Core genes for general and secondary metabolism

Core genes involved in general and secondary metabolism, showed high degree of conservation when compared to those present in other Sordariomycetes. Among all, including those involved in energy metabolism (Table S16), only two genes (F-type H+-transporting ATPase subunit C and nitrite reductase NAD(P)H large subunit) were simultaneously present in S. schenckii and were not identified in the Eurotiomycetes genomes. The gene encoding subunit C of FoF1-ATPsynthase was present in both S. schenckii and S. brasiliensis as well as in the reference Sordariomycetes, but it was not conserved in the pathogenic dimorphic fungi. Probably, subunit C is not critical for the assembly and function of the FoF1-ATPsynthase in these dimorphic fungi, because their oxidative phosphorylation chain is functional and capable of oxidative ATP synthesis [1-4]. The nitrite reductase gene has also been correlated to the survival of filamentous fungi and persistence in hypoxic environments [5]. The nitrite reductase gene was present only in S. schenckii and in the class of Sordariomycetes fungi. Intriguingly, S. brasiliensis has no gene related to reduction or oxidation of both nitrate and nitrite. Hence, S. schenckii could have certain advantages compared to S. brasiliensis and other dimorphic fungi regarding environmental survival capabilities.

Genes involved in lipid metabolic pathways in S. schenckii and S. brasiliensis were identified based on previously annotated Sordariomycetes genomes (Table S17). Genome analysis of both species allowed us to identify several genes encoding phospholipases. Accordingly, a gene encoding for phospholipase A2 (PL2A) was identified in both Sporothrix species. The PLA2 homolog gene of S. schenckii was previously reported by [6] and is supposed to interact with a G-protein subunit playing a role in signal transduction and fungal pathogenesis. We have found four different phospholipase C (PLC) homologous in S. schenckii and S. brasiliensis. Several studies
have shown that PLC activation plays an important role in intracellular signal transduction by regulating intracellular calcium concentrations. In *Magnaporthe grisea* genes encoding PLC had an important role in growth, morphology, sporulation, appressorium development and pathogenesis [7, 8]. Finally, we identified four candidate homologous genes encoding phospholipase D (PLD) isoforms in both *Sporothrix* species which seems to occur in a wide range of fungi belonging to Sordariomycetes. Although the role of PLD in *Sporothrix* is unclear, in *Aspergillus fumigatus* it was shown to regulate conidial internalization into lung epithelial cells. Additionally, a mutant strain for *pld* was less virulent in immunosuppressed mice, suggesting that this gene may encode a virulence factor of *A. fumigatus* [9].

We have observed that only three enzymes of amino acid metabolism pathways identified in the genomes of *S. schenckii* and *S. brasiliensis* were absent in most of the dimorphic fungi (Table S16). The first is a monoamine oxidase enzyme belonging to the tryptophan, arginine, proline, phenylalanine and tyrosine metabolism pathways. It is a flavoenzyme, widely distributed in fungi, bacteria and mammals, [10, 11] and involved in the oxidative deamination of primary amines, using ammonia as nitrogen source [11, 12]. In the tyrosine metabolism pathway, the enzyme maleylacetoacetate isomerase is coded by two ORFs in *S. schenckii*. This enzyme is present in dimorphic fungi, but absent in sordariomycete species such as *G. clavigera* and *M. grisea*. This enzyme participates in the degradation of phenylalanine to fumarate and acetoacetate [13]. The enzyme enolase-phosphatase E1, belonging to the cysteine and methionine metabolism pathways, is absent in all dimorphic fungi and present in all Sordariomycetes, suggesting that it is probably involved in situations of environmental stress.

Genes encoding enzymes of vitamins and cofactors metabolism (Table S18) were found in both genomes, except for only a single enzyme of lipoic acid metabolism, a
homologue of *Escherichia coli* lipoate protein ligase A (LplA). Interestingly, this enzyme appears to be absent in the genome of *S. schenckii* and *S. brasiliensis*, but has been found in all Sordariomycetes and dimorphic fungi analyzed. The formyltetrahydrofolate deformylase is present in both *Sporothrix* species, as well as in Sordariomycetes and other members of the subphylum Pezizomycotina. In contrast, this enzyme is absent from the subphylum Saccharomycotina, from Basidiomycota (except *Ustilago maydis*) and even from dimorphic fungi. This enzyme has been described in bacteria, such as *E. coli*, in which it metabolizes formyl-THF to formate and THF in purine and glycine biosynthesis [14]. There are two other genes associated with the metabolism of riboflavin, nicotinate and nicotinamide which are present in both *Sporothrix* species and Sordariomycetes, but absent from all dimorphic fungi analyzed. One of them encodes an acid phosphatase that participates in the riboflavin metabolism pathway, and the other encodes a 5’-nucleotidase, an acid phosphatase that participates in the nicotinate and nicotinamide metabolism. It has been suggested that 5’-nucleotidase could be involved in stress response in *E. coli* [15].

**Transport and catabolism**

Autophagy in fungi is essential for survival during starvation, but has also been involved in various processes such as morphogenesis, virulence, survival upon phagocytosis, and metal ion homeostasis. The analysis of the genomes of *S. schenckii* and *S. brasiliensis* supports the observation that these fungi probably have fully functional autophagy, peroxisomes and endocytosis pathways. We have focused on the analysis of 20 genes, which include those shown on the KEGG category of “Regulation of autophagy” and others necessary for autophagosome biogenesis in *S. cerevisiae* [16]. Of these 20 genes, 16 were readily identifiable in both *S. schenckii* and *S. brasiliensis* (Table S19). Atg4 was only found in *S. schenckii*, whereas Atg10, Atg13 and Atg14
orthologous were not identified in either species. Atg4 is a protease that is involved in
cleavage of Atg8, a step that is essential during autophagosome formation. Atg10 is
part of an ubiquitin-like conjugation system that is essential in the early steps of
autophagosome formation [17]. Atg13 is a regulatory subunit of the Atg1 kinase
complex, which is involved in autophagosome expansion [18]. Atg14 is a subunit of a
phosphatidylinositol 3-kinase complex involved in regulating autophagy initiation [19].
The category “Peroxisome” includes 19 genes involved in the biogenesis of this
organelle. Of these genes, 15 were readily identifiable both in S. schenckii and in S.
brasiliensis. Pex12 was only found in S. brasiliensis, while also Pex26, Pmp70 and
Pxmp2 were absent from all fungal genomes compared. Pex12 is part of a complex
including Pex10 and Pex2 that is found in the peroxisomal membrane and is involved in
the translocation of peroxisomal proteins from the cytoplasm to the organelle matrix
[20]. Both Pex10 and Pex2 were found in the S. schenckii genome. Further
experimental work is necessary to understand the significance of Atg4 lack in S.
brasiliensis and Pex12 in S. schenckii.

In S. cerevisiae, 55 genes are involved in endocytosis [21]. Of these, 46 genes were
found in both S. schenckii and S. brasiliensis, whereas App1, Arc18 were only found in
S. schenckii and Aim3, Aim21, Bsp1, Gts1and Scd5 were not found from either species.
Little is known about App1 except that it has phosphatide phosphatase activity and that
it interacts with proteins in the endocytic pathway [22]. Arc18 is part of the multi-subunit
Arp2/3 complex [23], of which all other subunits are found in both Sporothrix genomes.
It is thus hard to find any biological significance to the specific lack of these genes in S.
brasiliensis. Of interest might be the lack of Gts1 and Scd5 from the Sporothrix
genomes. Both of these genes can be found in the genome sequences of several fungi
in the class Saccharomycotina, including pathogenic Candida species. The lack of
these two genes in the genomes of Sporothrix species indicates that despite the conservation of most of the endocytic machinery in fungi, a few differences do exist, which could be important in understanding Sporothrix physiology.

References


