

MiniReview

Manganese superoxide dismutase in pathogenic fungi: An issue with pathophysiological and phylogenetic involvements

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Abstract

Manganese-containing superoxide dismutases (MnSODs) are ubiquitous metalloenzymes involved in cell defence against endogenous and exogenous reactive oxygen species. In fungi, using this essential enzyme for phylogenetic analysis of *Pneumocystis* and *Ganoderma* genera, and of species selected among Ascomycota, Basidiomycota and Zygomycota, provided interesting results in taxonomy and evolution. The role of mitochondrial and cytosolic MnSODs was explored in some pathogenic Basidiomycota yeasts (*Cryptococcus neoformans* var. *grubii*, *Cryptococcus neoformans* var. *gattii*, *Malassezia sympodialis*), Ascomycota filamentous fungi (*Aspergillus fumigatus*), and Ascomycota yeasts (*Candida albicans*). MnSOD-based phylogenetic and pathogenic data are confronted in order to evaluate the roles of fungal MnSODs in pathophysiological mechanisms.

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1. Introduction

Diversity in clinical manifestations of mycoses (superficial, allergic, primary or opportunistic systemic infections), and fungal pathogenic species repartition among Zygomycota, Basidiomycota, or Ascomycota, indicate both a clinical and a phylogenetic diversity in human pathogenic fungi.

In spite of this diversity, all these primary or opportunistic pathogenic fungi have to face the first line of host defence against fungal infection: oxidative stress in phagocytes. Evolution of antioxidant system could therefore be a key process determining fungal adaptation and resistance in the host environment, contributing to the emergence of pathogenicity among fungi.

Many enzymatic, e.g., catalase, glutathione peroxidase and superoxide dismutase (SOD), and non-enzymatic systems, e.g., melanin and mannitol, participate to reactive oxygen species (ROS) elimination [1]. In enzymatic antioxidant systems, one monofunctional enzyme is often encoded by multigene families, with each isoform differently regulated or located. For example, in *Aspergillus fumigatus*, three catalases have been reported: the conidial catalase, encoded by the *catA* genes [2], and the two mycelial catalases encoded by *catI/catB*

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[3] and *cat2* [2], respectively. Four more catalases homologues to *cat1/catB* and *cat2* have been detected in the genome of *A. fumigatus*.

In SODs (EC 1.15.1.1), redundancy is even more evident.

These ubiquitous metalloenzymes, catalyzing the dismutation of toxic superoxide anions into hydrogen peroxide, have three main isoforms, depending on their metal cofactor: manganese- (MnSOD), iron- (FeSOD), or copper/zinc- (Cu/ZnSOD) SODs. Mn- and Fe-SODs share a similar sequence and structure [4], but they are unrelated to Cu/ZnSODs [5,6]. MnSODs (product of the *SOD2* gene) are located in the mitochondrial matrix, while Cu/ZnSODs (product of the *SOD1* gene) were initially thought to reside in the cytosol [7]. Recently, Cu/Zn has also been found to reside in the mitochondrial intermembrane space of some fungi as well [8,9]. Fungal SOD diversity is based on: (i) the structure and metal cofactor: fungi typically have both a mitochondrial MnSOD and a cytosolic Cu/ZnSOD, (ii) the gene redundancy in each SOD family. For example, two MnSOD and four Cu/ZnSOD genes were recently found in *Candida albicans* completely sequenced genome [10,11].

2. MnSOD: a major role in oxidative or temperature-induced stress

In eukaryotes, MnSODs are usually homotetrameric mitochondrial metalloenzymes [12]. Expression of *sod2* and phenotype of *sod2* mutants explored in model organisms (*Saccharomyces cerevisiae*, *Saccharomyces pombe*), and in some pathogenic fungi (*Cryptococcus neoformans*, *C. albicans*) supported the role of MnSOD in oxidative stress and high temperature conditions, two major stresses encountered by pathogenic fungi in the host environment.

2.1. MnSOD: scavenging endogenous and exogenous superoxide anions produced in oxidative stress

Fungi, like all aerobic organisms, are exposed to endogenous superoxide anions generated by the leakage of electrons onto O₂ from various components of the cellular electron transport chain during natural metabolic conditions. Mitochondria, where 90% of the O₂ is consumed, and the endoplasmic reticulum are the two major compartments where endogenous superoxide accumulation occurs [13]. Therefore, usual mitochondrial localization of MnSOD makes endogenous ROS the primary target of this SOD isoform. In *C. neoformans* var. *gattii*, *SOD2* rather than *SOD1* was shown to be essential for survival during the stationary phase, when mitochondrial formation of ROS is especially increased. In fact, 98% of *C. neoformans* var. *gattii* *sod2* deletional mutant in stationary phase, versus 10% of

wild-type cells, lost viability in 3 days [14], whereas *sod1* mutant cells did not show viability loss in the same conditions [15]. In *C. albicans*, both mitochondrial (*SOD2*) and cytosolic MnSOD (*SOD3*) could also play a role during the stationary phase since their expression was increased, while *SOD1* expression decreased [10,16,17].

MnSOD role in organisms facing exogenous oxidative ROS, and especially superoxide overproduction, were also explored, using artificial oxidative stressors such as menadione, paraquat or H₂O₂. The role of MnSOD was deduced from the *SOD2* increased expression after exposure to menadione in *S. pombe* [18,19], *Penicillium chrysogenum* [20], or *S. cerevisiae* [21]. Furthermore, *sod2* mutants were more sensitive than wild-type to menadione [14,18,21,22], paraquat [14,18,22–25], and other oxidant agents, such as plumbagin, a menadione derivative [18]. After exposure to hydrogen peroxide, neither an enhanced MnSOD expression or a *sod2* mutant growth inhibition were observed in *S. pombe* [18], suggesting *SOD2* is not involved in resistance to H₂O₂ induced stress.

Even if they are experimentally useful, these agents provide just a simulation of oxidative stress undertaken by fungal cells in natural conditions. In vitro exposure of fungi to the phagocytic activity of macrophages or neutrophils, which is rarely reported, is closer to the in vivo model. Results from the two methods can be divergent. In *C. neoformans* var. *gattii*, *sod2* mutant did not show an increased sensitivity to killing by human neutrophils, even if *sod2* mutant was sensitive to menadione or paraquat [14].

2.2. Heat shock

Growth at 37 °C is a determinant character in fungal pathogenicity. Fungi are often exposed to several stresses simultaneously, like in the host, where they are exposed to both an increased temperature, and to ROS generated by phagocytes. The development of common response pathways could explain the close correlation between heat shock and oxidative stress responses [26,27]. In addition, at higher temperature, an increased metabolism could lead to a greater consumption of molecular oxygen and greater production of ROS, with accumulation in mitochondria [27].

In *C. neoformans* var. *gattii* and *C. neoformans* var. *grubii*, *SOD2* essential role in adaptation to grow at elevated temperature was clearly demonstrated by (i) an induced *SOD2* expression in *C. neoformans* var. *gattii* upon shift from 30 to 37 °C (fivefold upregulation) [14], (ii) a marked growth inhibition of a *sod2* mutant of both species at 37 °C [14,23]. No loss of viability was observed in *sod2* mutant strains incubated at 37 °C under low oxygen concentration [14] or anaerobic conditions [23], showing *sod2* mutant increased thermo-

sensitivity was oxygen dependent. This observation confirmed the close relationship between oxidative and temperature stress pathways.

SOD2 participation to the heat shock-induced SOD response is dominant in *C. neoformans* var. *gattii* and *C. neoformans* var. *grubii*, since *sod1* mutant did not show any growth defect at 37 °C [15,28]. *C. albicans* *sod2* mutant sensitivity to high temperature (43 °C) was also reported [22].

3. MnSOD for fungal phylogeny: taxonomic and evolutionary data

Fungal taxonomy, for a long time, was mainly based on morphological characters, leading to a complex, controversial and incomplete classification. This resulted mostly from: (i) the fact that macroscopic and microscopic characters could fail to differentiate closely related species (e.g., *Trichophyton mentagrophytes* complex, *Ganoderma lucidum* complex); (ii) the existence of two morphological forms, asexual (anamorph) and sexual (teleomorph, frequently unknown), with different names, in the same species; (iii) the difficulty to determine the taxonomic position within Asco-, Basidio-, or Zygomycota of species whose teleomorph is unknown, which were classified on the basis of their anamorph within the Deuteromycota; (iv) the impossibility to determine the taxonomic position of fungi whose culture in vitro is difficult (e.g., *Lacazia loboi* [29]).

Phylogenetic studies using molecular sequence data, mainly ribosomal RNA (rRNA) sequences, provided an alternative to morphological-based taxonomy and highly contributed to clarify the complex taxonomy and evolution of these eukaryotes [30–32]. Furthermore, they provided precious information on fungal rRNA gene sequences, further used for the development of molecular methods for identification of fungi [33–36].

However, since congruence between several phylogenies is needed to confirm classical rRNA genes data, many other phylogenetic markers were explored: internal transcribed regions (ITS) [37], or protein-coding genes such as cytochrome *b* [38], RPB1, RPB2, EF2 or ACT1 [39], translation elongation factor 1 α , actin-1, RNA polymerase II, cytochrome oxidase II [40]. MnSOD is one of the protein-coding genes used in taxonomy and phylogenetic fungal studies. Interesting results were obtained in *Ganoderma* [41], and *Pneumocystis* genera [42], and in fungi selected in Ascomycota, Basidiomycota and Zygomycota [43, Frealle et al., 2005, manuscript submitted]. Furthermore, the molecular phylogeny of essential enzymes helped to understand their evolution, interrelationships and functions [43, Frealle et al., 2005, manuscript submitted].

The 99 MnSOD amino acid sequences, corresponding to these studies, were aligned using the BioEdit

v7.0.1 program (<http://www.mbio.ncsu.edu/BioEdit/bioedit.html>), and the MnSOD-based phylogenetic tree synthesizing the results of these studies (Fig. 1) was built using maximum parsimony in MrBAYES 3.0b4 program [44], as previously described [Frealle et al., 2005, manuscript submitted].

3.1. MnSOD sequences: alignment and phylogenetic information

MnSOD sequence variability is much higher than 18S rRNA one, probably reflecting a weaker selective pressure on the MnSOD gene. For example, identity between homologous *A. fumigatus*, *Aspergillus niger*, *Aspergillus nidulans*, *Aspergillus flavus* MnSOD gene sequences ranges from 73.1% to 79.9%, whereas SSU rRNA sequence identity exceeds 98.4% [Frealle et al., 2005, manuscript submitted].

With protein-coding genes, either nucleotide or protein sequences can be used. In the *Pneumocystis* study, trees inferred from 15 protein or nucleotide sequences yielded the same topology [42]. cDNA nucleotide sequences were used to build the *Ganoderma* tree [41], and protein sequences were used to build the two others MnSOD trees [43, Frealle et al., 2005, manuscript submitted]. Using protein rather than nucleotide sequences avoid (i) intron interference: exons and introns undergo different evolutionary pressures, leading to a different rate of incorporation of base substitutions, which does not reflect the protein evolution, and (ii) wobble base degeneracy interference. Aligning deduced amino acid sequences (20 amino acids) rather than DNA sequences (4 nucleotides) will also improve the quality of the alignment since sequences are shorter and much easier to align, and the information will sharpen up considerably [45]. When nucleotide sequences have high identity rates, like in the *Ganoderma* study, where sequences U56106–U56137 (GenBank Accession Nos.) had over 79.5% identity, unambiguous alignment is possible [41].

Interestingly, although MnSOD protein sequences are shorter than 18S rRNA nucleotide sequences they exhibit higher variability and enough informative sites for phylogenetic analysis. For example, in the recent study comparing MnSOD and SSU rRNA-based phylogenies of pathogenic fungi, 131 and 1260 sites from MnSOD and SSU rRNA sequences were used, respectively, showing that shorter sequences could provide more phylogenetic information [Frealle et al., 2005, manuscript submitted].

3.2. High variability of MnSOD sequences: usefulness for taxonomy of closely related species and genotyping

Another advantage of using MnSOD for phylogeny is that highly divergent MnSOD sequences allow closely related species differentiation. In the *Pneumocystis*

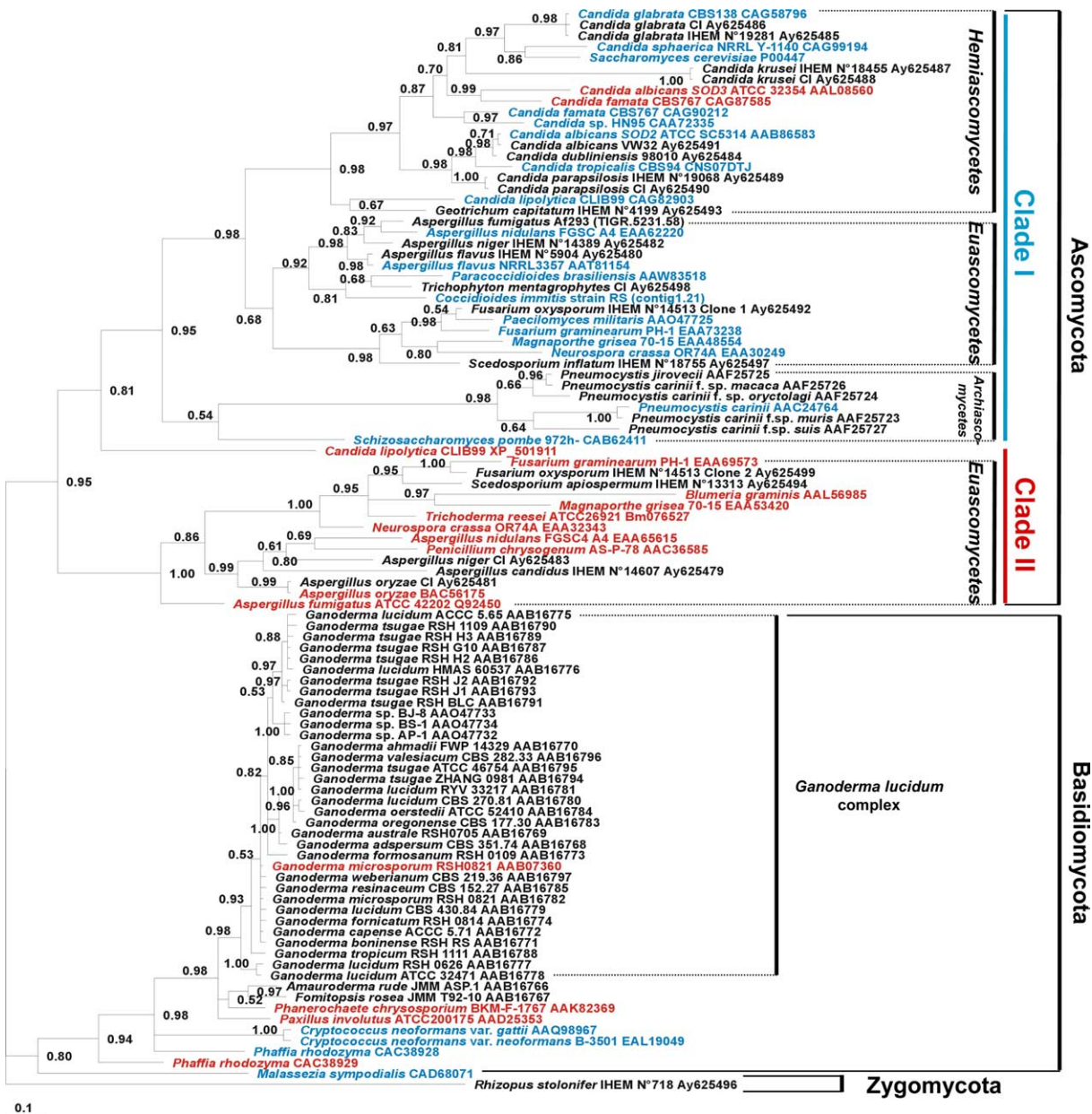


Fig. 1. Consensus Bayesian tree of fungi inferred from MnSOD protein sequences. *R. stolonifer* served as outgroup. Accession numbers or references of the sequences retrieved from genome sequencing projects are shown after each species. The predicted cytosolic or mitochondrial subcellular localization of fungal MnSODs is indicated in red and blue, respectively. Numbers near the individual nodes indicate Bayesian posterior probabilities given as percentages. Nodes with values of <50% are not shown. Scale bar, 0.1 substitutions (corrected) per site.

genus, MnSOD-based phylogeny confirmed the existence of host-specific species, already supported by 18S rRNA sequence heterogeneity, together with phenotypic variability among the different host-derived *Pneumocystis carinii* isolates [46–48]. In Denis et al., *Pneumocystis carinii* f. sp. *hominis*, *P. carinii* f. sp. *macacae*, *P. carinii* f. sp. *oryctolagi*, *P. carinii* f. sp. *muris*, *P. carinii* f. sp. *suis*, and rat-derived *P. carinii* formed a monophyletic group within fungi. Grouping of *P. carinii* f. sp. *hominis* (= *P. jirovecii*) and *P. carinii* f. sp. *macacae* with *P. carinii* f. sp. *oryctolagi* was even clearer using the MnSOD than the 18S rRNA se-

quences. However, the position of *P. carinii* f. sp. *suis* was not better resolved (BPP 40%) [42]. In Fig. 1, using more MnSOD sequences resulted in *P. carinii* f. sp. *suis* clustering with *P. carinii* f. sp. *muris* and rat-derived *P. carinii* (BPP 64%).

Within the Basidiomycota *Ganoderma* genus, regarded as an herb of longevity in Orient, MnSOD-based phylogenetic analysis of 28 *Ganoderma* isolates of the *G. lucidum* complex (whose taxonomic relationships failed to be clarified by conventional taxonomy) exhibited 5 groups, like in ITS- and LSU rRNA-based analysis [41]. Fig. 1 confirms morphological and cultural charac-

ters based-species identification fails to recognize monophyletic groups.

A polymorphism of the MnSOD gene in *G. lucidum* and *G. tsugae* was observed [41]. In *P. jirovecii*, two genotypes were identified at the SOD locus: one with C/T and the second with T/C at positions 110/215 [49–51].

3.3. MnSOD for large taxonomic studies

A phylogenetic analysis of MnSODs mainly including plants (18 sequences), fungi (15 sequences) and prokaryotes (58 sequences) [43], and a recent comparison of MnSOD- and SSU rRNA-based phylogenies including Zygomycota, Basidiomycota and Ascomycota species [Fréalle et al., 2005, manuscript submitted] confirmed MnSOD could be used for large phylogenetic studies in fungi. In the first study, 15 fungal MnSOD sequences (8 Basidiomycota and 7 Ascomycota) were included. Basidiomycota species clustered together. In Ascomycota, Euascomycetes (3 species) and Hemi/Archiascomycetes (4 species) were in 2 different clades. The overall topology of the tree closely reflected the taxonomic classification [43]. The second study only targeted fungi and included 61 MnSOD sequences: 1 Zygomycota, 9 Basidiomycota, and 51 Ascomycota sequences [Fréalle et al., 2005, manuscript submitted]. On this tree, like in Fig. 1, the ancestral Zygomycota *Rhizopus stolonifer* was used as an outgroup, and Basidiomycota and Ascomycota both formed strongly supported monophyletic groups. In Ascomycota, two highly supported clades were found. The topology of the “clade I” was largely congruent with the Ascomycota classical SSUrRNA tree, suggesting MnSOD could be used for further phylogenetic studies in fungi. In this clade, the MnSOD-based phylogeny confirmed the early emergence of *Pneumocystis* spp. within Ascomycota. The “clade II” in Ascomycota was clearly resolved, and contained paralogous Euascomycetes MnSOD sequences (Fig. 1). Predicted localization of these MnSOD sequences supported an unusual cytosolic localization for the corresponding proteins, suggesting that many Euascomycetes had both a mitochondrial and a cytosolic MnSOD. Some Hemiascomycetes also had both a mitochondrial and a cytosolic MnSOD, resulting either from an early (*Candida lipolytica*) or a late duplication (*C. albicans* and *Candida famata*), as shown by low (41.9%) or high (63.3% and 74.0%, respectively) identity rates between the two paralogous sequences.

3.4. Cytosolic/mitochondrial MnSODs in fungi: a complex evolutionary history

The MnSOD-based phylogenetic analysis of fungi contributed to understand the complex evolutionary history of this gene within fungi, where (i) multiple early or

late duplication of the MnSOD genes, (ii) loss of the mitochondrial targeting peptide in the course of evolution in numerous fungi, leading to a cytosolic localization of the MnSOD, occurred. Consequently, many fungi express both Cu/ZnSOD and MnSOD in the cytosolic compartment, whereas a second MnSOD protects the mitochondrial compartment against ROS [Fréalle et al., 2005, manuscript submitted].

4. From MnSOD genes to protein characterization; pathophysiological involvements

Among these 99 MnSOD sequences currently reported in the databases and used for the previously cited phylogenetic analyses, only 14 have been characterized so far (Table 1). Other sequences are putative MnSOD sequences, predicted to encode MnSOD proteins, based on sequence homology and presence of characteristic residues: tetrameric and iron/manganese-binding amino acids [4,52].

Among these 14 characterized fungal MnSODs, two were reported to be homodimeric enzymes [18,53], which is rather the prokaryotic MnSOD structure, or to have a putative cytosolic localization, since they were lacking the 20–30 AA mitochondrial amino terminal targeting peptide [10,16,54, Fréalle et al., 2005, manuscript submitted].

Proteins were either purified from cultures or obtained by molecular recombination (Table 1). The MnSOD gene was either cloned and transfected in wild-type or mutant fungal strains, in order to study its regulation [10,14,17,18,23,54,55], or cloned in *Escherichia coli*, producing recombinant proteins whose MnSOD allergenic properties were evaluated [54,56]. Great variations in SOD activity were observed, ranging from 2 to 7500 U mg⁻¹ (Table 1). SOD activity was not inhibited by cyanide (CN⁻) or hydrogen peroxide (H₂O₂), indicating these proteins were members of the MnSOD family. Actually, FeSODs are sensitive to H₂O₂, and Cu/ZnSODs are sensitive to both H₂O₂ and KCN [57].

In some pathogenic fungi, MnSOD was reported to be involved in fungal virulence [14,23,54,56]. Linking these data with MnSOD-based phylogeny could contribute to understand the different roles MnSODs play in pathogenic fungi.

4.1. SOD2 in the Basidiomycota yeast *C. neoformans*: a clear role in virulence

C. neoformans is a basidiomycetous yeast causing cryptococcosis, a neurotropic mycosis with pulmonary route of entry. *C. neoformans* var. *neoformans* (synonym: *C. neoformans* var. *grubii*) and *C. neoformans* var. *gattii* are two variants, diverging by their geo-

Table 1
Characterized fungal MnSODs

Fungal strain (GenBank accession number)	Protein purification, gene cloning or expression	Protein length (AA)	ORF (bp)	CDS (bp)	Intron number (size in bp)	Molecular mass (kDa), subunit/polymer	Mitochondrial targeting peptide (predicted localization)	Activity (U mg ⁻¹)	References
<i>Aspergillus fumigatus</i> ATCC 42202 (U53561)	Gene cloned into <i>Bam</i> HI/ <i>Hind</i> III-restricted p(His) ₆ -DHFR vector and expressed in <i>E. coli</i> M15	210	ND	630	ND	26.7/ND	Lacking (cytosolic)	2.32–2.61 ^a	[54]
<i>Candida albicans</i> <i>SOD2</i> (AF031478)	<ul style="list-style-type: none"> • Protein purified from culture • Gene cloned into pGEM-T 	234	702	702	0	26/106	Present (mitochondrial)	ND	[17]
<i>Candida albicans</i> ATCC 32354 <i>SOD3</i> (AF416340)	<ul style="list-style-type: none"> • Gene cloned into pQE30 and expressed in <i>E. coli</i> M15 • Gene cloned into pVT102U and expressed in <i>S. cerevisiae</i> 	206	678	618	1 (60)	22.7/85	Lacking (cytosolic)	370 ^b	[10]
<i>Colletrichum graminicola</i> M1.001 (AF430836)	No	208	794	624	3 (51, 60, 59)	23/ND	Lacking (cytosolic)	ND	[76]
<i>Cryptococcus neoformans</i> var. <i>gattii</i> ATCC 32609 (AY423630)	<ul style="list-style-type: none"> • Protein purified from culture • Gene cloned into pCR2.1 	225	956	675	4 (56, 120, 55, 50)	ND	Present (mitochondrial)	ND	[14]
<i>Cryptococcus neoformans</i> var. <i>grubii</i> H99 (AY737799)	No	234	1010	702	6 (55, 58, 62, 54, 51, 28)	19.5/80	Present (mitochondrial)	ND	[23,77]
<i>Ganoderma microporum</i> RSH 0821 (U56403, U56127)	Protein purified from culture	200	>708	600	≥2 (65, 217)	25/98	Lacking (cytosolic)	398–433 ^c	[78]
<i>Humicola lutea</i> 110	Protein purified from culture	ND	ND	ND	ND	18.9/76	Present (mitochondrial)	ND	[79]
<i>Malassezia sympodialis</i> ATCC 42132 (AJ548421)	Gene cloned into the pCR4 TOPO vector, subcloned into pQE30, and expressed in <i>E. coli</i> M15	237	ND	711	ND	22.4/ND	Present (mitochondrial)	ND	[56]
<i>Paxillus involutus</i> ATCC 200175 (AF114848)	<ul style="list-style-type: none"> • Protein purified from culture • Gene cloned into pET3d and expressed in <i>E. coli</i> BL21(DE3) 	206	859	618	3 (113, 66, 62)	26.2/53.2	Lacking (cytosolic)	ND	[53]
<i>Penicillium chrysogenum</i> AS-P-78 (AF026790, AF026523)	<ul style="list-style-type: none"> • Protein purified from culture • Gene cloned into pBluescript I-KS 	210	747	630	2 (60,57)	23.1/ND	Lacking (cytosolic)	ND	[16]

<i>Pneumocystis carinii</i> (AF036321)	• Protein purified from culture	220	1094	660	7 (45, 54, 144, 50, 48, 47, 46)	ND	Present (mitochondrial)	2 ^d	[80,81]
<i>Saccharomyces cerevisiae</i> YHR008C, ChrVIII (X02156, AY557821)	• Gene cloned into pCR-Script SK								
	• Protein purified from culture	233	699	699	0	24/97	Present (mitochondrial)	7500 ^b	[82]
<i>Schizosaccharomyces pombe</i> 972h-, ChrI (AL133357, AF069292)	• Protein purified from culture	219	780	657	1 (123)	22/50	Present (mitochondrial)	940 ^b	[18,83]
	• Gene cloned into pET-15b and expressed in <i>E. coli</i>								

ND: Not determined.

^a SOD activity determined according to Elstner et al. protocol [84].

^b SOD activity determined according to Beauchamp and Fridovich protocol [85].

^c SOD activity determined according to Oberley and Spitz protocol [86].

^d SOD activity determined according to Marklund and Marklund protocol [87].

graphic repartition (cosmopolite and subtropical, respectively), and antigenic properties [58].

Only one MnSOD gene was found in *C. neoformans* var. *neoformans* JEC21 and B-3501A completely sequenced genomes [59]. It encodes for a classical mitochondrial MnSOD whose role in virulence was clearly demonstrated in *C. neoformans* var. *gattii* and *C. neoformans* var. *grubii*, in two independent experiments [14,23].

In vitro, a growth inhibition of *sod2* mutant at 37 °C (host temperature), or facing an oxidative or osmotic stress (supposed to occur in phagocytic cells phagosomes) was observed [14,23]. In *C. neoformans* var. *gattii* SOD2 is not required for protection against human neutrophils in vitro [14]. However, SOD2 plays an important role in the virulence of both *C. neoformans* var. *gattii* and *C. neoformans* var. *grubii*, depending on the route of infection. In the intranasal infection model, SOD2 is absolutely essential for virulence since no mortality or morbidity was observed in mice infected with *C. neoformans* var. *gattii* *sod2* mutant at 120 days post-infection, whereas mice infected with wild-type or *sod2* Δ SOD2 reconstituted strains died by 45 days post-infection. *C. neoformans* var. *grubii* *sod2* mutant was avirulent too: *sod2* mutant infected mice completely cleared the fungi by day 38 post-infection and survived for up to 80 days post-infection, whereas all wild-type and *sod2* Δ SOD2 infected mice died 25 and 38 days post-infection, respectively [23]. In a high-dose intravenous infection model, only attenuated virulence was observed, with *C. neoformans* var. *gattii* *sod2* mutant producing typical meningoencephalitis, but a delayed course of the infection (31 days compared with 12–13 days with wild-type or reconstituted strains). In a low-dose intravenous infection model, *sod2* mutant was avirulent [14,15].

C. neoformans var. *gattii* *sod1sod2* double knockout strains were even more incapacitated since they were avirulent in intranasal and incapable of producing disease in intravenous infection models, in spite of long-term survival in the brain, where a lower partial pressure of oxygen (5 kPa) than in the lung (21 kPa) may have provided advantage to its growth [14].

The results of these two studies agree with the close phylogenetic relationships of these two *C. neoformans* variants (the two protein MnSOD sequences AAQ98967–EAL19049 have 96% identity). MnSOD probably conserved exactly the same function in both variants.

4.2. *Aspergillus fumigatus* and *Malassezia sympodialis* MnSODs: major allergens

A. fumigatus is an Ascomycota filamentous fungi, and *M. sympodialis* is a Basidiomycota yeast. *A. fumigatus* cytosolic and *M. sympodialis* mitochondrial complete

protein MnSOD sequences (Q92450 and CAD68071, respectively) have 45.5% identity. However, surprisingly, in these two phylogenetically distant species, MnSOD was found to have similar allergenic properties: (i) recombinant *A. fumigatus* MnSOD (r*Asp f6*) binds serum IgE from allergic patients, and more specifically in ABPA (allergic broncho-pulmonary aspergillosis), which is a severe pulmonary complication of cystic fibrosis (CF) (1–15%) or asthma (1–2%) [54,60,61], and (ii) recombinant *M. sympodialis* MnSOD (r*Mala s11*) binds serum IgE from AEDS (atopic eczema/dermatitis syndrome) patients, a common inflammatory skin disease that often develops in early childhood [56].

Among the 23 detected and proposed allergens of *A. fumigatus* [60,62], MnSOD is the only one whose cross-reactivity with human MnSOD (hMnSOD) was demonstrated, both by *A. fumigatus*-IgE binding inhibition when recombinant hMnSOD was added (humoral cross-reactivity), and by *A. fumigatus* or recombinant hMnSOD ability to provoke allergic reaction when injected to anti-MnSOD IgE producing individuals (cell-mediated cross-reactivity) [54]. Putative cross-reactive epitopes were identified by comparing the crystal structures of the two MnSODs [63]. Of the identical residues between *A. fumigatus* and hMnSODs, 4 were at least 30% solvent exposed in both crystal structures. Two more than 50% solvent exposed residues were identified as suitable targets for substitution by site-directed mutagenesis [63]. Cross-reactivity with MnSODs from other organisms (*Drosophila melanogaster*, *Hevea brasiliensis*, *E. coli* and *S. cerevisiae*) confirmed that MnSOD is a family of cross-reactive allergens [64,65]. *M. sympodialis* MnSOD (*Mala s11*) allergenic role supports the hypothesis of a panallergen MnSOD family [64,66]. Humoral and cell-mediated cross-reactivity with human and *A. fumigatus* homologues was recently found on 29 out of 69 atopic dermatitis (AD) patients, who had IgE-specific response to hMnSOD and r*Asp f6*, and reacted positively to skin challenges with both recombinant proteins [67].

In addition, interestingly, *A. fumigatus*-MnSOD targeted IgE response was found to be specific of ABPA in CF patients [61,68], since r*Asp f6* was recognized by IgE from ABPA patient's sera, but not by IgE from *A. fumigatus*-sensitized patient's sera [69]. Colonization of the respiratory tract and sensitization by *A. fumigatus* (up to 60% of CF patients) has to be clearly distinguished from ABPA in CF patients, since a specific prophylaxis and treatment of acute exacerbations, and prevention of end-stage fibrotic disease with corticosteroid and antifungal therapy is critical [70]. r*Asp f6*, like r*Asp f4*, a 30-kDa cytosolic protein of unknown function, could discriminate allergic patients (no specific IgE response) and ABPA patients (significant specific IgE response) [61]. However, other allergic diseases involving MnSOD can provoke *Asp f6* specific IgE sig-

nificant levels, like in AD where similar mean levels were found in AD and ABPA patients [67].

Specific IgE response to the cytosolic r*Asp f6* in patients with ABPA, and to *Mala s11* in patients with AD suggests these two allergens are involved in specific physiopathological mechanisms [67,69]. However, when it was investigated on a murine model of ABPA, r*Asp f6* failed to show any airway hyperreactivity. r*Asp f6* provoked an attenuated immune and inflammatory response compared to the response of *Asp f1*, *Asp f2* and *Asp f4*, three other *A. fumigatus* major allergens [71].

The role of *A. fumigatus* cytosolic and *M. sympodialis* mitochondrial MnSODs in defence against ROS was not explored.

4.3. Mitochondrial (SOD2) and cytosolic (SOD3) MnSODs in *C. albicans*

In *C. albicans*, an Ascomycota yeast, no virulence attenuation was observed with a *sod2* mutant on a murine model of candidiasis [72], suggesting *C. albicans* SOD2, unlike *C. neoformans* SOD2, is not required for virulence. The presence of six SOD genes in this species, two MnSODs and four Cu/ZnSODs [10,11], instead of two in *C. neoformans* var. *gattii*, one mitochondrial MnSOD and one cytosolic Cu/ZnSOD, could explain the differences in SOD regulation.

Four out of these six SODs have been characterized so far: SOD1, a cytosolic Cu/ZnSOD [72], SOD2, a mitochondrial MnSOD [17], SOD3, a cytosolic MnSOD [10], and SOD5, a cytosolic Cu/ZnSOD [11]. SOD1, but not SOD2, is required for pathogenicity [73]. This was supported by the gene expression profile of *C. albicans* in human blood, which showed that SOD1 was highly expressed in blood containing leucocytes, but not in plasma, and that SOD2 was not expressed in cells exposed to blood, but only in cultures [74]. In vitro, the advantage for *C. albicans* to express both a MnSOD (SOD3) and a Cu/ZnSOD (SOD1) in the cytosolic compartment could be (i) to improve resistance to oxidative stress, (ii) to modulate SOD expression according to metal ions availability in growth phases. Since no difference in paraquat or menadione sensitivity was observed, and since the two SOD regulation is inversely coordinated, resulting in a constant rather than an increased cytosolic SOD activity, no improved resistance to oxidative stress could be hypothesized. The second hypothesis seems more likely: SOD1 decreased expression during stationary phase could be related to *C. albicans* eliminating copper to avoid copper deleterious effects, and result in an increased SOD3 expression, as seen in *Podospira anserina*, where copper-depleted conditions increased SOD2 transcript levels [75]. Therefore, increased SOD3 expression could substitute for SOD1 to provide protection against ROS during stationary phase. SOD3

role in pathogenicity has not been explored yet. It could be involved in the commensal pathogen *C. albicans* survival in the host environment [10].

Many other pathogenic fungi express both a cytosolic and a mitochondrial MnSOD. Clustering of *C. albicans* and *C. famata* cytosolic MnSOD sequences on the MnSOD tree (66.5% identity) suggest these enzymes could have similar functions. Other Ascomycota cytosolic MnSODs (gathered in the Euascomycetes and *C. lipolytica* “cytosolic” clade) probably result from an early duplication, as suggested by low identity rates between paralogous sequences (41.9–49.6%) [Fréalle et al., 2005, manuscript submitted]. Resulting proteins could have acquired functional specialization. *A. fumigatus* cytosolic MnSOD showed allergenic properties, but the role of both a Cu/ZnSOD and a MnSOD in the cytosolic compartment has not been explored. Further studies should be directed to determine the role of these two MnSODs in so many phylogenetically distant species, and if they are involved in pathogenicity.

5. Conclusion

MnSOD role in pathogenic fungi is poorly known. It was only explored in four phylogenetically distant pathogenic fungi. Differences in MnSOD gene regulation, function, subcellular localization, or role in fungal virulence were not correlated to the phylogenetic position of the fungi. Since defence against ROS is determinant for pathogenicity, MnSOD evolutionary history and pathophysiological roles in invasive or allergic mycoses agree with the hypothesis that pathogenicity emerged multiple times within fungi.

References

- [1] Hamilton, A.J. and Holdom, M.D. (1999) Antioxidant systems in the pathogenic fungi of man and their role in virulence. *Med. Mycol.* 37, 375–389.
- [2] Paris, S., Wysong, D., Debeaupuis, J.P., Shibuya, K., Philippe, B., Diamond, R.D. and Latge, J.P. (2003) Catalases of *Aspergillus fumigatus*. *Infect. Immun.* 71, 3551–3562.
- [3] Calera, J.A., Paris, S., Monod, M., Hamilton, A.J., Debeaupuis, J.P., Diaquin, M., Lopez-Medrano, R., Leal, F. and Latge, J.P. (1997) Cloning and disruption of the antigenic catalase gene of *Aspergillus fumigatus*. *Infect. Immun.* 65, 4718–4724.
- [4] Parker, M.W., Blake, C.C., Barra, D., Bossa, F., Schinina, M.E., Bannister, W.H. and Bannister, J.V. (1987) Structural identity between the iron- and manganese-containing superoxide dismutases. *Protein Eng.* 1, 393–397.
- [5] Tainer, J.A., Getzoff, E.D., Beem, K.M., Richardson, J.S. and Richardson, D.C. (1982) Determination and analysis of the 2 A-structure of copper, zinc superoxide dismutase. *J. Mol. Biol.* 160, 181–217.
- [6] Smith, M.W. and Doolittle, R.F. (1992) A comparison of evolutionary rates of the two major kinds of superoxide dismutase. *J. Mol. Evol.* 34, 175–184.
- [7] Fridovich, I. (1995) Superoxide radical and superoxide dismutases. *Annu. Rev. Biochem.* 64, 97–112.
- [8] Nedeva, T.S., Petrova, V.Y., Zamfirova, D.R., Stephanova, E.V. and Kujumdzieva, A.V. (2004) Cu/Zn superoxide dismutase in yeast mitochondria – a general phenomenon. *FEMS Microbiol. Lett.* 230, 19–25.
- [9] Sturtz, L.A., Diekert, K., Jensen, L.T., Lill, R. and Culotta, V.C. (2001) A fraction of yeast Cu,Zn-superoxide dismutase and its metallochaperone, CCS, localize to the intermembrane space of mitochondria. A physiological role for SOD1 in guarding against mitochondrial oxidative damage. *J. Biol. Chem.* 276, 38084–38089.
- [10] Lamarre, C., LeMay, J.D., Deslauriers, N. and Bourbonnais, Y. (2001) *Candida albicans* expresses an unusual cytoplasmic manganese-containing superoxide dismutase (*SOD3* gene product) upon the entry and during the stationary phase. *J. Biol. Chem.* 276, 43784–43791.
- [11] Martchenko, M., Alarco, A.M., Harcus, D. and Whiteway, M. (2004) Superoxide dismutases in *Candida albicans*: transcriptional regulation and functional characterization of the hyphal-induced *SOD5* gene. *Mol. Biol. Cell* 15, 456–467.
- [12] Borgstahl, G.E., Parge, H.E., Hickey, M.J., Beyer Jr., W.F., Hallewell, R.A. and Tainer, J.A. (1992) The structure of human mitochondrial manganese superoxide dismutase reveals a novel tetrameric interface of two 4-helix bundles. *Cell* 71, 107–118.
- [13] Bai, Z., Harvey, L.M. and McNeil, B. (2003) Oxidative stress in submerged cultures of fungi. *Crit. Rev. Biotechnol.* 23, 267–302.
- [14] Narasipura, S.D., Chaturvedi, V. and Chaturvedi, S. (2005) Characterization of *Cryptococcus neoformans* variety *gattii* SOD2 reveals distinct roles of the two superoxide dismutases in fungal biology and virulence. *Mol. Microbiol.* 55, 1782–1800.
- [15] Narasipura, S.D., Ault, J.G., Behr, M.J., Chaturvedi, V. and Chaturvedi, S. (2003) Characterization of Cu,Zn superoxide dismutase (*SOD1*) gene knock-out mutant of *Cryptococcus neoformans* var. *gattii*: role in biology and virulence. *Mol. Microbiol.* 47, 1681–1694.
- [16] Diez, B., Schleissner, C., Moreno, M.A., Rodriguez, M., Collados, A. and Barredo, J.L. (1998) The manganese superoxide dismutase from the penicillin producer *Penicillium chrysogenum*. *Curr. Genet.* 33, 387–394.
- [17] Rhie, G.E., Hwang, C.S., Brady, M.J., Kim, S.T., Kim, Y.R., Huh, W.K., Baek, Y.U., Lee, B.H., Lee, J.S. and Kang, S.O. (1999) Manganese-containing superoxide dismutase and its gene from *Candida albicans*. *Biochim. Biophys. Acta* 1426, 409–419.
- [18] Jeong, J.H., Kwon, E.S. and Roe, J.H. (2001) Characterization of the manganese-containing superoxide dismutase and its gene regulation in stress response of *Schizosaccharomyces pombe*. *Biochem. Biophys. Res. Commun.* 283, 908–914.
- [19] Jung, H.I., Lee, Y.Y., Lim, H.W., Ahn, K.S., Park, E.H. and Lim, C.J. (2002) Regulation of the manganese-containing superoxide dismutase gene from fission yeast. *Mol. Cells* 14, 300–304.
- [20] Emri, T., Poci, I. and Szentirmai, A. (1999) Analysis of the oxidative stress response of *Penicillium chrysogenum* to menadione. *Free Radic. Res.* 30, 125–132.
- [21] Cyrne, L., Martins, L., Fernandes, L. and Marinho, H.S. (2003) Regulation of antioxidant enzymes gene expression in the yeast *Saccharomyces cerevisiae* during stationary phase. *Free Radic. Biol. Med.* 34, 385–393.
- [22] Hwang, C.S., Baek, Y.U., Yim, H.S. and Kang, S.O. (2003) Protective roles of mitochondrial manganese-containing superoxide dismutase against various stresses in *Candida albicans*. *Yeast* 20, 929–941.
- [23] Giles, S.S., Batinic-Haberle, I., Perfect, J.R. and Cox, G.M. (2005) *Cryptococcus neoformans* mitochondrial superoxide dismutase: an essential link between antioxidant function and high-temperature growth. *Eukaryot. Cell.* 4, 46–54.

- [24] Longo, V.D., Liou, L.L., Valentine, J.S. and Gralla, E.B. (1999) Mitochondrial superoxide decreases yeast survival in stationary phase. *Arch. Biochem. Biophys.* 365, 131–142.
- [25] Gralla, E.B. and Valentine, J.S. (1991) Null mutants of *Saccharomyces cerevisiae* Cu,Zn superoxide dismutase: characterization and spontaneous mutation rates. *J. Bacteriol.* 173, 5918–5920.
- [26] Davidson, J.F. and Schiestl, R.H. (2001) Mitochondrial respiratory electron carriers are involved in oxidative stress during heat stress in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 21, 8483–8489.
- [27] Davidson, J.F., Whyte, B., Bissinger, P.H. and Schiestl, R.H. (1996) Oxidative stress is involved in heat-induced cell death in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* 93, 5116–5121.
- [28] Cox, G.M., Harrison, T.S., McDade, H.C., Taborda, C.P., Heinrich, G., Casadevall, A. and Perfect, J.R. (2003) Superoxide dismutase influences the virulence of *Cryptococcus neoformans* by affecting growth within macrophages. *Infect. Immun.* 71, 173–180.
- [29] Herr, R.A., Tarcha, E.J., Taborda, P.R., Taylor, J.W., Ajello, L. and Mendoza, L. (2001) Phylogenetic analysis of *Lacazia lobii* places this previously uncharacterized pathogen within the dimorphic Onygenales. *J. Clin. Microbiol.* 39, 309–314.
- [30] Bruns, T.D., Vilgalys, R., Barns, S.M., Gonzalez, D., Hibbett, D.S., Lane, D.J., Simon, L., Stickel, S., Szaro, T.M., Weisburg, W.G. and Sogin, M.L. (1992) Evolutionary relationships within the fungi: analyses of nuclear small subunit rRNA sequences. *Mol. Phylogenet. Evol.* 1, 231–241.
- [31] De Hoog, G.S., Guarro, J., Gene, J. and Figueras, M.J. (2000) Atlas of Clinical Fungi, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands, and Universitat Rovira i Virgili, Reus, Spain.
- [32] Kurtzman, C.P. (1994) Molecular taxonomy of the yeasts. *Yeast* 10, 1727–1740.
- [33] Henry, T., Iwen, P.C. and Hinrichs, S.H. (2000) Identification of *Aspergillus* species using internal transcribed spacer regions 1 and 2. *J. Clin. Microbiol.* 38, 1510–1515.
- [34] Voigt, K., Cigelnik, E. and O'Donnell, K. (1999) Phylogeny and PCR identification of clinically important Zygomycetes based on nuclear ribosomal-DNA sequence data. *J. Clin. Microbiol.* 37, 3957–3964.
- [35] Vanittanakom, N., Vanittanakom, P. and Hay, R.J. (2002) Rapid identification of *Penicillium marneffeii* by PCR-based detection of specific sequences on the rRNA gene. *J. Clin. Microbiol.* 40, 1739–1742.
- [36] Hinrikson, H.P., Hurst, S.F., Lott, T.J., Warnock, D.W. and Morrison, C.J. (2005) Assessment of ribosomal large-subunit d1–d2, internal transcribed spacer 1, and internal transcribed spacer 2 regions as targets for molecular identification of medically important *Aspergillus* species. *J. Clin. Microbiol.* 43, 2092–2103.
- [37] Graser, Y., El Fari, M., Vilgalys, R., Kuijpers, A.F., De Hoog, G.S., Presber, W. and Tietz, H. (1999) Phylogeny and taxonomy of the family Arthrodermataceae (dermatophytes) using sequence analysis of the ribosomal ITS region. *Med. Mycol.* 37, 105–114.
- [38] Yokoyama, K., Wang, L., Miyaji, M. and Nishimura, K. (2001) Identification, classification and phylogeny of the *Aspergillus* section *Nigri* inferred from mitochondrial cytochrome *b* gene. *FEMS Microbiol. Lett.* 200, 241–246.
- [39] Diezmann, S., Cox, C.J., Schonian, G., Vilgalys, R.J. and Mitchell, T.G. (2004) Phylogeny and evolution of medical species of *Candida* and related taxa: a multigenic analysis. *J. Clin. Microbiol.* 42, 5624–5635.
- [40] Kurtzman, C.P. and Robnett, C.J. (2003) Phylogenetic relationships among yeasts of the 'Saccharomyces complex' determined from multigene sequence analyses. *FEMS Yeast Res.* 3, 417–432.
- [41] Wang, H.F. (1996) Studies of Manganese Superoxide Dismutase Gene of *Ganoderma*. Graduate Institute of Agricultural Chemistry, National Taiwan University, Taipei, Taiwan, quoted in Hseu, R.S. (1997) Recent advances in molecular systematics of the *Ganoderma lucidum* complex, presented during the 1st international symposium on *Ganoderma lucidum* in Tokyo, Japan.
- [42] Denis, C.M., Mazars, E., Guyot, K., Odberg-Ferragut, C., Viscogliosi, E., Dei-Cas, E. and Wakefield, A.E. (2000) Genetic divergence at the SODA locus of six different *formae speciales* of *Pneumocystis carinii*. *Med. Mycol.* 38, 289–300.
- [43] Fink, R.C. and Scandalios, J.G. (2002) Molecular evolution and structure–function relationships of the superoxide dismutase gene families in angiosperms and their relationship to other eukaryotic and prokaryotic superoxide dismutases. *Arch. Biochem. Biophys.* 399, 19–36.
- [44] Huelsenbeck, J.P. and Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17, 754–755.
- [45] Opperdoes, F.R. (1997) Molecular Evolution and Phylogenetic Analysis of Proteins. Available from: <<http://www.icp.ucl.ac.be/~opperd/private/arguments.html>>.
- [46] Dei-Cas, E., Mazars, E., Ferragut, C.O., Durand, I., Aliouat, E.M., Dridba, M., Palluault, F., Cailliez, J.C., Seguy, N., Tibayrenc, M., Mullet, C., Creusy, C. and Camus, D. (1994) Ultrastructural, genomic, isoenzymatic and biological features make it possible to distinguish rabbit *Pneumocystis* from other mammal *Pneumocystis* strains. *J. Eukaryot. Microbiol.* 41, 84S.
- [47] Kovacs, J.A., Halpern, J.L., Lundgren, B., Swan, J.C., Parrillo, J.E. and Masur, H. (1989) Monoclonal antibodies to *Pneumocystis carinii*: identification of specific antigens and characterization of antigenic differences between rat and human isolates. *J. Infect. Dis.* 159, 60–70.
- [48] Wakefield, A.E. (1998) Genetic heterogeneity in *Pneumocystis carinii*: an introduction. *FEMS Immunol. Med. Microbiol.* 22, 5–13.
- [49] Wakefield, A.E., Lindley, A.R., Ambrose, H.E., Denis, C.M. and Miller, R.F. (2003) Limited asymptomatic carriage of *Pneumocystis jirovecii* in human immunodeficiency virus-infected patients. *J. Infect. Dis.* 187, 901–908.
- [50] Miller, R.F., Lindley, A.R., Ambrose, H.E., Aliouat-Denis, C.M. and Wakefield, A.E. (2003) Multilocus genotyping of *Pneumocystis jirovecii* from adult HIV-infected patients with *Pneumocystis pneumonia*. *J. Eukaryot. Microbiol.* 50 (Suppl), 654–655.
- [51] Miller, R.F., Lindley, A.R., Malin, A.S., Ambrose, H.E. and Wakefield, A.E. (2005) Isolates of *Pneumocystis jirovecii* from Harare show high genotypic similarity to isolates from London at the superoxide dismutase locus. *Trans. R. Soc. Trop. Med. Hyg.* 99, 202–206.
- [52] Wintjens, R., Noel, C., May, A.C., Gerbod, D., Dufernez, F., Capron, M., Viscogliosi, E. and Rooman, M. (2004) Specificity and phenetic relationships of iron- and manganese-containing superoxide dismutases on the basis of structure and sequence comparisons. *J. Biol. Chem.* 279, 9248–9254.
- [53] Jacob, C., Courbot, M., Brun, A., Steinman, H.M., Jacquot, J.P., Botton, B. and Chalot, M. (2001) Molecular cloning, characterization and regulation by cadmium of a superoxide dismutase from the ectomycorrhizal fungus *Paxillus involutus*. *Eur. J. Biochem.* 268, 3223–3232.
- [54] Cramer, R., Faith, A., Hemmann, S., Jaussi, R., Ismail, C., Menz, G. and Blaser, K. (1996) Humoral and cell-mediated autoimmunity in allergy to *Aspergillus fumigatus*. *J. Exp. Med.* 184, 265–270.
- [55] Van Loon, A.P., Pesold-Hurt, B. and Schatz, G. (1986) A yeast mutant lacking mitochondrial manganese-superoxide dismutase is hypersensitive to oxygen. *Proc. Natl. Acad. Sci. USA* 83, 3820–3824.
- [56] Andersson, A., Rasool, O., Schmidt, M., Kodzius, R., Fluckiger, S., Zargari, A., Cramer, R. and Scheynius, A. (2004) Cloning, expression and characterization of two new IgE-binding proteins from the yeast *Malassezia sympodialis* with sequence similarities

- to heat shock proteins and manganese superoxide dismutase. *Eur. J. Biochem.* 271, 1885–1894.
- [57] Misra, H.P. and Fridovich, I. (1978) Inhibition of superoxide dismutases by azide. *Arch. Biochem. Biophys.* 189, 317–322.
- [58] Neuville, S., Dromer, F., Chretien, F., Gray, F. and Lortholary, O. (2002) Physiopathologie des méningoencéphalites dues à *Cryptococcus neoformans*. *Ann. Med. Intern. (Paris)* 153, 323–328.
- [59] Loftus, B.J., Fung, E., Roncaglia, P., Rowley, D., Amedeo, P., Bruno, D., Vamathevan, J., Miranda, M., Anderson, I.J., Fraser, J.A., Allen, J.E., Bosdet, I.E., Brent, M.R., Chiu, R., Doering, T.L., Donlin, M.J., D'Souza, C.A., Fox, D.S., Grinberg, V., Fu, J., Fukushima, M., Haas, B.J., Huang, J.C., Janbon, G., Jones, S.J., Koo, H.L., Krzywinski, M.I., Kwon-Chung, J.K., Lengeler, K.B., Maiti, R., Marra, M.A., Marra, R.E., Mathewson, C.A., Mitchell, T.G., Pertea, M., Riggs, F.R., Salzberg, S.L., Schein, J.E., Shvartsbeyn, A., Shin, H., Shumway, M., Specht, C.A., Suh, B.B., Tenney, A., Utterback, T.R., Wickes, B.L., Wortman, J.R., Wye, N.H., Kronstad, J.W., Lodge, J.K., Heitman, J., Davis, R.W., Fraser, C.M. and Hyman, R.W. (2005) The genome of the basidiomycetous yeast and human pathogen *Cryptococcus neoformans*. *Science* 307, 1321–1324.
- [60] Stevens, D.A., Moss, R.B., Kurup, V.P., Knutsen, A.P., Greenberger, P., Judson, M.A., Denning, D.W., Cramer, R., Brody, A.S., Light, M., Skov, M., Maish, W. and Mastella, G. (2003) Allergic bronchopulmonary aspergillosis in cystic fibrosis-state of the art: Cystic Fibrosis Foundation Consensus Conference. *Clin. Infect. Dis.* 37 (Suppl 3), S225–S264.
- [61] Cramer, R. (1998) Recombinant *Aspergillus fumigatus* allergens: from the nucleotide sequences to clinical applications. *Int. Arch. Allergy Immunol.* 115, 99–114.
- [62] Rementeria, A., Lopez-Molina, N., Ludwig, A., Vivanco, A.B., Bikandi, J., Ponton, J. and Garaizar, J. (2005) Genes and molecules involved in *Aspergillus fumigatus* virulence. *Rev. Iberoam. Micol.* 22, 1–23.
- [63] Fluckiger, S., Mittl, P.R., Scapozza, L., Fijten, H., Folkers, G., Grutter, M.G., Blaser, K. and Cramer, R. (2002) Comparison of the crystal structures of the human manganese superoxide dismutase and the homologous *Aspergillus fumigatus* allergen at 2-Å resolution. *J. Immunol.* 168, 1267–1272.
- [64] Mayer, C., Hemmann, S., Faith, A., Blaser, K. and Cramer, R. (1997) Cloning, production, characterization and IgE cross-reactivity of different manganese superoxide dismutases in individuals sensitized to *Aspergillus fumigatus*. *Int. Arch. Allergy Immunol.* 113, 213–215.
- [65] Wagner, S., Sowka, S., Mayer, C., Cramer, R., Focke, M., Kurup, V.P., Scheiner, O. and Breiteneder, H. (2001) Identification of a *Hevea brasiliensis* latex manganese superoxide dismutase (Hev b 10) as a cross-reactive allergen. *Int. Arch. Allergy Immunol.* 125, 120–127.
- [66] Fluckiger, S., Scapozza, L., Mayer, C., Blaser, K., Folkers, G. and Cramer, R. (2002) Immunological and structural analysis of IgE-mediated cross-reactivity between manganese superoxide dismutases. *Int. Arch. Allergy Immunol.* 128, 292–303.
- [67] Schmid-Grendelmeier, P., Fluckiger, S., Disch, R., Trautmann, A., Wuthrich, B., Blaser, K., Scheynius, A. and Cramer, R. (2005) IgE-mediated and T cell-mediated autoimmunity against manganese superoxide dismutase in atopic dermatitis. *J. Allergy Clin. Immunol.* 115, 1068–1075.
- [68] Kurup, V.P., Banerjee, B., Hemmann, S., Greenberger, P.A., Blaser, K. and Cramer, R. (2000) Selected recombinant *Aspergillus fumigatus* allergens bind specifically to IgE in ABPA. *Clin. Exp. Allergy* 30, 988–993.
- [69] Hemmann, S., Nikolaizik, W.H., Schoni, M.H., Blaser, K. and Cramer, R. (1998) Differential IgE recognition of recombinant *Aspergillus fumigatus* allergens by cystic fibrosis patients with allergic bronchopulmonary aspergillosis or *Aspergillus* allergy. *Eur. J. Immunol.* 28, 1155–1160.
- [70] Stevens, D.A., Kan, V.L., Judson, M.A., Morrison, V.A., Dummer, S., Denning, D.W., Bennett, J.E., Walsh, T.J., Patterson, T.F. and Pankey, G.A. (2000) Practice guidelines for diseases caused by *Aspergillus*. *Infectious Diseases Society of America. Clin. Infect. Dis.* 30, 696–709.
- [71] Kurup, V.P., Xia, J.Q., Cramer, R., Rickaby, D.A., Choi, H.Y., Fluckiger, S., Blaser, K., Dawson, C.A. and Kelly, K.J. (2001) Purified recombinant *A. fumigatus* allergens induce different responses in mice. *Clin. Immunol.* 98, 327–336.
- [72] Hwang, C.S., Rhie, G., Kim, S.T., Kim, Y.R., Huh, W.K., Baek, Y.U. and Kang, S.O. (1999) Copper- and zinc-containing superoxide dismutase and its gene from *Candida albicans*. *Biochim. Biophys. Acta* 1427, 245–255.
- [73] Hwang, C.S., Rhie, G.E., Oh, J.H., Huh, W.K., Yim, H.S. and Kang, S.O. (2002) Copper- and zinc-containing superoxide dismutase (Cu/ZnSOD) is required for the protection of *Candida albicans* against oxidative stresses and the expression of its full virulence. *Microbiology* 148, 3705–3713.
- [74] Fradin, C., Kretschmar, M., Nichterlein, T., Gaillardin, C., d'Enfert, C. and Hube, B. (2003) Stage-specific gene expression of *Candida albicans* in human blood. *Mol. Microbiol.* 47, 1523–1543.
- [75] Borghouts, C., Werner, A., Elthon, T. and Osiewacz, H.D. (2001) Copper-modulated gene expression and senescence in the filamentous fungus *Podospora anserina*. *Mol. Cell. Biol.* 21, 390–399.
- [76] Fang, G.C., Hanau, R.M. and Vaillancourt, L.J. (2002) The SOD2 gene, encoding a manganese-type superoxide dismutase, is up-regulated during conidiogenesis in the plant-pathogenic fungus *Colletotrichum graminicola*. *Fungal Genet. Biol.* 36, 155–165.
- [77] Tesfa-Selase, F. and Hay, R.J. (1995) Superoxide dismutase of *Cryptococcus neoformans*: purification and characterization. *J. Med. Vet. Mycol.* 33, 253–259.
- [78] Pan, S.M., Ye, J.S. and Hseu, R.S. (1997) Purification and characterization of manganese superoxide dismutase from *Ganoderma microsporium*. *Biochem. Mol. Biol. Int.* 42, 1035–1043.
- [79] Dolashka-Angelova, P., Genova, L., Stoeva, S., Stefanov, B., Angelova, M., Hristova, R., Pashova, S. and Voelter, W. (1999) Isolation and characterization of a novel superoxide dismutase from fungal strain *Humicola lutea* 110. *J. Pept. Res.* 54, 279–289.
- [80] Denis, C.M., Guyot, K., Wakefield, A.E., Dive, D., Dei-Cas, E., Camus, D. and Odberg-Ferragut, C. (1998) Molecular cloning and characterization of a superoxide dismutase (sod) gene in *Pneumocystis carinii*. *J. Eukaryot. Microbiol.* 45, 475–483.
- [81] Denis, C.M., Odberg-Ferragut, C., Guyot, K., Dei-Cas, E., Camus, D. and Dive, D. (1996) Manganese-cofactored superoxide dismutase activity in rat-derived *Pneumocystis carinii*. *J. Eukaryot. Microbiol.* 43, 26S.
- [82] Ravindranath, S.D. and Fridovich, I. (1975) Isolation and characterization of a manganese-containing superoxide dismutase from yeast. *J. Biol. Chem.* 250, 6107–6112.
- [83] Wood, V., Gwilliam, R., Rajandream, M.A., Lyne, M., Lyne, R., Stewart, A., Sgouros, J., Peat, N., Hayles, J., Baker, S., Basham, D., Bowman, S., Brooks, K., Brown, D., Brown, S., Chillingworth, T., Churcher, C., Collins, M., Connor, R., Cronin, A., Davis, P., Feltwell, T., Fraser, A., Gentles, S., Goble, A., Hamlin, N., Harris, D., Hidalgo, J., Hodgson, G., Holroyd, S., Hornsby, T., Howarth, S., Huckle, E.J., Hunt, S., Jagels, K., James, K., Jones, L., Jones, M., Leather, S., McDonald, S., McLean, J., Mooney, P., Moule, S., Mungall, K., Murphy, L., Niblett, D., Odell, C., Oliver, K., O'Neil, S., Pearson, D., Quail, M.A., Rabinowitsch, E., Rutherford, K., Rutter, S., Saunders, D., Seeger, K., Sharp, S., Skelton, J., Simmonds, M., Squares, R., Squares, S., Stevens, K., Taylor, K., Taylor, R.G., Tivey, A., Walsh, S., Warren, T., Whitehead, S., Woodward, J., Volckaert, G., Aert, R., Robben, J., Grymonprez, B., Weltjens, I., Vanstreels, E., Rieger, M., Schafer, M., Muller-Auer, S., Gabel, C., Fuchs, M., Dusterhoft, A., Fritze, C., Holzer, E., Moestl, D., Hilbert, H., Borzym, K., Langer, I., Beck, A., Lehrach, H.,

- Reinhardt, G., Pohl, T.M., Eger, P., Zimmermann, W., Wedler, H., Wambutt, R., Purnelle, B., Goffeau, A., Cadieu, E., Dreano, S., Gloux, S., Lelaure, V., Mottier, S., Galibert, F., Aves, S.J., Xiang, Z., Hunt, C., Moore, K., Hurst, S.M., Lucas, M., Rochet, M., Gaillardin, C., Tallada, V.A., Garzon, A., Thode, G., Daga, R.R., Cruzado, L., Jimenez, J., Sanchez, M., del Rey, F., Benito, J., Dominguez, A., Revuelta, J.L., Moreno, S., Armstrong, J., Forsburg, S.L., Cerutti, L., Lowe, T., McCombie, W.R., Paulsen, I., Potashkin, J., Shpakovski, G.V., Ussery, D., Barrell, B.G. and Nurse, P. (1974) The genome sequence of *Schizosaccharomyces pombe*. *Nature* 415, 871–880.
- [84] Elstner, E.F., Youngman, R.J. and Osswald, W. (1983) Superoxide dismutase In: *Methods of Enzymatic Analysis* (Bergmeyer, M., Bergmeyer, J. and Grasol, M., Eds.), pp. 293–302. Verlag Chemie, Weinheim.
- [85] Beauchamp, C. and Fridovich, I. (1971) Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Anal. Biochem.* 44, 276–287.
- [86] Oberley, L.W. and Spitz, D.R. (1985) Assay of superoxide dismutase using nitroblue tetrazolium In: *CRC Handbook of Methods for Oxygen Radical Research* (Greenwald, R.A., Ed.), pp. 217–221. CRC Press, Boca Raton, FL.
- [87] Marklund, S. and Marklund, G. (1974) Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur. J. Biochem.* 47, 469–474.