Secretomes of medically important fungi reflect morphological and phylogenetic diversity

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ABSTRACT

Secretome represents a main target for understanding the mechanisms of fungal adaptation. In the present study, we focus on the secretomes of fungi associated with infections in humans and other mammals in order to explore relationships between the diverse morphological and phylogenetic groups. Almost all the mammalian pathogenic fungi analyzed have secretome sizes smaller than 1000 proteins and, secreted proteins comprise between 5 % and 10 % of the total proteome. As expected, the correlation pattern between the secretome size and the total proteome was similar to that described in previous secretome studies of fungi. With regard to the morphological groups, minimum secretome sizes of less than 250 secreted proteins and low values for the fraction of secreted proteins are shown in mammalian pathogenic fungi with reduced proteomes such as microsporidia, atypical fungi and some species of yeasts and yeast-like fungi (*Malassezia*). On the other hand, filamentous fungi have significantly more secreted proteins and the highest numbers are present in species of filamentous fungi that also are plant or insect pathogens (*Fusarium verticilloides*, *Fusarium oxysporum* and *Basidiobolus meristosporus*). With respect to phylogeny, there are also variations in secretome size across fungal subphyla: *Microsporidia*, *Taphrinomycotina*, *Ustilagomycotina* and *Saccharomycotina* contain small secretomes; whereas larger secretomes are found in *Agaricomycotina*, *Pezizomycotina*, *Mucoromycotina* and *Entomophthoromycotina*. Finally, principal component analysis (PCA) was conducted on the complete secretomes. The PCA results revealed that, in general, secretomes of fungi belonging to the same morphological group or subphyla cluster together. In conclusion, our results point out that in medically important fungi there is a relationship between the secretome and the morphological group or phylogenetic classification.

1. Introduction

Humans and animals are highly resistant to most invasive infections by fungi (Köhler et al., 2015; Köhler et al., 2017), and only a small number of species in the fungal kingdom can cause human and animal infections (Heitman, 2011; Brown et al., 2012; Gostin ar et al., 2018). Medically important fungi cover true pathogens, commensals and opportunistic pathogens (Sexton and Howlett, 2006; Mayer et al., 2013; Gostin ar et al., 2018). However, a limited proportion of the clinically relevant species of fungi can be considered true pathogens that are able to infect healthy mammal hosts. Commensal fungi typically survive without tissue invasion and can emerge to cause occasional infections. Most opportunistic fungi are environmental species with a saprophytic lifestyle that are not specialized for the mammal host, and usually cause invasive life-threatening systemic infections in immunocompromised hosts (de Hoog et al., 2014). Moreover, many environmental filamentous pathogenic fungi also elicit toxic effects upon ingestion and produce allergic responses (Wanger et al., 2017). Although some fungal pathogens such as *Pneumocystis* species are directly transmitted between individuals, direct transmission is not usually a regular part of the disease cycle of fungal pathogens (Sexton and Howlett, 2006).

Fungi are exodigesters that require secreted proteins to alter their environment and the organisms they colonize (Girard et al., 2013) and, consequently, secretome represents a main target for understanding the mechanisms of fungal adaptation. Secreted proteins play important roles in the development of fungal diseases in mammalian hosts, enabling invasive infection and nutrient acquisition, establishing interactions with hosts and directly modulating the host immune response (Ranganathan and Garg, 2009; Wartenberg et al., 2011; Lowe and Howlett, 2012).

Secretome analysis may have clinical applications toward a better control of fungal diseases of humans and animals providing new information for the identification of potential diagnostic biomarkers, therapeutic targets, vaccine development and antifungal strategies (Girard et al., 2013; Sorgo et al., 2013).

Bioinformatic approaches make possible to the large-scale prediction and analysis of the entire set of secreted proteins in a fungal species (Choi et al., 2010; Lum and Min, 2011; Girard et al., 2013; Cortázar et al., 2014; Cortázar et al., 2015). However, up to now there is only limited knowledge about the diversity, composition and evolution of secretomes of fungi that infect humans and other mammals. The rapid accumulation of complete genome sequences now provide us the opportunity to analyze exclusively the predicted secretomes from fungal species associated with mammalian diseases. In this study, we explore relationships between the secretomes and the different morphological and phylogenetic groups of medically important fungi.

2. Materials and methods

2.1. Organisms and sequence data

To delineate relationships between the secretomes of fungi that are commonly associated with infections in humans and other mammals, publicly available proteomes of fungal species were retrieved from NCBI (as July of 2019). The proteome dataset was composed of 80 representative pathogenic species of the following fungal phyla: *Ascomycota*, *Basidiomycota*, *Mucoromycota*, *Zoopagomycota*, *Microsporidia* and *Chytridiomycota* (Table 1). Only one species per genus was included in the proteome dataset in order to minimize data duplication. The pathogenic fungi analyzed comprised predominantly species belonging to *Ascomycota* (58 species), followed by

Basidiomycota (7 species), *Microsporidia* (7 species), *Mucoromycota* (5 species), *Zoopagomycota* (2 species), and *Chytridiomycota* (1 species). As outgroup species, this proteome dataset contained three fungi that are not pathogenic of mammals: *Batrachochytrium dendrobatidis*, *Encephalitozoon romaleae* and *Pochonia chlamydosporia*. The chytridiomycete *B. dendrobatidis* causes a skin infection in amphibians (Fisher et al., 2012) and currently none of the *Chytridiomycota* are not known to infect mammals (Köhler et al., 2017). The microsporidia *E. romaleae* infects grashoppers and its genome is strikingly similar to those of *Encephalitozoon* species infecting humans (Pombert et al., 2012). Finally, the ascomycete *P. clamydosporia* infects eggs and females of economically important plant-parasitic nematodes (Braga and de Araújo, 2014).

2.2. Secretome analysis: prediction of secreted proteins

The secretomes of fungal species (Table S1) were obtained using the pipeline SECRETOOL, a bioinformatic resource for prediction of secreted proteins in fungi (Cortázar et al., 2014). SECRETOOL is a web-based analysis tool (http://genomics.cicbiogune.es/SECRETOOL/Secretool.php) that comprises a group of widely used bioinformatics tools, such as SignalP, TargetP, TMHMM, PredGPI, and WoLF PSORT. Sperschneider et al. (2015) have already evaluated the sensitivity and specificity of these individual bioinformatics tools applied to predictions of secreted proteins in fungi. In addition, Vivek-Ananth et al. (2018) have carried out a comparative analysis of computational pipelines for secretome prediction in fungi, including SECRETOOL. Only soluble secreted proteins through a classical secretion pathway and satisfying the following parameters were analyzed: proteins that possess an N-

terminal signal peptide for entering the classical secretion pathway, without the presence of internal transmembrane domains, complete absence of GPI-anchor sites and extracellular secretion of the proteins outside the cell. The identification of secreted proteins using SECRETOOL was designed to be extra stringent, and exclusion of some proteins can occur in the predicted secretomes due to the stringency of the combined default values: SignalP (cut-off 0.8), TargetP (cut-off 0.8), TMHMM (0 or 1 transmembrane domains), PredGPI (cut-off 0.005), and WoLF PSORT (cut-off 14).

2.3. Machine learning protocols

Full sets of secreted proteins in medically important fungi were compared using an all-versus-all BLAST type approach, and a set of unique ortholog proteins was then used to create the reference column to generate the final table. The set of unique ortholog proteins could be defined as a list consisting of all the secreted orthologs, including those that can be found in different species, and those only present in a particular phylum, genera or species, thus, trying to comprise all the possible candidates derived from the predicted secretomes. A set of Perl an R scripts was designed to carry out BLASTp searches to detect putative orthologs (E-value cut-off e-5). BLASTp output was parsed via an in house Perl script, and a presence/absence (P/A) table was generated: the presence of an ortholog of the protein X in the strain Y was represented as “1”, while its absence was considered as “0”. The P/A table was remodeled and filtered using “unique” function from R data.table package to enable removal of replicated ortholog relations. The rows of the table represented all the unique secreted proteins and the columns represented the analyzed species. The P/A table was the input for unsupervised machine learning protocol via principal component analysis (PCA) to cluster fungal species based on their secretome content in an

unsupervised manner so clustering was not biased by any kind of categorization. Data was processed for PCA analysis using R base function "prcomp" for the PCA analysis itself. Then “caret” package was used to remove zero variance variables and "factoextra" package (https://CRAN.R-project.org/package=factoextra) determined dimension and variable contributions, and plotted the corresponding graphics. Three-dimensional PCA (PCA 3D) were plotted using “rgl” R package (https://CRAN.R-project.org/package=rgl).

3. Results and discussion

3.1. Morphological and phylogenetic diversity

Morphology is an important characteristic used for the differentiation of fungal species, and medically important fungi can be separated into different morphological groups without regard to phylogenetic classification (Sciortino, 2017; Wanger et al., 2017). The principal morphological difference between mammalian pathogenic fungi is that one group grows as yeast or yeast-like single cells, and other group has a multicellular filamentous growth. Moreover, the thermally dimorphic fungi pathogenic to humans and mammals overlap between these two morphological groups, and include *Ascomycota* species that are capable of temperature dependent alterations in morphological state. A common attribute of all thermally dimorphic fungi is the response to shifts in temperature by transforming from mycelial growth in the environment to yeast cells within the mammalian host (Gauthier, 2017). On the other hand, both atypical fungi and microsporidia are obligate intracellular parasites in humans and animals. Atypical fungi comprises *Pneumocystis* species with at least two different life cycle forms, the trophic form and the cyst (Ma et al., 2018). Microsporidia

are highly host dependent fungi with an extreme genome reduction (Han and Weiss, 2017; Wadi and Reinke, 2020).

For the purposes of this study, fungal species that cause infections in mammals were assigned into the above groups based on their morphological characteristics: yeasts and yeast-like fungi, filamentous fungi, thermally dimorphic fungi, atypical fungi, and microsporidia (Table 1). Pathogenic yeasts and yeast-like fungi comprise species of *Ascomycota* belonging to *Saccharomycotina*, and *Basidiomycota* belonging to *Ustilagomycotina* and *Agaromycotina* (Table 1). Filamentous fungal pathogens are mainly species of *Ascomycota* belonging to *Pezyzomycotina*, except for zygomycete fungi and the basidiomycete *Schizophyllum commune*. Besides that, the pathogenic filamentous fungi are a broad group of species that can be assorted into different morphological subgroups: dermatophyte fungi, hyaline fungi, dematiaceous fungi and zygomycete fungi (Table 1). Dermatophyte fungi cause a variety of skin diseases in animals and humans. Dermatophyte fungi and thermally dimorphic fungi are closely related *Ascomycota*, all belonging to *Pezizomycotina* order *Onygenales* (Martinez et al., 2012). Hyaline fungi are characterized by narrow, septate and colorless hyphae; dematiaceous fungi contain melanin in their cell wall, which causes the brown and black coloured pigmentation of the septate hyphae; and zygomycete fungi usually form aseptate hyphae (Sciortino, 2017; Wanger et al., 2017). The basidiomycete *S. commune* hyaline fungus belongs to *Agarocomycotina*, and is a wood decomposer that has also been demonstrated to be an opportunistic pathogen of humans and animals (Ohm et al., 2010). Dematiaceous fungi are mainly ubiquitous saprophytic fungi found in soil and plants (Chowdhary et al., 2015). Zygomycete fungi are clinically relevant opportunistic species that have been separated into two phyla, the *Mucoromycota* and *Zoopagomycota* (Spatafora et al., 2016). The subphylum

Mucoromycotina of *Mucoromycota* comprises important opportunistic pathogens of humans and animals (de Hoog et al., 2014), and the subphylum *Entomophthoromycotina* of *Zoopagomycota* includes species that cause infection in insects and mammalian hosts (Spatafora et al., 2016; Vilela and Mendoza, 2018). Finally, some morphological groups correlate with their phylogenetic classification: the morphological group of atypical fungi contains only *Ascomycota* species belonging to *Taprinomycotina*, and the morphological group of microsporidia only species of the phylum *Microsporidia* (Table 1).

3.2. Secretome sizes

Proteome sizes of the pathogenic fungi analyzed in this study varied significantly ranging from 1831 to 27347 total proteins in *Encephalitozoon romaleae* and *Fusarium oxysporum*, respectively (Table 1). In terms of the number of total proteins that were part of the bioinformatically predicted secretome, there was also an extensive variation among the pathogenic fungi investigated: *Encephalitozoon* species had the smallest secretomes containing 61 to 88 proteins, and the largest were the *Fusarium verticilloides*, *F. oxysporum* and *Basidiobolus meristosporus* secretomes with 1535, 1846 and 1894 proteins, respectively (Table 1). As observed previously (Lowe and Howlett, 2012; Krijger et al., 2014; Lo Presti et al., 2015), for most of the fungal species secreted proteins comprised between 5 % and 10 % of the total proteome (Table 1). The lowest proportion of secreted proteins was present in the grasshopper pathogen *E. romaleae* (3.3 %), and the highest in the entomopathogen *B. meristosporus* (11.8 %) (Table 1).

Furthermore, previous studies have revealed that fungal secretome size correlates with lifestyle, with human and animal pathogens having smaller secretomes

than plant and insect pathogens (Lowe and Howlett, 2012; Sorgo et al., 2013; Krijger et al., 2014; Kim et al., 2016; Le Marquer et al., 2019). The presence of small secretomes in human and animal pathogenic fungi can be related to environments or niches with compounds or nutrients that are easier to obtain and metabolize, and may be a strategy to evade the host immune system (Krijger et al., 2014). In correspondence with this, the majority of the mammalian pathogenic fungi analyzed contained predicted secretome sizes smaller than 1000 proteins (Table 1). Remarkably, only six of the mammalian pathogens were filamentous fungi with predicted secretomes containing more than 1000 proteins: the *Pezizomycotina Aspergillus flavus*, *F. oxysporum*, *F. verticilloides*, and *Alternaria alternata*, and the *Entomophthoromycotina B. meritosporum* and *Conidiobolus coronatus* (Table 1). The presence of high numbers of secreted proteins in fungi is most likely associated with to their pathogenicity in plants or insects (Kim et al., 2016; Le Marquer et al., 2019). Accordingly, the aforementioned *Pezizomycotina* filamentous fungi are also important plant pathogens and, in addition, *A. flavus* causes infection in insects (St. Leger et al., 2000; Thomma, 2003; Nucci and Anaissie, 2007). The *Entomophthoromycotina B. meritosporum* and *C. coronatus* are entomopathogenic fungi that parasitize and kill arthropods (Vilela and Mendoza, 2018).

3.3. Secretome size and fungal morphology

Multiple studies have analyzed the repertoires of secreted proteins in fungal species. In general, it has been pointed out that secretome size correlates with proteome size, but also that variations in secretome size can be related with different fungal lifestyles (Lowe and Howlett, 2012; Girard et al., 2013; Sorgo et al., 2013; Meinken et al., 2014; Lo Presti et al., 2015; Kim et al., 2016; Le Marquer et al., 2019).

However, a comparative analysis including mostly *Ascomycota* secretomes proposed a correlation of secretome size with phylogeny rather than with fungal lifestyle (Krijger et al., 2014). Besides that, other analyses mainly performed on *Basidiomycota* supported different hypotheses, such as that secretome size is not related to the fungal lifestyle (Pellegrin et al., 2015) or that secretomes reflect fungal lifestyles rather than phylogenetic classifications (Alfaro et al., 2016). Furthermore, yeasts and yeast-like fungi tend to have considerably reduced numbers of secreted proteins than filamentous fungi suggesting a correlation between secretome size and the complexity of the life cycle (Lowe and Howlett, 2012; Sorgo et al., 2013; Le Marquer et al., 2019).

First, we examined the differences in the number of secreted proteins among the morphological groups of medically important fungi. As expected, the correlation pattern between the secretome size and the total proteome was similar to that observed in other previous studies of fungal secretomes (Lowe and Howlett, 2012; Krijger et al., 2014; Kim et al., 2016) (Fig. 1). In addition, we observed a remarkable variation in the abundance of secreted proteins across morphological groups (Figs. 1 and 2A). In particular, filamentous fungi tended to have significantly more secreted proteins (430-1894) than microsporidia (61-243), atypical fungi (136-147), yeasts and yeast-like fungi (180-591) and thermally dimorphic fungi (381-704) (Fig. 2A; Table 1). The presence of higher numbers of secreted proteins in filamentous fungi may be associated to the filamentous lifestyle.

However, there were not significant variations among the morphological groups for the fraction of predicted proteins that are secreted, with only two exceptions: the *Entomophthoromycotina B. meritosporum* and *C. coronatus*, and the mammalian pathogenic fungi with reduced proteomes (below 5000 proteins) (Table 1). *B. meritosporum* and *C. coronatus* also cause infection in insects and exhibited the

highest values for the fraction of secreted proteins with 11.8 % and 10 %, respectively (Table 1). On the other hand, minimum secretome sizes (less than 250 secreted proteins) and low values for the fraction of secreted proteins were found in mammalian pathogenic fungi with reduced proteomes (below 5000 proteins) such as microsporidia (3.3-7.6 %), atypical fungi (3.7-4.1 %) and members of the genus *Malassezia* (4.3-4.9 %) (Table 1). Microsporidia and atypical fungi are obligate fungal pathogens depending on the presence of eukaryotic cells to complete their life cycle. *Malassezia* species are basidiomycete yeasts associated with mammals as superficial commensals that can cause multiple skin disorders. With the exception of *Malassezia pachydermatis*, all the other *Malasezzia* species have an obligate lipophilic lifestyle and require fatty acids for growth (Xu et al., 2007; Schuster et al., 2018). Low values for the fraction of secreted proteins might also be related with the adaptation to an obligate intracellular parasitic lifestyle in microsporidia and atypical fungi or to a lipophilic lifestyle in *Malassezia* yeasts.

Finally, the pathogenic filamentous fungi analyzed are a very large group of species exhibiting considerable variation in their secretome sizes (430-1894) (Fig. 2A; Table 1). In contrast, the subgroup of dermatophyte fungi showed only limited differences in their number of predicted secreted proteins (512-647) (Fig. 2A; Table 1). Dermatophytes are closely phylogenetically related filamentous fungi highly specialized to infect keratinized tissues such as hair, nails and skin (Köhler et al., 2015). Besides, it is also particularly noteworthy that *Pseudogymnoascus destructans* can be distinguished by the smallest secretome size (430) among the filamentous fungi investigated (Table 1). *P. destructans* is a psychrophilic fungus that grows in a strict temperature range of ~4-20°C and is associated with the white-nose syndrome (WNS) skin infection in bats. Although bats are mammal animals, they are susceptible to WNS

during their hibernation period (Palmer et al., 2018). Likewise *P. destructans* exhibited a largely reduced secretome (~50 %) in comparison to nonpathogenic *Pseudogymnoascus* species (Palmer et al., 2018). Altogether, our analysis showed a correlation between secretome size and the morphological groups of medically important fungi (Figs. 1 and 2A).

3.4. Secretome size and fungal phylogeny

Krijger et al., (2014) carried out a comparative analysis of 36 fungal and *Oomycota* secretomes, and proposed that secretome size correlates with phylogeny and to a lesser extent with fungal lifestyle. The wide analysis of 136 fungal species by Kim et al. (2016) revealed a high variation of secretome sizes across phylogenetic lineages. *Microsporidia* contained the smallest secretomes; and in *Ascomycota*, species belonging to the subphylum *Pezizomycotina* had significantly larger secretomes than those of *Taphrinomycotina* and *Saccharomycotina*; and in *Basidiomycota*, species belonging to *Pucciniomycotina* and *Agaricomycotina* had larger secretomes than those of *Ustilaginomycotina* (Kim et al., 2016). With regard to the mammalian pathogenic fungi compared here, we have also observed similar variations in secretome size across fungal subphyla: small secretomes were present in *Microsporidia* (61-243), *Taphrinomycotina* (136-147), *Ustilagomycotina* (180-222) and *Saccharomycotina* (263-463). Whereas larger secretomes with a considerably size variation were found in *Agaricomycotina* (342-891), *Pezizomycotina* (381-1846), *Mucoromycotina* (628-865) and *Entomophthoromycotina* (1061-1894) (Fig. 2B; Table 1). Accordingly, our analysis indicated that there is also a relationship between the secretome size and the phylogenetic classification of medically important fungi.

3.5. Principal component analysis

To corroborate the relationships based on secretome sizes, principal component analysis (PCA) was performed to compare the complete secretomes (Figs. 3 and S1). Our expectation was to observe clustering of secretomes according to morphology and phylogenetic classification of mammalian pathogenic fungi. As a result, PCA highlighted that secretomes of species belonging to the same morphological group or subphyla cluster together, excepting the three species of filamentous fungi with the largest secretomes: the entomopathogen *B. meristosporus*, and the plant pathogens *F. oxysporum* and *F. verticilloides* (Figs. 3 and Fig. S1). With respect to morphological groups, secretomes of yeasts and yeast-like fungi, thermally dimorphic fungi, atypical fungi, and microsporidia formed separate clusters in the PCA; whereas secretomes of filamentous fungi did not grouped together and occupied distinct positions. Noteworthy, secretomes of each morphological subgroup of filamentous fungi (dermatophyte fungi, hyaline fungi, dematiaceous fungi and zygomycete fungi) were arranged in separate clusters (Figs. 3A and S1).

4. Conclusion

Secreted proteins play important roles in the interaction of a fungal pathogen with its mammalian host. In the present study, we bioinformatically predicted and compared the secretomes of fungi that infect humans and other mammals. Among the clinically relevant fungi analyzed, there was an extensive variation of secretome sizes (61-1894 secreted proteins), although in almost all the species secretome sizes were smaller than 1000 proteins. Only some species of filamentous fungi that also are plant or insect pathogens showed considerably larger secretomes. Overall, our results are in accordance with previous studies of fungal secretomes stating the correlation

between the secretome size and the total proteome (Lowe and Howlett, 2012; Krijger et al., 2014; Kim et al., 2016). In addition, our observations point out that in medically important fungi there is a relationship between the secretome and the morphological group or phylogenetic classification. However, at present it is not possible to define whether the morphology, the phylogeny or the lifestyle produces the strongest effect on the secretomes of fungal species that cause infections in mammals. Further functional and comparative secretome analyses are needed to advance in the understanding of the role of secreted proteins in the mammalian pathogenic lifestyle of fungi.

Conflicts of interest

The authors state that there are no conflicts of interest related to this publication.

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Appendix A. Supplementary data

The following are the Supplementary data to this article:

TableS1.xlsx	Table S1. Secretomes of medically important fungi
FigureS1.7z	Figure S1. PCA 3D of the secretomes of medically important fungi

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Figure captions

Fig.1. Correlation between the sizes of the secretome and the total proteome in medically important fungi. Points are coloured according to the classification to morphological groups of the analyzed species.

Fig. 2. Number of secreted proteins with regard to morphological group and phylogenetic classification of medically important fungi. (A) The number of secreted proteins among species in different morphological groups and subgroups. Points are coloured by phylum. (B) The number of secreted proteins among species in different fungal phyla and subphyla. Points are coloured by morphological group. The phylum *Chytridiomycota*, represented by only one species (*Batrachochytrium dendrobatidis*), is not included. Sac, *Saccharomycotina*; Pez, *Pezyzomycotina*; Tap, *Taphrinomycotina*; Aga, *Agaromycotina*; Ust, *Ustilagomycotina*; Muc, *Mucoromycotina*; Ent, *Entomophthoromycotina*.

Fig. 3. Principal component analysis (PCA) of the secretomes of medically important fungi. The PCA scores plot for PC1 and PC2 are shown in (A) with points coloured by morphological group or subgroup, and (B) with points coloured by phylum or subphylum. The percentage of the total variance explained by each principal component is shown in brackets on each axis. Additional information of PCA analysis in Supplementary material, Fig. S1.

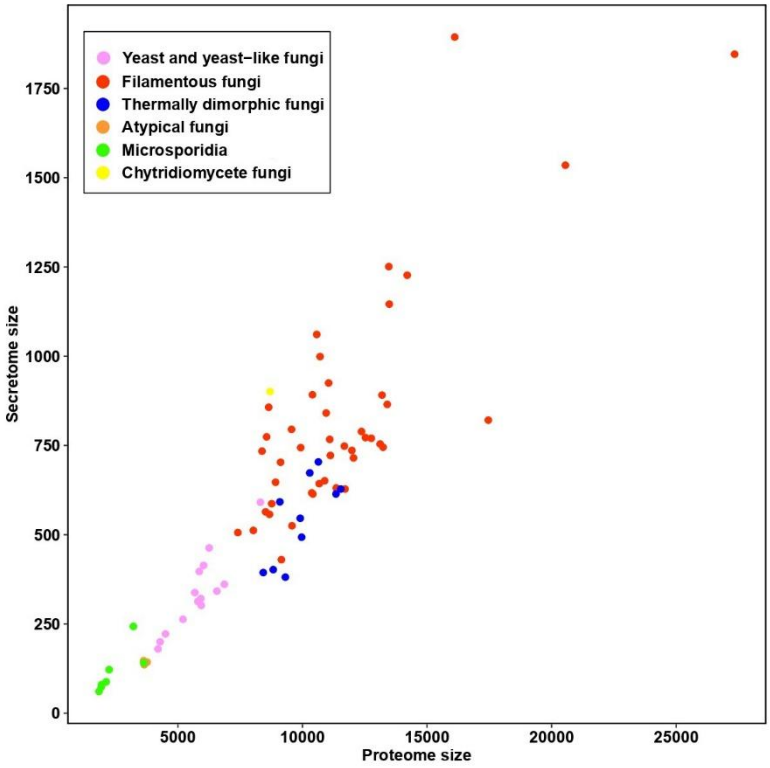
Table 1

Fungal species analyzed in this study.

Species	Phylum	Subphylum	Proteome	Secretome	% Proteome
YEASTS AND YEAST-LIKE FUNGI					
Candida glabrata CBS 138	*Ascomycota*	*Saccharomycotina*	5202	263	5.1
Candida orthopsilosis Co 90-125	*Ascomycota*	*Saccharomycotina*	5678	338	6.0
Candida tropicalis MYA-3404	*Ascomycota*	*Saccharomycotina*	6254	463	7.4
Candida dubliniensis CD36	*Ascomycota*	*Saccharomycotina*	5860	397	6.8
Candida albicans SC5314	*Ascomycota*	*Saccharomycotina*	6030	414	6.9
Lodderomyces elongisporus NRRL YB-4239	*Ascomycota*	*Saccharomycotina*	5799	313	5.4
Meyerozyma guilliermondii ATCC 6260	*Ascomycota*	*Saccharomycotina*	5920	321	5.4
Clavispora lusitaniae ATCC 42720	*Ascomycota*	*Saccharomycotina*	5936	302	5.1
Malassezia sympodialis ATCC 42132	*Basidiomycota*	*Ustilaginomycotina*	4502	222	4.9
Malassezia globosa CBS 7966	*Basidiomycota*	*Ustilaginomycotina*	4286	200	4.7
Malassezia pachydermatis CBS 1879	*Basidiomycota*	*Ustilaginomycotina*	4202	180	4.3
Cryptococcus neoformans var. neoformans JEC21	*Basidiomycota*	*Agaricomycotina*	6863	361	5.3
Cryptococcus gattii WM276	*Basidiomycota*	*Agaricomycotina*	6565	342	5.2
Trichosporon asahii var. asahii CBS 2479	*Basidiomycota*	*Agaricomycotina*	8311	591	7.1
FILAMENTOUS FUNGI					
Dermatophyte fungi					
Trichophyton tonsurans CBS 112818	*Ascomycota*	*Pezizomycotina*	8521	564	6.6
Trichophyton equinum CBS 127.97	*Ascomycota*	*Pezizomycotina*	8676	557	6.4
Trichophyton soudanense CBS 452.61	*Ascomycota*	*Pezizomycotina*	10671	643	6.0
Trichophyton rubrum CBS 118892	*Ascomycota*	*Pezizomycotina*	10418	614	5.9
Nannizzia gypsea CBS 118893	*Ascomycota*	*Pezizomycotina*	8921	647	7.3
Microsporum canis CBS 113480	*Ascomycota*	*Pezizomycotina*	8765	587	6.7
Trichophyton verrucosum HKI 0517	*Ascomycota*	*Pezizomycotina*	8028	512	6.4
Trichophyton benhamiae CBS 112371	*Ascomycota*	*Pezizomycotina*	7405	506	6.8
Hyaline fungi					
Aspergillus niger ATCC 1015	*Ascomycota*	*Pezizomycotina*	10950	841	7.7
Aspergillus parasiticus SU-1	*Ascomycota*	*Pezizomycotina*	8645	857	9.9
Aspergillus flavus NRRL 3357	*Ascomycota*	*Pezizomycotina*	13485	1146	8.5
Aspergillus nidulans FGSC A4	*Ascomycota*	*Pezizomycotina*	9561	795	8.3
Aspergillus terreus NIH2624	*Ascomycota*	*Pezizomycotina*	10401	892	8.6
Aspergillus fumigatus A1163	*Ascomycota*	*Pezizomycotina*	9929	744	7.5
Aspergillus clavatus NRRL 1	*Ascomycota*	*Pezizomycotina*	9121	703	7.7
Fusarium oxysporum f. sp. *lycopersici* 4287	*Ascomycota*	*Pezizomycotina*	27347	1846	6.8
Fusarium verticillioides 7600	*Ascomycota*	*Pezizomycotina*	20553	1535	7.5
Chaetomium globosum CBS 148.51	*Ascomycota*	*Pezizomycotina*	11048	925	8.4
Pochonia chlamydosporia 170	*Ascomycota*	*Pezizomycotina*	14204	1227	8,6
Scedosporium apiospermum IHEM 14462	*Ascomycota*	*Pezizomycotina*	8376	734	8.8
Lomentospora prolificans JHH-5317	*Ascomycota*	*Pezizomycotina*	8560	774	9.0
Pseudogymnoascus destructans 20631-21	*Ascomycota*	*Pezizomycotina*	9153	430	4.7
Madurella mycetomatis mm55	*Ascomycota*	*Pezizomycotina*	10707	999	9.3
Schizophyllum commune H4-8	*Basidiomycota*	*Agaricomycotina*	13194	891	6.8
Dematiaceous fungi					
Exophiala dermatitidis NIH/UT8656	*Ascomycota*	*Pezizomycotina*	9578	525	5.5
Exophiala aquamarina CBS 119918	*Ascomycota*	*Pezizomycotina*	13118	754	5.7
Exophiala oligosperma CBS 72588	*Ascomycota*	*Pezizomycotina*	13234	745	5.6
Exophiala spinifera CBS 89968	*Ascomycota*	*Pezizomycotina*	12049	715	5.9
Cladophialophora bantiana CBS 173.52	*Ascomycota*	*Pezizomycotina*	12762	770	6.0
Cladophialophora carrionii CBS 160.54	*Ascomycota*	*Pezizomycotina*	10373	617	5.9
Fonsecaea monophora CBS 269.37	*Ascomycota*	*Pezizomycotina*	11984	736	6.1
Fonsecaea multimorphosa CBS 102226	*Ascomycota*	*Pezizomycotina*	12369	789	6.4
Fonsecaea nubica CBS 269.64	*Ascomycota*	*Pezizomycotina*	11681	748	6,4
Fonsecaea pedrosoi CBS 271.37	*Ascomycota*	*Pezizomycotina*	12527	772	6.2
Verruconis gallopava CBS 43764	*Ascomycota*	*Pezizomycotina*	11357	631	5.6
Cyphellophora europaea CBS 101466	*Ascomycota*	*Pezizomycotina*	11094	767	6.9
Rhinocladiella mackenziei CBS 650.93	*Ascomycota*	*Pezizomycotina*	11382	627	5.5
Alternaria alternata SRC1lrK2f	*Ascomycota*	*Pezizomycotina*	13466	1251	9.3

Zygomycete fungi					
Lichtheimia corymbifera JMRC:FSU:9682	*Mucoromycota*	Mucoromycotina	13404	865	6.5
Rhizopus delemar RA 99-880	*Mucoromycota*	Mucoromycotina	17459	821	4.7
Rhizopus microsporus ATCC 52813	*Mucoromycota*	Mucoromycotina	10891	651	6.0
Mucor circinelloides CBS 277.49	*Mucoromycota*	Mucoromycotina	11709	628	5.4
Syncephalastrum racemosum NRRL 2496	*Mucoromycota*	Mucoromycotina	11121	722	6.5
Basidiobolus meristosporus CBS 931.73	*Zoopagomycota*	Entomophthoromycotina	16110	1894	11.8
Conidiobolus coronatus NRRL 28638	*Zoopagomycota*	Entomophthoromycotina	10572	1061	10.0
THERMALLY DIMORPHIC FUNGI					
Coccidioides posadasii RMSCC 3488	*Ascomycota*	Pezizomycotina	9964	493	4.9
Coccidioides immitis RS	*Ascomycota*	Pezizomycotina	9910	546	5.5
Histoplasma capsulatum NAm1	*Ascomycota*	Pezizomycotina	9313	381	4.1
Paracoccidioides lutzii Pb01	*Ascomycota*	Pezizomycotina	8826	402	4.6
Paracoccidioides brasiliensis Pb03	*Ascomycota*	Pezizomycotina	8427	394	4.7
Blastomyces gilchristii SLH14081	*Ascomycota*	Pezizomycotina	11343	614	5,4
Blastomyces dermatitidis ER-3	*Ascomycota*	Pezizomycotina	11539	628	5.4
Sporothrix schenckii 1099-18	*Ascomycota*	Pezizomycotina	10293	673	6.5
Talaromyces marneffei ATCC 18224	*Ascomycota*	Pezizomycotina	10638	704	6.6
Sporothrix brasiliensis 5110	*Ascomycota*	Pezizomycotina	9091	592	6.5
ATYPICAL FUNGI					
Pneumocystis jirovecii RU7	*Ascomycota*	Taphrinomycotina	3761	143	3.8
Pneumocystis murina B123	*Ascomycota*	Taphrinomycotina	3623	147	4.1
Pneumocystis carinii B80	*Ascomycota*	Taphrinomycotina	3646	136	3.7
MICROSPORIDIA					
Encephalitozoon intestinalis ATCC 50506	*Microsporidia*	n.a.	1939	80	4.1
Encephalitozoon hellem ATCC 50504	*Microsporidia*	n.a.	1928	74	3.8
Encephalitozoon romaleae SJ-2008	*Microsporidia*	n.a.	1831	61	3.3
Encephalitozoon cuniculi GB-M1	*Microsporidia*	n.a.	2120	88	4.2
Enterocytozoon bieneusi H348	*Microsporidia*	n.a.	3632	141	3.9
Vittaforma corneae ATCC 50505	*Microsporidia*	n.a.	2239	122	5.4
Trachipleistophora hominis	*Microsporidia*	n.a.	3212	243	7.6
CHYTRIDIOMYCETE FUNGI					
Batrachochytrium dendrobatidis JAM81	*Chytridiomycota*	n.a.	8700	901	10.4

Figure 1


Yeast and yeast−like fungi
Filamentous fungi
Thermally dimorphic fungi
Atypical fungi
Microsporidia
Chytridiomycete fungi
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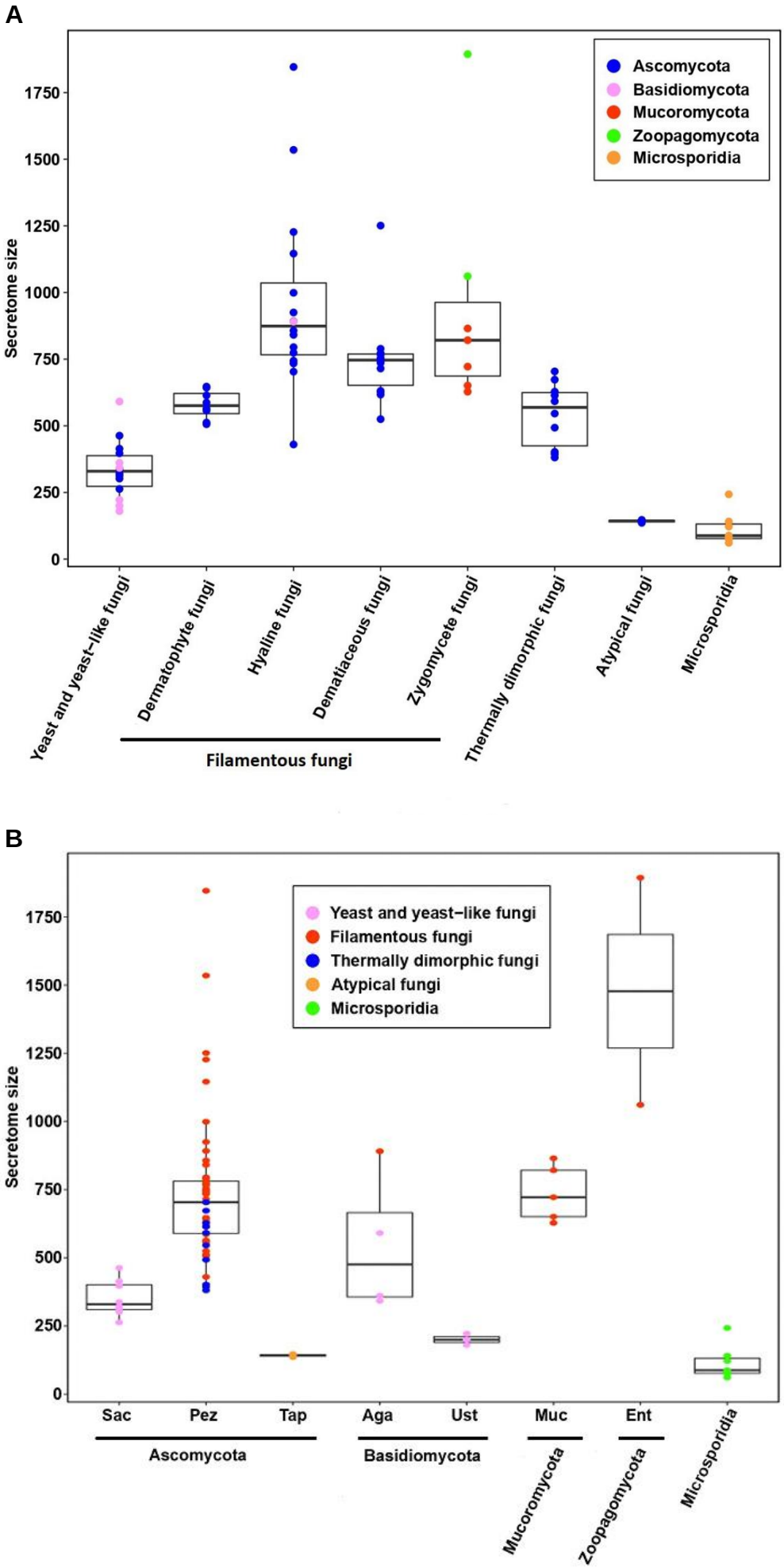
750
1000
1250
1500
1750
5000
10000
15000
20000
25000
Proteome size

Figure 2


A
Secretome size
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250
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1500
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Ascomycota
Basidiomycota
Mucoromycota
Zoopagomycota
Microsporidia
Yeast and yeast−like fungi
Dermatophyte fungi
Hyaline fungi
Dematiaceous fungi
Zygomycete fungi
Thermally dimorphic fungi
Atypical fungi
Microsporidia
Filamentous fungi
B
Secretome size
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Yeast and yeast−like fungi
Filamentous fungi
Thermally dimorphic fungi
Atypical fungi
Microsporidia
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Microsporidia
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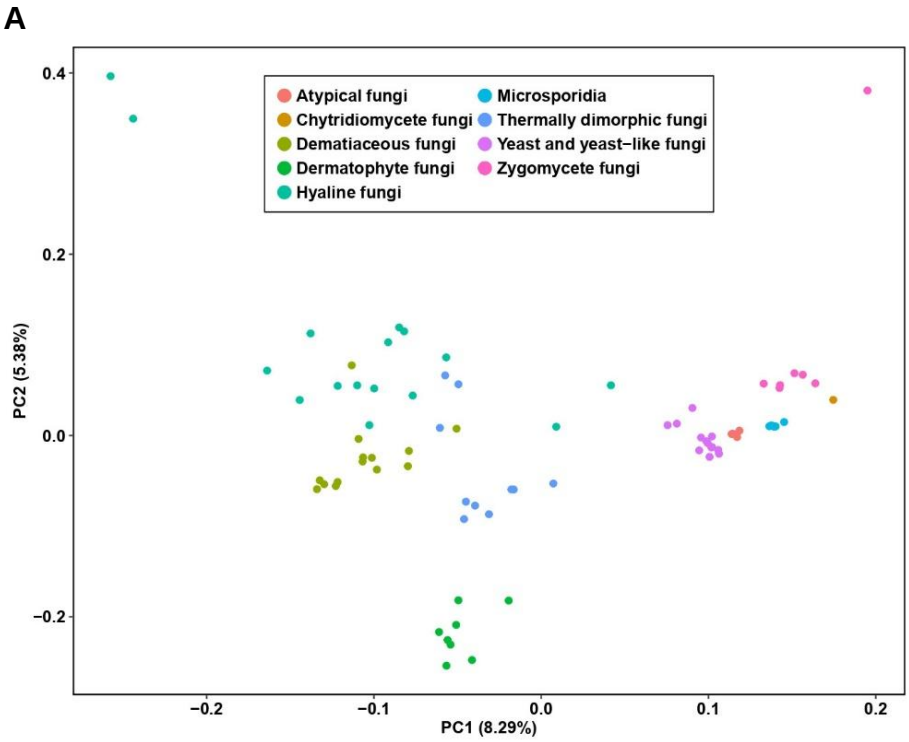
Basidiomycota
Mucoromycota
Zoopagomycota

Figure 3


A
Atypical fungi
Chytridiomycete fungi
Dematiaceous fungi
Dermatophyte fungi
Hyaline fungi
Microsporidia
Thermally dimorphic fungi
Yeast and yeast−like fungi
Zygomycete fungi
PC2 (5.38%)
PC1 (8.29%)
0.4
0.2
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−0.2
−0.2
−0.1
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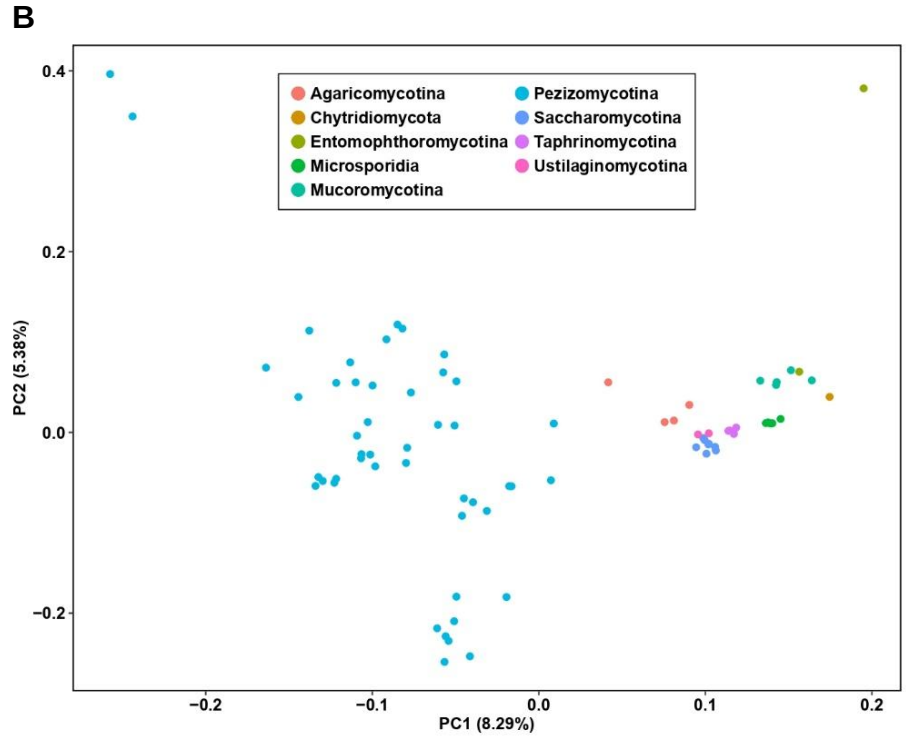
B
Agaricomycotina
Chytridiomycota
Entomophthoromycotina
Microsporidia
Mucoromycotina
Pezizomycotina
Saccharomycotina
Taphrinomycotina
Ustilaginomycotina
PC2 (5.38%)
PC1 (8.29%)
0.4
0.2
0.0
−0.2
−0.2
−0.1
0.0
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