fig. 4).

Sub2 produced by gene overexpression had the same electrophoretic mobility as the enzyme secreted by strain GS115 with an estimated mass of 37 kDa (Fig. 5). Identification of the heterologously produced Sub2 was also confirmed using ESI–LC-MS-MS on tryptic digestion of the detected 37-kDa protein in a gel slice (Fig. 3). MS analysis allowed the detection of several different peptides which matched from residues 169 to 468 but not from residues 1 to 168 of the prepropeptide Sub2 amino acid sequence. These results confirmed that cleavage of the prosequence occurred after residue 168 or few residues before, as it was suggested from alignment with other subtilisins.

Purification assays of recombinant Sub2 were attempted by ion-exchange chromatography. However, we observed that Sub2 underwent self-digestion at high concentrations. Therefore, further characterization of Sub2 was performed using enzyme from desalted culture supernatant. The enzyme was found to be active between pH 4.0 and 11.0, with a broad peak of optimum activity between pH 6.0 and 8.0 on resorufin-labeled casein and *N*-Suc-Ala-Ala-Pro-Phe-pNA. The specific activity of Sub2 and that of proteinase K used for comparison for *N*-Suc-Ala-Ala-Pro-Phe-pNA as a substrate at a pH of 7.0 were measured as 6.0 U and 26 mU/μg protein, respectively. Like other subtilisins (29), recombinant Sub2 was totally inhibited by PMSF, antipain, and chymostatin. Aprotinin and leupeptin inhibited the enzymatic activity by 15 to 20% at a concentration of 2 to 5 μg/ml. The enzyme was not inhibited by pepstatin, E64, bestatin, EDTA, and phosphoramidon.

**Amount of Sub2 secreted by *P. pastoris* GS115.** Resorufin-labeled casein substrate as well as *N*-Suc-Ala-Ala-Pro-Phe-pNA was not sensitive enough to detect proteolytic activity in *P. pastoris* culture supernatants. Therefore, proteolytic activities of GS115 and KM71 culture supernatants were compared with serial dilutions of purified recombinant Sub2 extracts to assess the amount of protease secreted by the yeast (Fig. 5). One milliliter of culture supernatant was found to contain approximately 3 ng of Sub2.

***SUB2* disruption in various *P. pastoris* strains producing secreted proteases and use of GS115-*sub2* for heterologous protease production.** *Pichia pastoris* GS115-*DPPIV* and GS115-*LAP2*, producing AfuDppIV and AfuLap2, respectively, were previously constructed (2, 29). Both strains were transformed by pKS1 cut at its unique Bsu96I restriction site, and Zeocin-resistant transformants were screened for *SUB2* disruption. One clone producing AfuDppIV and one clone producing AfuLap2 which no longer produced Sub2 were retained and called GS115-*DPPIV-sub2* and GS115-*LAP2-sub2*, respectively (Fig. 6). Identical results were obtained by transformation of *P. pastoris* GS115-*sub2* with previously used expression plasmids encoding recombinant AfuLap2 and AfuDppIV (2, 31).

**Phylogenetic analyses.** Most selected yeast sequences branched into two derived phylogenetic groups (Fig. 7). *Pichia pastoris* PrB could be unambiguously identified as the ortho-

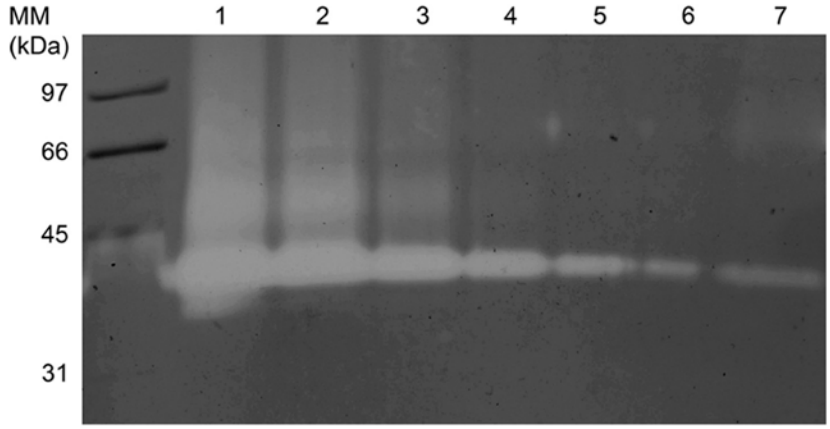



FIG. 5. Assessment of the amount of Sub2 secreted by *P. pastoris* GS115. In lanes 1 to 6, 10 ng, 3 ng, 1 ng, 300 pg, 100 pg, and 30 pg recombinant Sub2, respectively, were loaded on the gel. Lane 7, 10 μl of *P. pastoris* GS115 culture supernatant was loaded onto the gel.

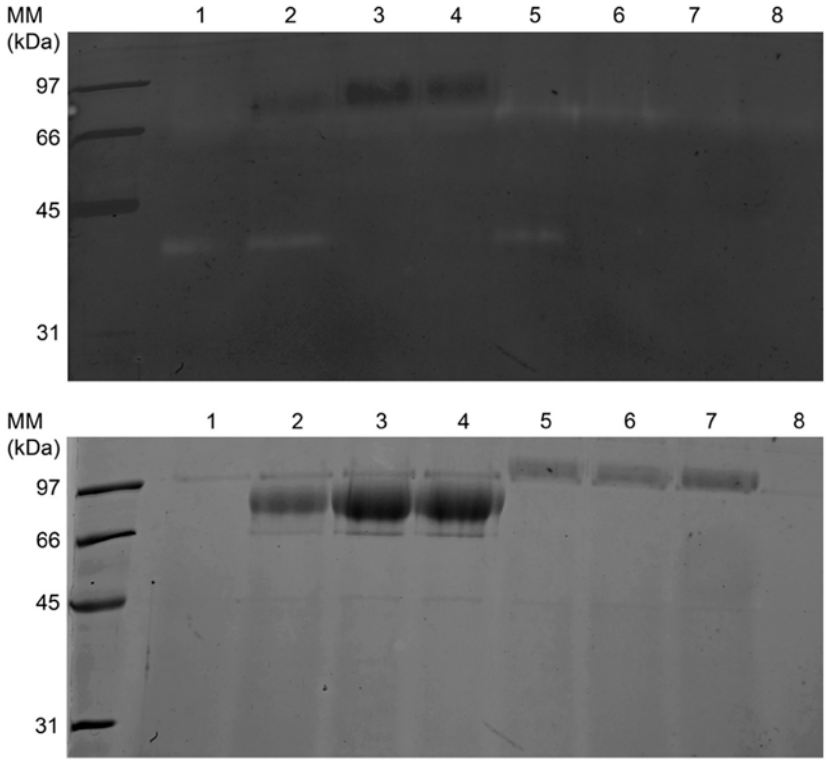



FIG. 6. Use of GS115-*sub2* for heterologous protease production (lanes 3 and 6) and *SUB2* disruption in *P. pastoris* strains producing secreted proteases (lanes 4 and 7). Lane 1, GS115; lane 2, GS115-*LAP2*; lane 3, GS115-*sub2* transformed with pKJ113-*LAP2*; lane 4, GS115-*LAP2* transformed with pKS1 (*SUB2* disruption in an AfuLap2-producing strain); lane 5, GS115-*DPPIV*; lane 6, GS115-*sub2* transformed with pKJ113-*DPPIV*; lane 7, GS115-*DPPIV* transformed with pKS1 (*SUB2* disruption in a DppIV-producing strain); lane 8, GS115-*sub2*. Ten microliters of *P. pastoris* GS115 culture supernatant was loaded onto the gel. The samples were analyzed by SDS-PAGE with a separation gel of 12% polyacrylamide stained with Coomassie blue (lower panel). To detect endoproteolytic activity of culture supernatant, gelatin at a concentration of 0.1% was added to the gel (upper panel).

logue of cerevisin proteases from *S. cerevisiae*, *Y. lipolytica*, *C. albicans*, and *P. guilliermondii* (clade 1; Fig. 7). Depending on the tree reconstruction methods used, *P. pastoris* Sub2 showed slight differences in branching. It formed a monophyletic group (clade 2; Fig. 7) together with hypothetical subtilisins from *Candida* spp. and *P. guilliermondii* in Bayesian (MrBayes) and distance (BIONJ) analyses, whereas it branched at the base of the group formed by clades 1 and 2 in maximum likelihood (PhyML) and parsimony (TNT) analyses. Clade 2 also comprised single Sub2 orthologues from other yeasts, namely, *Debaryomyces hansenii*, *Lodderomyces elongisporus*, *Pichia stipitis*, and *Clavispora lusitaniae* (data not shown). *Pichia pastoris* Sub2 showed 44 to 46% identity similarity with putative orthologues in other yeast species from clade 2. Both clades 1 and 2 are clearly yeast specific: vacuolar and secreted subtilisins from basidiomycetes and from filamentous ascomycetes (*Aspergillus* spp. and *Trichophyton rubrum*) formed the basal groups of the tree (Fig. 7).

## DISCUSSION

The *P. pastoris* genome harbors two genes encoding serine proteases of the subtilisin family, *PRB* coding for the vacuolar proteinase B and *SUB2* coding for the protease which gained particular attention in the present work. We have shown that Sub2 is secreted by *P. pastoris* in methanol medium in a high-cell-density process but not in the same medium containing glycerol as a carbon source instead of methanol (Fig. 1). While *P. pastoris* PrB is clearly orthologous to other vacuolar proteases (cerevisins) found in most yeast genomes, Sub2 belongs to another group of proteases which contains other putative yeast subtilisins (Fig. 7). Putative orthologues of Sub2 were found in only 7 out of 17 yeast genomes (subphylum *Saccharomycotina*) available. *Pichia pastoris* Sub2 is believed to be the first of these subtilisins identified in yeast culture supernatant. The derived phylogenetic positions of most yeast subtilisins compared to those of the basidiomycetes and of filamentous ascomycetes suggest a relatively recent radiation through gene duplication.

During the production of heterologous proteins in a high-cell-density process, some of the secreted heterologous proteins produced by *P. pastoris* were found to be subjected to substantial proteolysis (21, 22, 38). The major store of proteolytic activity in yeasts is located within the lumen of the vacuolar compartment (23), and previous reports disclosed that the proteolytic degradation of secreted recombinant proteins in *P. pastoris* was due to the release of proteases in the culture medium caused by cell lysis (27, 38). While growth at high cell density enables the production of heterologous proteins in remarkably high yields, it also increases the level of vacuolar

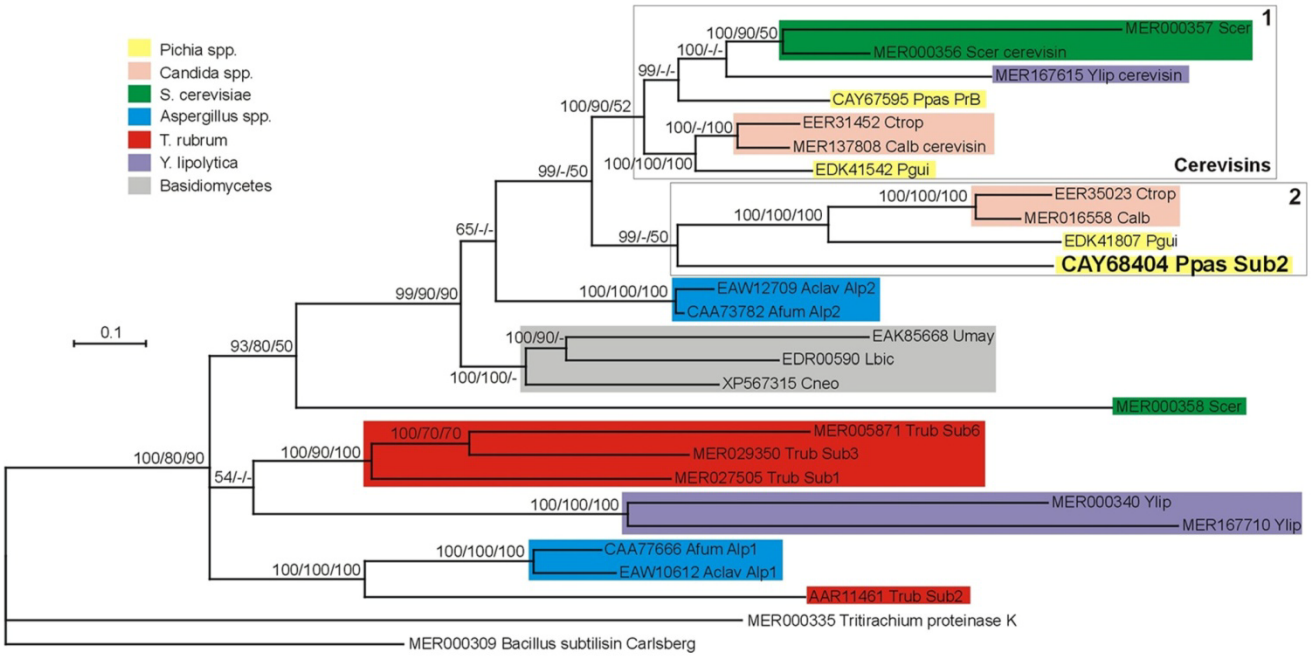



FIG. 7. Phylogenetic position of *Pichia pastoris* Sub2 (CAY68404) relative to other fungal proteases of the S8 family inferred from a Bayesian analysis of amino acid sequences. The numbers at nodes represent clade credibility or bootstrap values (100 replicates) obtained with MrBayes/PhyML/BIONJ methods, respectively. Nodes with credibility values lower than 50 were collapsed. Subtilisin Carlsberg from *Bacillus licheniformis* was used as a potential outgroup. Each sequence is identified by its GenBank or Merops accession number, species abbreviation, and protease name (if any). The colors of the rectangles correspond to specific species or taxonomic groups. Clade 1 comprises all cerevisin sequences; clade 2 comprises *Pichia pastoris* (Ppas) Sub2 and its putative orthologues from other yeast species. The scale bar indicates the number of expected changes per site. Scer, *S. cerevisiae*; Ylip, *Y. lipolytica*; Ctrop, *C. tropicalis*; Calb, *Candida albicans*; Pgui, *P. guilliermondii*; Aclav, *A. clavatus*; Afum, *A. fumigatus*; Umay, *U. maydis*; Lbic, *Laccaria bicolor*; Cneo, *C. neoformans*; Trub, *T. rubrum*.

proteases in the fermentation media (14). Using *P. pastoris* as an expression system to produce heterologous secreted protein, one should also be aware of the presence of secreted Sub2 in culture supernatant. The nature of the 55- to 80-kDa proteolytic activity was not elucidated, but similar 55- to 80-kDa activity was located in the periplasmic fraction of *P. pastoris* (38). It is possible that the protease is released in the culture supernatant either after cell lysis or by diffusion though the cell wall.

Apparently, Sub2 was insensitive to 55- to 80-kDa proteolytic activity. It is likely that an organism adapts to secrete proteins which are immune to degradation by its own proteases. For instance, in other fungi, such as *Aspergillus* spp. and dermatophytes, multiple proteases are concomitantly secreted in culture supernatant and are not degraded (13, 33). In addition, the acquisition of the correct three-dimensional structure may protect secreted proteins from proteolysis. However, various attempts to suppress proteolytic degradation of secreted recombinant proteins in the *P. pastoris* culture supernatant were undertaken. For example, conditions under which recombinant strains are grown were modified so as to inhibit protease activity. Adjusting the pH of the culture to 6.0 or lowering the temperature of the medium allowed a substantial reduction of proteolytic activity (21, 26). Determining the optimum induction time in methanol medium for recombinant protein production was also found to be important (22). To inhibit proteolysis in culture supernatant, vacuolar protease-deficient strains of *P. pastoris* were also constructed (14). Although vacuolar proteases are required for several developmental transitions in the life cycle of yeast cells (e.g., sporulation), they are dispensable for vegetative growth. The same is true for Sub2, as *SUB2* disruption does not alter the viability of *P. pastoris*. Therefore, *P. pastoris sub2*-deficient strains could be used to suppress one of the causes of secreted heterologous enzyme proteolysis. Although secreted recombinant proteases were not subject to proteolysis, GS115-*sub2* was used as a tool for the production of exoproteases without endogenous subtilisin contamination.

## ACKNOWLEDGMENTS

We thank Massimo Lurati for critical review of the manuscript and assistance with the English and Marina Fratti for helpful discussions and technical assistance.

This work was supported by the Swiss National Foundation for Scientific Research, grant 320030-1179641.

## REFERENCES

1. **Beauvais, A., M. Monod, J. P. Debeaupuis, M. Diaquin, H. Kobayashi, and J. P. Latgé.** 1997. Biochemical and antigenic characterization of a new dipeptidyl-peptidase isolated from *Aspergillus fumigatus*. J. Biol. Chem. **272:** 6238–6244.
2. **Beauvais, A., M. Monod, J. Wyniger, J. P. Debeaupuis, E. Grouzmann, N. Brakch, J. Svab, A. G. Hovanessian, and J. P. Latge.** 1997. Dipeptidyl-peptidase IV secreted by *Aspergillus fumigatus*, a fungus pathogenic to humans. Infect. Immun. **65:**3042–3047.
3. **Beggah, S., B. Léchenne, U. Reichard, S. Foundling, and M. Monod.** 2000. Intra- and intermolecular events direct the propeptide-mediated maturation of the *Candida albicans* secreted aspartic proteinase Sap1p. Microbiology **146:**2765–2773.
4. **Borelli, C., E. Ruge, J. H. Lee, M. Schaller, A. Vogelsang, M. Monod, H. C.**

**Korting, R. Huber, and K. Maskos.** 2008. X-ray structures of Sap1 and Sap5: structural comparison of the secreted aspartic proteinases from *Candida albicans*. Proteins **72:**1308–1319.
5. **Borg-von Zepelin, M., S. Beggah, K. Boggian, D. Sanglard, and M. Monod.** 1998. The expression of the secreted aspartyl proteinases Sap4 to Sap6 from *Candida albicans* in murine macrophages. Mol. Microbiol. **28:**543–554.
6. **Couderc, R., and J. Baratti.** 1980. Oxidation of methanol by the yeast *Pichia pastoris*: purification and properties of alcohol oxidase. Agric. Biol. Chem. **44:**2279–2289.
7. **Cregg, J. M., K. R. Madden, K. J. Barringer, G. P. Thill, and C. A. Stillman.** 1989. Functional characterization of the two alcohol oxidase genes from the yeast *Pichia pastoris*. Mol. Cell. Biol. **9:**1316–1323.
8. **DelMar, E. G., C. Largman, J. W. Brodrick, and M. C. Geokas.** 1979. A sensitive new substrate for chymotrypsin. Anal. Biochem. **99:**316–320.
9. **De Schutter, K., Y. C. Lin, P. Tiels, A. Van Hecke, S. Glinka, J. Weber-Lehmann, P. Rouzé, Y. Van de Peer, and N. Callewaert.** 2009. Genome sequence of the recombinant protein production host *Pichia pastoris*. Nat. Biotechnol. **6:**561–566.
10. **De Viragh, P. A., D. Sanglard, G. Togni, R. Falchetto, and M. Monod.** 1993. Cloning and sequencing of two *Candida parapsilosis* genes encoding acid proteases. J. Gen. Microbiol. **139:**335–342.
11. **Ellis, S. B., P. F. Brust, P. J. Koutz, A. F. Waters, M. M. Harpold, and T. R. Gingeras.** 1985. Isolation of alcohol oxidase and two other methanol regulatable genes from the yeast *Pichia pastoris*. Mol. Cell. Biol. **5:**1111–1121.
12. **Gascuel, O.** 1997. BIONJ: an improved version of the NJ algorithm based on a simple model of sequence data. Mol. Biol. Evol. **14:**685–695.
13. **Giddey, K., M. Monod, J. Barblan, A. Potts, P. Waridel, C. Zaugg, and M. Quadroni.** 2007. Comprehensive analysis of proteins secreted by *Trichophyton rubrum* and *Trichophyton violaceum* under in vitro conditions. J. Proteome Res. **6:**3081–3092.
14. **Gleeson, M. A., and B. D. Howard.** November 1997. Genes which influence *Pichia* proteolytic activity, and uses therefor. U.S. patent 5,691,166.
15. **Goloboff, P., J. Farris, and K. Nixon.** 2008. TNT: a free program for phylogenetic analysis. Cladistics **24:**774–786.
16. **Grossberger, D.** 1987. Minipreps of DNA from bacteriophage lambda. Nucleic Acids Res. **15:**6737.
17. **Guindon, S., and O. Gascuel.** 2003. A simple, fast and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol. **52:**696–704.
18. **Hall, T. A.** 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. **41:**95–98.
19. **Higgins, D. R., and J. M. Cregg.** 1998. *Pichia* protocols. Humana Press, Totowa, NJ.
20. **Huson, D. H., D. C. Richter, C. Rausch, T. Dezulian, M. Franz, and R. Rupp.** 2007. Dendroscope: an interactive viewer for large phylogenetic trees. BMC Bioinformatics **8:**460.
21. **Jahic, M., F. Wallberg, M. Bollok, P. Garcia, and S. O. Enfors.** 2003. Temperature limited fed-batch technique for control of proteolysis in *Pichia pastoris* bioreactor cultures. Microb. Cell Fact. **18:**6.
22. **Jahic, M., M. Gustavsson, A. K. Jansen, M. Martinelle, and S. O. Enfors.** 2003. Analysis and control of proteolysis of a fusion protein in *Pichia pastoris* fed-batch processes. J. Biotechnol. **102:**45–53.
23. **Jones, E. W.** 1991. Tackling the protease problem in *Saccharomyces cerevisiae*. Methods Enzymol. **194:**428–453.
24. **Jousson, O., B. Léchenne, O. Bontems, B. Mignon, U. Reichard, J. Barblan, M. Quadroni, and M. Monod.** 2004. Secreted subtilisin gene family in *Trichophyton rubrum*. Gene **339:**79–88.
25. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227:**680–685.
26. **Li, Z., F. Xiong, Q. Lin, M. d'Anjou, A. J. Daugulis, D. S. Yang, and C. L. Hew.** 2001. Low-temperature increases the yield of biologically active herring antifreeze protein in *Pichia pastoris*. Protein Expr. Purif. **21:**438–445.
27. **Mattanovich, D., A. Graf, J. Stadlmann, M. Dragosits, A. Redl, M. Maurer, M. Kleinheinz, M. Sauer, F. Altmann, and B. Gasser.** 2009. Genome, secretome and glucose transport highlight unique features of the protein production host *Pichia pastoris*. Microb. Cell Fact. **8:**29.
28. **Mignon, B., M. Swinnen, J. P. Bouchara, M. Hofinger, A. Nikkels, G. Pierard, C. Gerday, and B. Losson.** 1998. Purification and characterization of a 31.5 kDa keratinolytic subtilisin-like serine protease from *Microsporum canis* and evidence of its secretion in naturally infected cats. Med. Mycol. **36:**395–404.
29. **Monod, M., G. Togni, L. Rahalison, and E. Frenk.** 1991. Isolation and characterisation of an extracellular alkaline protease of *Aspergillus fumigatus*. J. Med. Microbiol. **35:**23–28.
30. **Monod, M., G. Togni, B. Hube, and D. Sanglard.** 1994. Multiplicity of genes encoding secreted aspartic proteinases in *Candida* species. Mol. Microbiol. **13:**357–368.
31. **Monod, M., B. Léchenne, O. Jousson, D. Grand, C. Zaugg, R. Stocklin, and E. Grouzmann.** 2005. Aminopeptidases and dipeptidyl-peptidases secreted by the dermatophyte *Trichophyton rubrum*. Microbiology **151:**145–155.
32. **Monod, M.** 2008. Secreted proteases from dermatophytes. Mycopathologia **166:**285–294.
33. **Monod, M., O. Jousson, and U. Reichard.** 2009. *Aspergillus fumigatus* secreted proteases, p. 87–106. *In* J.-P. Latgé and W. J. Steinbach (ed.), *Aspergillus fumigatus* and aspergillosis. ASM Press, Washington, DC.
34. **Reichard, U., B. Léchenne, A. R. Asif, F. Streit, E. Grouzmann, O. Jousson, and M. Monod.** 2006. Sedolisins, as new class of secreted proteases from *Aspergillus fumigatus* with endoprotease or tripeptidyl-peptidase activity at acidic pH. Appl. Environ. Microbiol. **72:**1739–1748.
35. **Ronquist, F., and J. P. Huelsenbeck.** 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics **19:**1572–1574.
36. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
37. **Shevchenko, A., M. Wilm, O. Vorm, and M. Mann.** 1996. Mass spectrometric sequencing of proteins silver-stained polyacrylamide gels. Anal. Chem. **68:**850–858.
38. **Sinha, J., B. A. Plantz, M. Inan, and M. M. Meagher.** 2005. Causes of proteolytic degradation of secreted recombinant proteins produced in methylotrophic yeast *Pichia pastoris*: case study with recombinant ovine interferon-tau. Biotechnol. Bioeng. **89:**102–112.
39. **von Heijne, G.** 1986. A new method for predicting signal sequence cleavage sites. Nucleic Acids Res. **14:**4683–4690.
40. **Wilm, M., A. Shevchenko, T. Houthaeve, S. Breit, L. Schweigerer, T. Fotsis, and M. Mann.** 1996. Femtomole sequencing of proteins from polyacrylamide gels by nano-electrospray mass spectrometry. Nature **379:**466–469.
41. **Zaugg, C., O. Jousson, B. Léchenne, P. Staib, and M. Monod.** 2008. *Trichophyton rubrum* secreted and membrane-associated carboxypeptidases. Int. J. Med. Microbiol. **298:**669–682.