

# Biological and Clinical Attributes of *Sporothrix globosa*, a Causative Agent of Sporotrichosis

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**Abstract:** Sporotrichosis is an important subcutaneous mycosis with high prevalence and threat to human and animal health worldwide. *Sporothrix schenckii*, *Sporothrix brasiliensis*, and *Sporothrix globosa* are the main etiological agents of this disease; and even though many efforts have been made recently to understand the *Sporothrix*-host interaction, little is known about *S. globosa*, an underestimated species. This organism shows the lowest virulence among the members of the *Sporothrix* pathogenic clade and represents an important pathogenic agent due to its global distribution. Here, we offer a review with all the known information about *S. globosa*, including its genome and proteomic information, and compare it with *S. schenckii* and *S. brasiliensis*, to explain the differences observed among these species, in terms of virulence, the host immune response, and the antifungal sensitivity. Also, we provide the gene prediction of some *S. globosa* putative virulence factors.

**Keywords:** antifungal drugs, fungal infection, host–fungus interplay, epidemiology, diagnosis, treatment

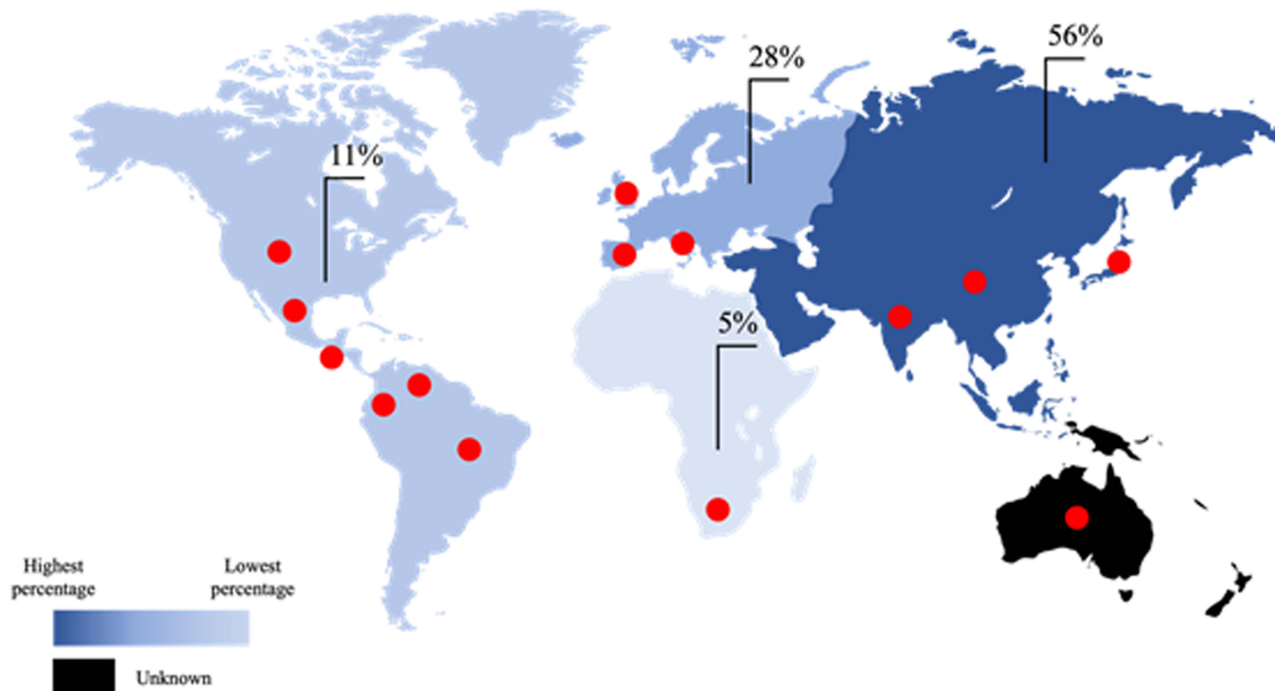
## Introduction

Sporotrichosis is a human and animal fungal infection that affects the epidermis and subcutaneous tissues, having as etiological agent members of the *Sporothrix* genus.<sup>1–3</sup> The classical infection route is the traumatic conidia implantation when interacting with decaying vegetal tissues, thus it is considered as a sapronosis.<sup>1</sup> However, epidemic sporotrichosis outbreaks of domestic animals, in the last years, have revealed that the fungus can be transmitted by close contact between infected animals and human beings, and thus is now considered as a zoonosis too.<sup>4–6</sup> The *Sporothrix* genus currently contains at least 51 species and phylogenetic analyses have grouped them within different clades, from which the pathogenic clade contains most of the sporotrichosis etiological agents: *Sporothrix schenckii*, *Sporothrix brasiliensis*, *Sporothrix globosa*, and *Sporothrix luriei*.<sup>7</sup> The first three species are the most common ones associated with the disease, and *S. schenckii* was described and recognized as the causative agent of the disease more than a century ago, and thus it is currently the most studied species of the *Sporothrix* pathogenic clade.<sup>1,3</sup> *S. brasiliensis* has attracted attention in recent years because of the zoonotic outbreaks in Brazil, and most recently in Argentina,<sup>1,8,9</sup> affecting both domestic cats and human beings.<sup>8,10</sup> In the case of *S. globosa*, the species has been recently associated with both sapronotic and zoonotic outbreaks in Asia, and to a lesser extent in some parts of America.<sup>11–13</sup> This organism is the most underestimated and poorly study *Sporothrix* species; and thus far, no review papers dealing with both its basic and clinical aspects are available in the scientific literature. Therefore, in this review, we focused on highlighting the similarities and differences of *S. globosa* with other medically relevant *Sporothrix* species, in terms of basic biological aspects, clinical manifestations, diagnosis, and infection treatment.

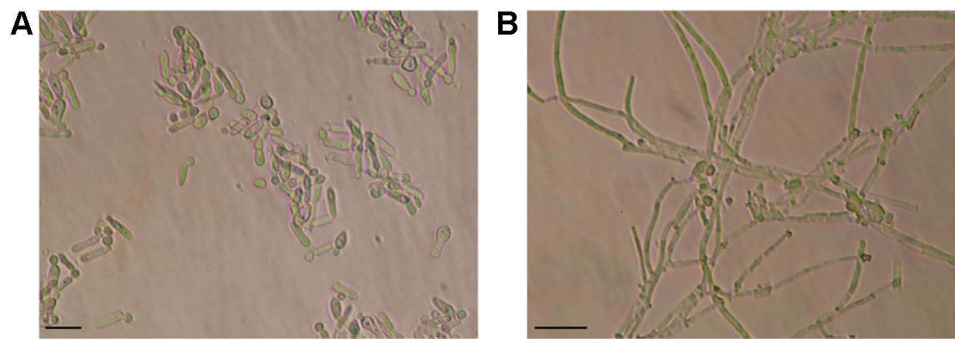
## Fundamental Aspects of *Sporothrix globosa*

Although *S. globosa* is considered a cosmopolitan species, there are geographical areas where it is most frequently isolated, with a prevalence rate of 56% in Asia (China, India, and Japan), where it represents 99.3% of the total *Sporothrix* species in this region, 28% in Europe (Spain, Italy, and United Kingdom), 11% in America (Brazil, United States, Venezuela, Guatemala, Colombia, and Mexico), and 5% in Africa (South Africa) (Figure 1).<sup>13–18</sup> It has also been reported that all the strains found in India so far belong to *S. globosa*, and it is the second main species found in Australia.<sup>19,20</sup>

Like other medically relevant *Sporothrix* species, *S. globosa* is a thermodimorphic fungus that in vitro grows as conidium-producer hypha at 25°C, and this is regarded as the saprophytic phase found in the environment; while the yeast-like phase grows in vitro at 37°C and is considered the parasitic morphology found in the host tissues.<sup>1</sup> *S. schenckii*, *S. brasiliensis*, and *S. globosa* have a similar conidial morphology when grown in the potato-dextrose-agar medium at 30°C, where terminal or intercalary on differentiated conidiophores conidiogenous cells are observed, and these may be of two kinds: sympodial conidia, which are usually hyaline to sub-hyaline, obovoidal and 2–5-µm-long by 1–3-µm-wide cells; and sessile conidia that are brown to dark brown, thick-walled, globose to subglobose, and 2.5–4-µm-long by 2–3.5-µm-wide cells (Figure 2).<sup>16,20,21</sup> However, unlike *S. schenckii* and *S. brasiliensis* that display normal growth at temperatures up to 37°C, *S. globosa* has shown to decrease in growth rate as the temperature increases, as demonstrated by the mean colony diameter of 16–42 mm at 30°C, 3–15 mm at 35°C, and with very limited growth at 37 °C for most *S. globosa* isolates, with colony sizes ranging from 1.5 to 5.5 mm in diameter.<sup>13,16,18,22</sup> The macroscopic morphologies of all three species, regardless of the culture medium, are also very similar, with creamy filamentous colonies, some of them darker to brown or black, oval or orbed with milky membranous edge and wrinkled surface.<sup>16,18</sup> In the case of the fungal growth as yeast-like cells, *S. globosa* cultured in brain-heart-infusion at 37°C grows like elongated, cigar-shaped or globose hyaline budding yeast cells, measuring about 5–7 µm long and 1–3 µm wide (Figure 2), whereas colonies are moist, glabrous, and with a light brown color,<sup>20,23</sup> similar to *S. schenckii* and *S. brasiliensis* grown under the same conditions.



**Figure 1** Geographic distribution of *Sporothrix globosa* isolates. This species is mainly found in Asia (China, India, and Japan), followed by Europe (Spain, Italy, and United Kingdom), America (Brazil, United States, Venezuela, Guatemala, Colombia, and Mexico), and Africa (South Africa). The red dots indicate the countries where *S. globosa* has been isolated.



**Figure 2** Microscopic morphology of *Sporothrix globosa* growing in the yeast–peptone–dextrose medium for 4 days. **(A)** Yeast-like cells growing at 35°C and pH 7.8, with the typical elongated cigar shape. **(B)** Mycelium growing at 28°C and pH 4.5, with globose conidia and branching septate hyphae. Scale bars = 10  $\mu$ m.

Since *S. globosa* morphology is similar to other *Sporothrix* species, this phenotypical trait is useless for identification purposes. However, some biochemical parameters can help in species identification. One of them is the carbohydrate assimilation, such as sucrose, ribitol, and raffinose, which is different among the medically relevant *Sporothrix* species: *S. globosa* has positive assimilation to sucrose and ribitol, and negative assimilation to raffinose; *S. schenckii* has positive assimilation for the three sugars; and *S. brasiliensis* has negative assimilation to sucrose and raffinose, and positive assimilation to ribitol.<sup>16,20</sup> These zymogram profiles are considered a tool for preliminary taxonomical identification, and molecular techniques are required for species confirmation, as discussed in the following sections.

The genome of two *S. globosa* strains has been sequenced and assembled, but not annotated. One strain was isolated from a facial lesion in Spain (CBS 120340) and the other was isolated from a Chinese patient with itraconazole-resistant sporotrichosis (SS01).<sup>24</sup> Both strains had similar results, with an estimated genome size of 33,473,551 and 33,493,113 bp with a G+C content of 54.37%, 7760, and 7719 predicted genes, respectively, and a total of 126 putative tRNA genes decoding 20 standard amino acids.<sup>25</sup> The mitochondrial genomes from the two strains were also very similar, consisting of a circular DNA of 26,288 and 26,672 bp, for CBS 1203740 and SS01, respectively, 44 unique genes subdivided in 24 tRNAs, 17 protein-coding genes, 2 rRNAs, and the *rpnB* gene that encodes for the RNA subunit of the RNaseP, a ribozyme responsible for tRNA maturation.<sup>26</sup> In addition, both *S. globosa* mitochondrial genomes are similar and have synteny with *S. schenckii* ATCC MYA-4821 (26,572 bp), and are smaller than that of *S. brasiliensis* ATCC MYA-4823 (36,054 bp).<sup>27–29</sup> Through in silico comparative genome analysis of the *Sporothrix* pathogenic species, it was found that *S. schenckii*, *S. brasiliensis* and *S. globosa* share 86–96% of genomic synteny, while the less virulent *S. pallida* species shares only 13%.<sup>30</sup> In addition, it was observed that *S. brasiliensis*, *S. globosa*, *S. pallida* and *S. schenckii* have 8715, 8983, 9845 and 8719 genes, respectively, with a similarity of 80–100% among the most virulent species *S. schenckii*, *S. brasiliensis* and *S. globosa*, and only of 40% for *S. pallida*.<sup>30</sup> When virulence associated genes were predicted using the pathogen–host interaction database, it was found that *S. brasiliensis*, *S. schenckii*, *S. globosa*, and *S. pallida* have a similar number of virulence genes in their genomes, with 1650, 1699, 1758 and 2020 genes, respectively, and most of these were cell wall associated genes, extracellular domains, stress response genes, iron acquisition genes, proteases and cell signalling molecules.<sup>30</sup>

Although the *S. schenckii*, *S. brasiliensis*, and *S. globosa* genome sequences are available, genomic, transcriptomic, and metabolomic comparative analyses are scarce and required to understand the molecular bases of the phenotypical differences shown by *S. globosa*. *Sporothrix* species have different levels of virulence and antifungal susceptibilities, which will be also further discussed, and these might be explained by molecular polymorphisms.<sup>31,32</sup>

*S. schenckii* undergoes recombination in nature, showing a high degree of genetic variability in its population, which has not been observed in *S. brasiliensis*, where the isolates from a feline outbreak are mainly clonal, conforming a homogenous population, which does not exclude the absence of sexual reproduction but does indicate the emergence of a successful genotype.<sup>15,24,33</sup> Regarding *S. globosa*, this species is also considered to have a low degree of genetic variation,<sup>15,24</sup> but some molecular studies suggest the existence of a sexual state, since the *MATI-1* and *MATI-2* genes, which are essential for sexual reproduction, were found within this species genome.<sup>34</sup> However, additional analyses of

these genes are required to determine the teleomorphs existence. It is also suggested that *S. globosa* and *S. brasiliensis* are clonal offshoots that differentiated from a common ancestor shared with *S. schenckii*, a hypothesis generated by phylogenetic analyses and genetic diversity using the calmodulin encoding gene sequences.<sup>35</sup> On the other hand, recent phylogenetic studies, discriminatory AFLP markers analysis and mating-type analysis from human, animal and environmental isolates suggest that *S. schenckii*, *S. brasiliensis* and *S. globosa* are closely related taxonomic entities with strong genetic introgression that come from a single ancestor,<sup>7,22,36</sup> being *S. brasiliensis* and *S. schenckii* sister species from a clade that is sister to *S. globosa*.<sup>36</sup>

The *S. schenckii* and *S. brasiliensis* cell walls have been thoroughly studied and it is known that chitin is found in the inner layer, followed by  $\beta$ -1,3- and  $\beta$ -1,6-glucans surrounded by peptidorhamnomannan fibrils in the outer layer.<sup>26,37,38</sup> The *S. globosa* cell wall composition is not well studied, but it has been reported that it shows lower rhamnose, mannose, and *O*- and *N*-linked glycans content than *S. schenckii* and *S. brasiliensis*, but similar levels of glucosamine.<sup>32</sup> Moreover, it was also observed that even when *S. globosa* was grown under carbon and nitrogen limitation, the cell wall rhamnose, glucose, glucosamine, and mannose content did not change, at a difference of *S. schenckii* and *S. brasiliensis*, suggesting species-specific metabolic plasticity.<sup>39</sup> In addition, *S. globosa* showed a higher  $\beta$ -1,3-glucan exposure at the cell surface,<sup>32,40</sup> which might be an advantage for the host's immunity.

The proteome of these three main species has been studied, which may also explain the differences in virulence among them. From 247 proteins identified, 137 were found to be differentially expressed among the three species, and most of them were related to the carbohydrate and amino acid metabolism, and the stress response.<sup>41</sup> Eight of the 10 enzymes involved in the glycolytic pathway (except phosphofructokinase-1 and phosphoglycerate mutase) were identified in *S. schenckii*, *S. brasiliensis*, and *S. globosa*, and seven of them (pyruvate kinase, fructose-bisphosphate aldolase, enolase, triosephosphate isomerase, glucose-6-phosphate isomerase, phosphoglycerate kinase, and glyceraldehyde 3 phosphate dehydrogenase) were upregulated in *S. schenckii* and *S. globosa*.<sup>41</sup> In *S. brasiliensis*, three alcohol dehydrogenases were identified, while only two were found in *S. schenckii* and *S. globosa*.<sup>41</sup> The enzyme pyruvate dehydrogenase E2 component dihydrolipoamide acetyltransferase, part of the pyruvate dehydrogenase complex, and the aconitate hydratase, malate dehydrogenase, citrate synthase, and fumarate hydratase, enzymes of the tricarboxylic acid cycle, were expressed by the three species, although with a higher abundance in *S. schenckii* and *S. globosa*. E1 and E2 proteins of the 2-oxoglutarate dehydrogenase complex were also found, but the first protein only in *S. brasiliensis* and the second in both *S. brasiliensis* and *S. schenckii*, while proteins such as thiamine biosynthetic enzyme, 6-phosphogluconate dehydrogenase, transaldolase, transketolase, and a UDP-*N*-acetylglucosamine pyrophosphorylase were found to be overexpressed in *S. schenckii* and *S. globosa*. On the other hand, glycosyl hydrolases like  $\beta$ -glucosidase, endo-1,3(4)-glucanase, and 1,3- $\beta$ -glucosidase; the 4-hydroxyphenylpyruvate dioxygenase, metalloproteinase MepB, peptide-methionine (S)-S-oxide reductase, Xaa-Pro aminopeptidase, dipeptidyl-peptidase III, urease, and the trehalose synthase were found only or with higher abundance in *S. brasiliensis*.<sup>41</sup> In the case of *S. globosa*, the three enzymes related to the lipid metabolism were downregulated, while the diphosphomevalonate decarboxylase was upregulated in *S. brasiliensis* and *S. schenckii*. The three *Sporothrix* species express enzymes involved in the oxidative stress response (catalase, superoxide dismutase, and peroxidase), but these were found upregulated in *S. globosa*.<sup>41</sup> Finally, some heat shock proteins (chaperonin GroES, heat shock 70 kDa protein, molecular chaperone HtpG, and heat shock 70 kDa protein 18) were found to be downregulated in *S. brasiliensis*, but upregulated in *S. schenckii* and *S. globosa*.<sup>41</sup> These proteomic differences suggest that *S. brasiliensis* metabolism is different from that of *S. schenckii* and *S. globosa*, which might explain the species-specific virulence.

## Virulence Factors

Many *Sporothrix* spp. pathogenic aspects are unknown, and only a few virulence factors in *S. schenckii* and *S. brasiliensis* have been experimentally demonstrated. For *S. globosa*, the information in this regard is even more scarce, being this an opportunity area to expand our limited knowledge on this organism. In this section, the already known *S. globosa* virulence factors are discussed and others will be hypothesized, based on the genomic comparison with other *Sporothrix* species and with *Candida albicans* and *Aspergillus fumigatus*, two opportunistic fungal pathogens associated with high morbidity and mortality rates (Table 1).<sup>42</sup>

**Table 1** Genes Encoding for Putative Virulence Factors in *Sporothrix globosa*

Putative Function	<i>S. globosa</i> Genomic Region	Best Hit	Similarity (%)	Coverage (%)	Ortholog in <i>S. schenckii</i>	Ortholog in <i>S. brasiliensis</i>
<b>Dimorphism</b>						
<b>Hypal G cyclin I</b>	Sequence ID: LVYW01000001.1 Range: 1,025,425 to 1,026,504	<i>A. fumigatus</i> GI/S-specific cyclin ClnIs	67	87	Yes	Yes
<b>Cell wall synthesis</b>						
<b>Chitin synthase</b>	Sequence ID: LVYW01000005.1 Range: 1,866,742 to 1,869,543	<i>A. fumigatus</i> chitin synthase A	73	95	Yes	Yes
	Sequence ID: LVYW01000004.1 Range: 3,601,706 to 3,604,024	<i>A. fumigatus</i> chitin synthase B	83	75	Yes	Yes
	Sequence ID: LVYW01000001.1 Range: 1,933,042 to 1,935,999	<i>A. fumigatus</i> chitin synthase F	75	91	Yes	Yes
	Sequence ID: LVYW01000004.1 Range: 659,665 to 662,220	<i>A. fumigatus</i> chitin synthase G	77	97	Yes	Yes
<b><math>\beta</math>-1,3-Glucanosyltransferase</b>	Sequence ID: LVYW01000002.1 Range: 622,419 to 627,869	<i>A. fumigatus</i> 1,3- $\beta$ -glucan synthase catalytic subunit FksP	85	94	Yes	Yes
<b>Dolichol phosphate synthase</b>	Sequence ID: LVYW01000004.1 Range: 4,120,034 to 4,120,861	<i>A. fumigatus</i> dolichol phosphate mannosyl-transferase	77	100	Yes	Yes
<b>Protein mannosyltransferase</b>	Sequence ID: LVYW01000002.1 Range: 3,642,281 to 3,643,684	<i>A. fumigatus</i> protein O-mannosyl-transferase	82	93	Yes	Yes
	Sequence ID: LVYW01000006.1 Range: 1,823,756 to 1,826,095	<i>A. fumigatus</i> protein mannosyl-transferase Pmt1	71	93	Yes	Yes
<b><math>\alpha</math>-1,2-Mannosyltransferase</b>	Sequence ID: LVYW01000005.1 Range: 2,631,677 to 2,632,711	<i>A. fumigatus</i> $\alpha$ -1,2-mannosyl-transferase (Kre2)	81	80	Yes	Yes
	Sequence ID: LVYW01000001.1 Range: 1,590,849 to 1,591,889	<i>A. fumigatus</i> $\alpha$ -1,2-mannosyltransferase (Ktr4)	77	87	Yes	Yes
	Sequence ID: LVYW01000003.1 Range: 48,707 to 49,552	<i>A. fumigatus</i> $\alpha$ -1,2-mannosyl-transferase (Kre5)	86	100	Yes	Yes

(Continued)

Table 1 (Continued).

Putative Function	<i>S. globosa</i> Genomic Region	Best Hit	Similarity (%)	Coverage (%)	Ortholog in <i>S. schenckii</i>	Ortholog in <i>S. brasiliensis</i>
<b><math>\alpha</math>-1,6-Mannosyltransferase</b>	Sequence ID: LYYW01000002.1 Range: 3,966,407 to 3,967,531	<i>A. fumigatus</i> glycosyl-transferase OchI	65	82	Yes	Yes
	Sequence ID: LYYW01000005.1 Range: 2,421,530 to 2,422,483	<i>A. fumigatus</i> $\alpha$ 1,6-mannosyl-transferase Mnn9	80	80	Yes	Yes
<b>Thermotolerance</b>						
<b>Suppresses thermotolerance and modulates capsule formation</b>	Sequence ID: LYYW01000005.1 Range: 1,893,011 to 1,894,318	<i>A. fumigatus</i> cAMP-dependent protein kinase-like Sch9	86	99	Yes	Yes
<b>Heat shock transcription factor</b>	Sequence ID: LYYW01000002.1 Range: 2,640,249 to 2,642,180	<i>A. fumigatus</i> heat shock transcription factor HsfI	49	65	Yes	Yes
<b>Heat shock-induced protein</b>	Sequence ID: LYYW01000005.1 Range: 1,292,334 to 1,294,616	<i>A. fumigatus</i> heat shock protein/chaperonin Hsp78	84	92	Yes	Yes
<b>Invasin, member of Hsp70</b>	Sequence ID: LYYW01000002.1 Range: 3,289,290 to 3,291,185	<i>A. fumigatus</i> molecular chaperone Hsp70	94	90	Yes	Yes
	Sequence ID: LYYW01000003.1 Range: 4,342,973 to 4,344,541	<i>A. fumigatus</i> Hsp70 chaperone BiP/Kar2	91	99	Yes	Yes
	Sequence ID: LYYW01000003.1 Range: 1,590,876 to 1,592,192	<i>A. fumigatus</i> Hsp70 chaperone HscA	88	99	Yes	Yes
	Sequence ID: LYYW01000003.1 Range: 1,425,780 to 1,427,675	<i>A. fumigatus</i> mitochondrial Hsp70 chaperone (Ssc70)	92	91	Yes	Yes
	Sequence ID: LYYW01000001.1 Range: 3,575,451 to 3,577,520	<i>A. fumigatus</i> Hsp70 chaperone Hsp88	72	88	Yes	Yes
	Sequence ID: LYYW01000006.1 Range: 1,235,654 to 1,237,159	<i>A. fumigatus</i> Hsp70 chaperone BiP	68	91	Yes	Yes
	Sequence ID: LYYW01000003.1 Range: 5,249,506 to 5,251,209	<i>A. fumigatus</i> Hsp70 family chaperone LhsI/Orp150	70	71	Yes	Yes
<b>Heat shock protein, mitochondrial chaperone (Hsp60)</b>	Sequence ID: LYYW01000002.1 Range: 857,724 to 859,505	<i>A. fumigatus</i> antigenic mitochondrial protein Hsp60	87	96	Yes	Yes

<b>Heat shock protein involved in thermotolerance (Hsp90)</b>	Sequence ID: LYYW01000005.1 Range: 880,214 to 881,443	<i>A. fumigatus</i> molecular chaperone and allergen Mod-E/Hsp90/Hsp1	94	87	Yes	Yes
<b>Adhesins</b>						
<b>Cell wall integrity, adherence, and biofilm formation</b>	Sequence ID: LYYW01000001.1 Range: 1,884,061 to 1,884,846	<i>A. fumigatus</i> cell wall glucanase Scw11	59	41	Yes	Yes
	Sequence ID: LYYW01000007.1 Range: 2,857,516 to 2,858,574	<i>A. fumigatus</i> cell wall glucanase	61	95	Yes	Yes
<b>Cell wall morphology, pH-dependent expression (Phr1)</b>	Sequence ID: LYYW01000004.1 Range: 4,497,069 to 4,498,358	<i>A. fumigatus</i> $\beta$ -1,3-glucanosyl-transferase	69	78	Yes	Yes
	Sequence ID: LYYW01000001.1 Range: 86,737 to 87,795	<i>A. fumigatus</i> $\beta$ -1,3-glucanosyl-transferase Gel1	76	78	Yes	Yes
	Sequence ID: LYYW01000003.1 Range: 3,681,474 to 3,682,763	<i>A. fumigatus</i> $\beta$ -1,3-glucanosyl-transferase Gel2	69	88	Yes	Yes
<b>Biofilm formation</b>						
<b>Transcription Factor (Ndt80)</b>	Sequence ID: LYYW01000002.1 Range: 4,774,368 to 4,775,234	<i>A. fumigatus</i> NDT80/PhoG like DNA-binding family protein	53	48	Yes	Yes
<b>Secreted hydrolases</b>						
<b>Secreted aspartyl protease (Sap2)</b>	Sequence ID: LYYW01000006.1 Range: 613,730 to 615,028	<i>A. fumigatus</i> aspartic-type endopeptidase OpsB	69	88	Yes	Yes
	Sequence ID: LYYW01000006.1 Range: 2,678,211 to 2,679,401	<i>C. albicans</i> Sap8	47	81	Yes	Yes
	Sequence ID: LYYW01000001.1 Range: 4,068,222 to 4,069,364	<i>A. fumigatus</i> aspartic endopeptidase Pep2	81	90	Yes	Yes
	Sequence ID: LYYW01000005.1 Range: 1,785,032 to 1,786,228	<i>A. fumigatus</i> aspartic endopeptidase Ap1	63	88	Yes	Yes
	Sequence ID: LYYW01000002.1 Range: 2,154,952 to 2,156,013	<i>A. fumigatus</i> aspartic-type endopeptidase CtsD	57	75	Yes	Yes
	Sequence ID: LYYW01000004.1 Range: 2,345,538 to 2,346,500	<i>A. fumigatus</i> extracellular aspartic endopeptidase	66	77	Yes	Yes

(Continued)

Table 1 (Continued).

Putative Function	<i>S. globosa</i> Genomic Region	Best Hit	Similarity (%)	Coverage (%)	Ortholog in <i>S. schenckii</i>	Ortholog in <i>S. brasiliensis</i>
Phospholipase	Sequence ID: LYYW01000001.1 Range: 4,901,960 to 4,903,879	<i>A. fumigatus</i> lysophospholipase Plb1	67	96	Yes	Yes
	Sequence ID: LYYW01000001.1 Range: 5,943,020 to 5,945,185	<i>A. fumigatus</i> phospholipase A2 PlaA	63	87	Yes	Yes
Lipase	Sequence ID: LYYW01000002.1 Range: 2,894,403 to 2,895,689	<i>A. fumigatus</i> secretory lipase	61	89	Yes	Yes
Urease	Sequence ID: LYYW01000001.1 Range: 6,005,160 to 6,007,553	<i>A. fumigatus</i> Urease Ure	84	87	Yes	Yes
<b>Melanin production</b>						
Polyketide synthase	Sequence ID: LYYW01000001.1 Range: 5,766,340 to 5,772,381	<i>A. fumigatus</i> conidial pigment polyketide synthase PksP/Alb1	60	94	Yes	Yes
Phenoloxidase	Sequence ID: LYYW01000004.1 Range: 3,463,454 to 3,465,208	<i>A. fumigatus</i> ferroxidoreduc-tase Fet3	67	96	Yes	Yes
	Sequence ID: LYYW01000001.1 Range: 5,252,734 to 5,254,242	<i>A. fumigatus</i> extracellular dihydrogeodin oxidase/laccase	48	88	Yes	Yes
	Sequence ID: LYYW01000003.1 Range: 3,378,591 to 3,380,357	<i>A. fumigatus</i> laccase TilA	60	94	Yes	Yes
<b>Nutrient uptake</b>						
GTPase	Sequence ID: LYYW01000001.1 Range: 2,496,580 to 2,497,089	<i>A. fumigatus</i> Rheb small monomeric GTPase RhbA	89	100	Yes	Yes
	Sequence ID: LYYW01000006.1 Range: 992,857 to 993,159	<i>A. fumigatus</i> RAS small monomeric GTPase Rsr1	86	72	Yes	Yes
Ornithine monooxygenase	Sequence ID: LYYW01000002.1 Range: 2,281,754 to 2,283,208	<i>A. fumigatus</i> L-ornithine N5-oxygenase SidA	59	90	Yes	Yes



<b>Zinc homeostasis</b>	Sequence ID: LVYW01000005.1 Range: 1,316,107 to 1,317,288	<i>A. fumigatus</i> high-affinity zinc ion transporter	67	99	Yes	Yes
	Sequence ID: LVYW01000005.1 Range: 1,316,173 to 1,317,216	<i>A. fumigatus</i> plasma membrane low-affinity zinc ion transporter	55	86	Yes	Yes
	Sequence ID: LVYW01000002.1 Range I: 1,172,630 to 1,173,124	<i>A. fumigatus</i> ZIP family zinc transporter	83	71	Yes	Yes
<b>Acetyl-CoA synthetase</b>	Sequence ID: LVYW01000002.1 Range: 1,643,562 to 1,644,794	<i>A. fumigatus</i> acetyl-coenzyme A synthetase FacA	88	92	Yes	Yes
<b>Serine/threonine kinase</b>	Sequence ID: LVYW01000003.1 Range: 3,006,373 to 3,007,410	<i>A. fumigatus</i> carbon catabolite derepressing protein kinase SnfI	83	69	Yes	Yes
<b>Phosphoenolpyruvate carboxykinase</b>	Sequence ID: LVYW01000003.1 Range I: 1,604,482 to 1,605,645	<i>A. fumigatus</i> pyruvate kinase	84	99	Yes	Yes
<b>Hexose kinase</b>	Sequence ID: LVYW01000001.1 Range I: 1,447,659 to 1,448,525	<i>A. fumigatus</i> hexokinase Kxk	84	95	Yes	Yes
	Sequence ID: LVYW01000002.1 Range I: 4,445,090 to 4,447,084	<i>A. fumigatus</i> hexokinase	64	68	Yes	Yes
	Sequence ID: LVYW01000005.1 Range I: 443,537 to 444,583	<i>A. fumigatus</i> glucokinase	68	82	Yes	Yes
<b>Isocitrate lyase</b>	Sequence ID: LVYW01000006.1 Range: 3,093,278 to 3,094,873	<i>A. fumigatus</i> isocitrate lyase AcuD	83	98	Yes	Yes
	Sequence ID: LVYW01000005.1 Range: 2,780,931 to 2,782,532	<i>C. albicans</i> Isocitrate lyase I	51	97	Yes	Yes
<b>Malate synthase</b>	Sequence ID: LVYW01000002.1 Range I: 3,545,499 to 3,546,767	<i>A. fumigatus</i> malate synthase AcuE	91	99	Yes	Yes
<b>Response to stress</b>						
<b>Superoxide dismutase</b>	Sequence ID: LVYW01000002.1 Range: 3,012,655 to 3,013,167	<i>A. fumigatus</i> Cu/Zn superoxide dismutase SOD1	78	97	Yes	Yes
	Sequence ID: LVYW01000003.1 Range: 2,047,916 to 2,048,404	<i>A. fumigatus</i> cytosolic Cu/Zn superoxide dismutase <i>C. albicans</i> SOD5	57	71	Yes	Yes

(Continued)

**Table I** (Continued).

Putative Function	<i>S. globosa</i> Genomic Region	Best Hit	Similarity (%)	Coverage (%)	Ortholog in <i>S. schenckii</i>	Ortholog in <i>S. brasiliensis</i>
<b>Catalase</b>	Sequence ID: LVYW01000002.1 Range: 4,168,516 to 4,169,937	<i>C. albicans</i> catalase A	74	97	Yes	Yes
	Sequence ID: LVYW01000004.1 Range: 3,095,593 to 3,097,812	<i>A. fumigatus</i> spore-specific catalase CatA	71	97	Yes	Yes
<b>Flavo-hemoglobin-related protein</b>	Sequence ID: LVYW01000005.1 Range 1: 3,049,448 to 3,050,809	<i>A. fumigatus</i> flavohemoprotein	56	98	Yes	Yes
<b>Serine/threonine phosphatase</b>	Sequence ID: LVYW01000003.1 Range: 4,774,748 to 4,776,373	<i>A. fumigatus</i> calcineurin catalytic subunit CnaA	85	96	Yes	Yes
<b>Thioredoxin</b>	Sequence ID: LVYW01000001.1 Range: 5,596,040 to 5,597,059	<i>A. fumigatus</i> thioredoxin reductase Trr1/ Trr2	77	82	Yes	Yes
	Sequence ID: LVYW01000001.1 Range: 1,734,081 to 1,734,710	<i>A. fumigatus</i> thioredoxin	61	96	Yes	Yes
<b>Toxins and secondary metabolites</b>						
<b>Non-ribosomal peptide</b>	Sequence ID: LVYW01000006.1 Range: 3,263,839 to 3,277,668	<i>A. fumigatus</i> nonribosomal siderophore peptide synthase SidC	45	93	Yes	Yes
<b>C6 finger domain protein</b>	Sequence ID: LVYW01000004.1 Range: 4,538,250 to 4,539,548	<i>A. fumigatus</i> C6 finger domain protein	50	80	Yes	Yes

Virulence is defined as the pathogenicity degree of an organism, and it is determined by the ability to adhere, disseminate, and damage cells, tissues, organs, systems, or the whole organism.<sup>43</sup> Some of these factors, already described in *Sporothrix*, include dimorphism, thermotolerance, the presence of melanin, secreted hydrolytic enzymes, and extracellular vesicles.<sup>44</sup> *S. brasiliensis* and *S. globosa* are the members of the *Sporothrix* pathogenic clade with the highest and lowest virulence, respectively.<sup>45</sup> In agreement with this observation, it was reported that different *S. globosa* strains were not capable of killing mice, in a murine model of experimental sporotrichosis, regardless of the inoculum size applied to animals; and the fungal burdens in spleen, lungs, liver, testicles, kidneys, and brain were considerably low in comparison with *S. brasiliensis*-infected animals.<sup>45</sup> Additionally, some *S. globosa* strains have been reported as non-virulent.<sup>46,47</sup> In one study, where 18 animals received subcutaneous and retroperitoneal *S. globosa* inoculums, none showed any symptoms and the inoculum site did not have any fungal growth after a 30-day observation period.<sup>46</sup> Thus, it has been suggested that *S. globosa* causes mainly benign, fixed, or lymphocutaneous infections. However, it can spread to other organs such as the liver, kidneys, and brain, but to a lesser extent when compared with *S. brasiliensis*- and *S. schenckii*-caused infections.<sup>45</sup> This low virulent phenotype could be mainly due to differences in the metabolism and cell wall structure, as already mentioned. The overexpression of enzymes involved in amino acids and lipids metabolism, and the upregulation of glycolytic enzymes and trehalose synthase in *S. brasiliensis*,<sup>41</sup> suggest that this species has better nutrient exploitation during parasitism than *S. globosa*, better resistance to osmotic stress, and better cell wall remodeling, important factors for the infection that can contribute to fungal virulence. Moreover, the *S. globosa* cell wall, with its high levels of  $\beta$ -1,3-glucan exposed at the cell surface, is also a disadvantage when interacting with the host immunity.<sup>32,48</sup> Furthermore, *S. brasiliensis* and *S. schenckii* tolerate growth at 37°C but *S. globosa* hardly grows above 35°C, with larger duplication times than the other two fungal species,<sup>16</sup> and thus representing a handicap when adapting to grow within the host milieu.

Heat shock proteins are important factors for thermotolerance and mycelium-to-yeast transition, and six of them have been identified in *S. globosa*.<sup>41</sup> Among them, an Hsp90 homolog is found, which has a key role in the resistance to heat stress in *A. fumigatus*.<sup>49</sup> As a result, when this protein activity is inhibited in both *S. schenckii* and *S. globosa*, the transition from hypha to the yeast-like cells morphology is blocked.<sup>50</sup> In *S. schenckii*, the Hsp90 interaction with the SsCmk1 enzyme, a  $\text{Ca}^{2+}$ /calmodulin-dependent protein kinase, is necessary for thermotolerance, and molecular silencing of its encoding gene led to a phenotype similar to that observed when Hsp90 was inhibited with geldanamycin, ie, cells arrested in the filament morphology.<sup>50</sup> Additionally, yeast two-hybrid assays suggested that SsCmk1 has a role in the fungus thermotolerance through its effects on Hsp90.<sup>50</sup> The bioinformatics analysis of the *S. globosa* genome showed it contains one putative functional ortholog for both *S. schenckii* HSP90 and SsCMK1 (Table 2). It is worthy of mentioning that the bioinformatics predictions provided in Table 2 require experimental validation.

Another protein kinase that participates in the *S. schenckii* morphological switch is the histidine kinase SsDrk1.<sup>51</sup> This protein is important for the virulence of other pathogenic fungi, such as *Blastomyces dermatitidis* and *Histoplasma capsulatum*, as well as in *S. schenckii*, but unlike what has been seen in the typical histidine kinases, SsDrk1 is a soluble protein without a transmembrane domain.<sup>52</sup> The SsDRK1 gene manipulation resulted in mutants with limited growth, which suggests a delay in asexual development; abnormalities in the plasma membrane and cell wall, such as short and abundant invaginations in the yeast morphology, and lack of the electron-dense zone in the cell wall surface; and abnormal sensitivity to cell wall perturbing agents, factors that suggest a defective cell wall components deposition, and thus, defective cell wall integrity.<sup>51</sup> Moreover, the fungus' ability to perform dimorphism and melanization was altered, as most of the cells displayed a conidium-like morphology.<sup>51</sup> Finally, the SsDrk1-deficient strains had attenuated virulence in a murine model of cutaneous infection,<sup>51</sup> which might be explained by (i) the defective cell wall, which makes the fungus more vulnerable to environmental stress, osmotic changes, and the immune recognition and response by the host; (ii) pigmentation decrease, since melanization is relevant in the cutaneous sporotrichosis pathogenesis, helping in granuloma formation, fungal dissemination, protecting the yeast-like cells against phagocytosis, and decreasing the efficacy of some treatments<sup>53–55</sup>; and (iii) decrease of dimorphism, a process that contributes to a rapid growth inside the host cells and slower fungal clearance from the tissues, by protecting the fungus from the immune response.<sup>51</sup> A putative SsDRK1 ortholog is predicted to be part of the *S. globose* genome (Table 2).

**Table 2** Prediction of Putative Virulence Factors in *S. globosa*, Based on *S. schenckii* and *S. brasiliensis* Known Virulence Factors

Protein	Function	<i>S. schenckii</i>			<i>S. brasiliensis</i>		
		E. value	Similarity (%)	Coverage (%)	E. value	Similarity (%)	Coverage (%)
<b>Hsp90</b>	Thermotolerance	0.0	99	100	0.0	99	100
<b>SsCMK I</b>	Thermotolerance	0.0	90	99	0.0	91	100
<b>SsDRK I</b>	Morphological switching	0.0	99	100	0.0	99	100
<b>PKC</b>	Signal transduction	0.0	85	98	0.0	93	92
<b>Gp70</b>	Adhesion	0.0	90	100	0.0	91	100
<b>Enolase</b>	Adhesion	2e-176	97	100	2e-176	97	100
<b>Cell wall glucanase</b>	Lysis of immune cells	8e-170	86	98	2e-167	85	98
<b>SOD</b>	Oxidative stress resistance	0.0	89	100	8e-180	89	100
<b>Proteinase I</b>	Hydrolysis of tissue	0.0	92	99	0.0	97	96
<b>Proteinase II</b>	Hydrolysis of tissue	0.0	98	93	0.0	98	100
<b>Hsp60</b>	Adhesin	0.0	95	96	0.0	95	91
<b>Pap I</b>	Adhesin	3e-25	94	36	1e-25	96	31

The *S. schenckii* protein kinase C (PKC), an enzyme important in signal transduction, was also found to be related to the fungus dimorphism.<sup>56</sup> The *pkcSs-2* gene, which codes for the PKC, was found to be expressed at all intervals tested during the yeast to mycelium transition. A curious characteristic of this PKC is its ability to induce the morphological transition even at low calcium concentrations, which suggests the existence of two different mechanisms for dimorphism: a calcium-dependent pathway and a calcium-independent pathway.<sup>56,57</sup> The *S. globosa* genome has a sequence very similar (85%) to that of *S. schenckii* *pkcSs-2*, being likely that similar dimorphism control occurs in this species (Table 2).

There is a predominant antigenic glycoprotein named Gp70 described by several authors, which is found in the cell wall, extracellular space, and extracellular vesicles of all clinical *Sporothrix* species, including *S. globosa* (Table 2).<sup>47,58-60</sup> This glycoprotein was predicted to be an enzyme with 3-carboxymuconate cyclase activity and was found to be the most immunogenic antigen in the *S. brasiliensis*, *S. schenckii*, and *S. globosa* cell wall.<sup>60</sup> Additionally, the characterization by 2D-DIGE exposed that Gp70 presents several isoforms (that can range from 55 to 70 kDa), probably due to post-translational modifications, which are present in the clinical clade but absent in the non-pathogenic species.<sup>60</sup> Amino acid modifications and glycosylation could be the possible post-translational modifications involved in isoforms production. Besides, Gp70 has low homology to proteins found in other pathogen and non-pathogen fungal species, and since is a shared molecule in the pathogenic clade, it can be used for sporotrichosis diagnosis.<sup>58,60</sup> Gp70 is also an important vaccine candidate, due to the strong humoral response produced during the interaction with the host, which is backed up by several investigations.<sup>47,58,61-65</sup> Due to its high immunogenicity, it was found that its expression is related to the virulence profile of the *Sporothrix* species and isolates since highly virulent *S. brasiliensis* isolates have a lower protein abundance when compared to *S. schenckii* low virulence strains.<sup>66</sup>

Some other cell wall and extracellular enzymes have been reported to be important for *Sporothrix* virulence. A cell wall glucanase, transported by extracellular vesicles, contributes to the fungal virulence by inducing host immunity evasion, due to cell wall remodeling.<sup>67</sup> An enolase, a moonlighting protein in the cell wall with high antigenicity, has a possible role in the adhesion to the extracellular matrix, contributing to the fungal virulence;<sup>68</sup> the superoxide dismutase, another cell wall protein, contributes to the growth and survival of the yeast under oxidative stress conditions,

such as inside of macrophages;<sup>69</sup> and, certain proteases, such as collagenase, gelatinase, and proteinase I and II, which hydrolyze human stratum corneum, type I collagen, and elastin,<sup>70,71</sup> play an essential role in the host–fungus interaction, helping in the fungal invasion of cutaneous tissues and avoiding the immune response.<sup>72–74</sup> However, these mentioned proteins have not been completely characterized in other *Sporothrix* species, but the *S. globosa* genome has sequences that encode for proteins with these enzyme activities.

Finally, a well-characterized polysaccharide found in the *S. schenckii* and *S. brasiliensis* yeast cell wall surface, the peptidorhamnomannan (PRM), influences macrophage function by inhibiting phagocytosis.<sup>75</sup> This glycoconjugate is composed of a protein core (16%), modified with mannose (50%), rhamnose (33%), and galactose (1%).<sup>76</sup> The identity of the proteins that belong to this complex was recently determined, and two of them were demonstrated to work as virulence factors, the chaperonin GroEL (Hsp60) and the Pap1 (peptidorhamnomannan-associated protein 1).<sup>77</sup> It was demonstrated that both proteins are moonlighting proteins with adhesive properties on the *S. schenckii* cell wall, which allow fungal binding to the host extracellular matrix components.<sup>77</sup> The Hsp60 is a highly conserved protein with intracellular chaperone activity, but on the *S. schenckii* surface binds to laminin, elastin, fibrinogen, and fibronectin.<sup>77</sup> In addition, this protein participates in the fungal virulence, since the opsonization of the yeast-like cells with anti-recombinant Hsp60 antibodies and their subsequent use to challenge *Galleria mellonella* larva caused a decrease in the fungal ability to kill the host, probably due to increased fungal clearance by the larvae immune response.<sup>77</sup> When the recombinant protein was inoculated in the larvae before the infection with *S. schenckii*, it stimulated immunological priming and increased the host survival.<sup>77</sup> In the same line, Pap1, an uncharacterized protein, binds to laminin, elastin, fibrinogen, fibronectin, and type I and II collagen, and also participates in *S. schenckii* virulence. In similar experiments like those already described for Hsp60, Pap1 was also involved in fungal virulence.<sup>77</sup> A BLAST search within the *S. globosa* genome found that both proteins are present in this pathogen, although Pap1 was found with a very low sequence coverage (Table 2), at the difference of what it was found in *S. brasiliensis*.<sup>77</sup> This might suggest that this virulence factor is exclusive for the high virulence species of the *Sporothrix* clinical clade.

Recently, by comparing the *A. fumigatus* and *C. albicans* genomes with the *S. schenckii* genome sequence, putative factors were identified in this sporotrichosis etiological agent.<sup>78</sup> The analysis suggested multiple proteins with putative functions that could serve as virulence factors in *S. schenckii*. Following the same strategy, we have searched for these putative virulence factors within the *S. globosa* genome (Table 1). The results suggest multiple proteins that could be involved in morphological switching, cell wall synthesis, thermotolerance, adhesion, biofilm formation, hydrolysis, melanin production, nutrition, response to stress conditions, and toxins and secondary metabolites production.

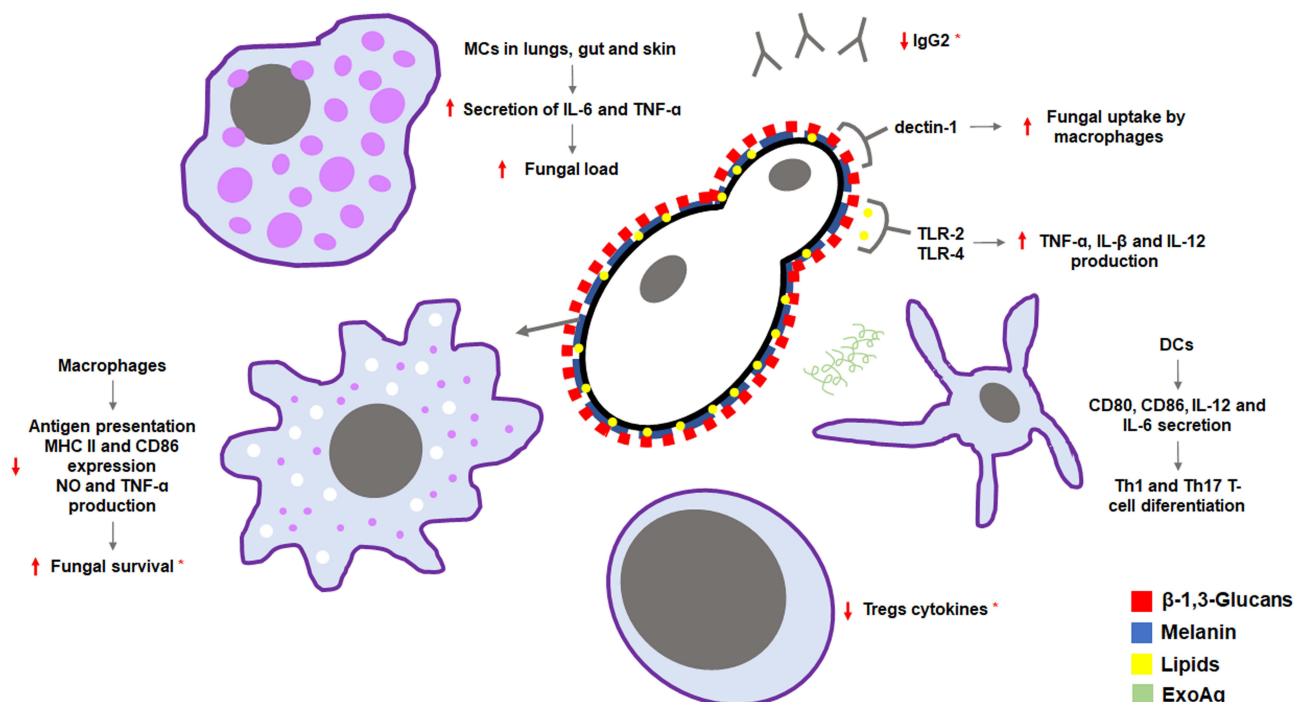
## Immune Response Against *Sporothrix globosa*

Even though the scientific community has made a significant effort trying to explain the host immune response against sporotrichosis, many details remain unclear regarding the *S. globosa* immune sensing, since most studies have been made in *S. schenckii* or *S. brasiliensis* (Figure 3).

### Innate Immune Response

As in viral and bacterial infections, the host reaction towards a fungal invader begins with the pathogen recognition by the innate immune system, a process in which pathogen-associated molecular patterns (PAMPs) and pattern recognition receptors (PRRs) are involved. PAMPs respond to evolutionary conserved molecular signatures of microorganisms, usually specific to the pathogen.<sup>79,80</sup> In *Sporothrix* spp., the main PAMPs source is the cell wall, where PRM, melanin, glycoconjugates, chitin, and  $\beta$ -1,3- and  $\beta$ -1,6-glucans are found.<sup>1,26,37</sup> On the other hand, PRRs, mainly expressed by antigen-presenting cells, such as macrophages and dendritic cells, are divided into four groups: Toll-like receptors (TLRs), nucleotide oligomerization domain (NOD)-like receptors, C-type lectin receptors (CLRs), and retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs).<sup>79</sup>

Even though *S. schenckii* and *S. brasiliensis* cell wall components are very similar, their abundance varies and suggests a difference in the immune response against them, since chitin and  $\beta$ -glucans exposure on the fungal surface favors their binding to PRRs.<sup>37,81</sup> It has been reported that dectin-1, a C-type lectin receptor, binds to *C. albicans* and *A. fumigatus*  $\beta$ -1,3-glucans, and this interaction triggers fungal uptake by murine and human macrophages.<sup>82</sup> *S. globosa* has higher  $\beta$ -1,3-glucan levels at the cell surface than *S. schenckii* and *S. brasiliensis*, an observation that is regardless of



**Figure 3** Immune response against *Sporothrix globosa*. Pathogen components trigger an innate immune response via dectin-1, TLR-2 and TLR-4 receptors, and macrophages and mast cells (MCs); an adaptative immune response via dendritic cells (DCs) and Tregs lymphocytes; and a humoral immune response by IgG antibodies. These responses have been observed in either *Sporothrix schenckii* or *Sporothrix brasiliensis* and are suggested for *S. globosa*, except those marked with\*, which have already been demonstrated in *S. globosa*.

the culture medium, while in *S. brasiliensis* and *S. schenckii* this depends on nutrients' availability.<sup>32</sup> This  $\beta$ -1,3-glucan exposure might explain the *S. globosa*'s lower virulence, due to a better recognition by the host.<sup>26,32</sup>

It has been demonstrated that TLR-2 and TLR-4 recognize the fungal lipid extracts and are relevant in sporotrichosis control, not only in *S. schenckii* infections but also in those caused by *S. brasiliensis* in mice; while in the human PBMCs-*Sporothrix* interaction, TLR-4 is dispensable, suggesting a host-dependent role for this PRR.<sup>26</sup> TLR-2 has been linked to TNF- $\alpha$  and IL- $\beta$  induction, whilst TLR-4 to TNF- $\alpha$ , IL-1 $\beta$ , and IL-12 production.<sup>83</sup> In *S. globosa*, it is possible to suggest that similar PRRs are involved in its immune sensing.

Melanin plays structural and protective roles in dematiaceous fungi and has been related to the *Sporothrix* spp. dissemination to internal organs in different cases.<sup>84</sup> It has been already demonstrated that *S. schenckii* melanin is involved in the antigen presentation inhibition by macrophages;<sup>54,85</sup> and recent studies have confirmed that *S. globosa* melanin increases fungal survival during the infection.<sup>86</sup> Twenty percent of mice infected with melanin-deficient *S. globosa* conidia developed spleen and kidney dissemination, while 80% of the animals infected with melanin producer conidia developed a lung infection and 100% developed liver, spleen, kidney, and testicle infections.<sup>86</sup> Furthermore, results also showed a negative impact on MHC II and CD86 expression in murine macrophages, as well as in nitric oxide (NO) and TNF- $\alpha$  production, which are important in digestive macrophage functions.<sup>86</sup> It is also known that conidia have a higher melanin content than yeast-like cells, and it has been suggested that accumulation of this component before yeast conversion delays the adaptative immunity induction.<sup>86</sup>

On the other hand, the role of mast cells (MCs) in the immune response has also been of special interest in the last years, as they are located in tissues at the interface with the environment (lungs, gut, skin). It has been reported that MCs may have a positive or negative immunoregulatory function within fungal infections; therefore, a recent study using MC-competent wild-type mice and an MC-deficient strain showed that infected mice with *S. schenckii* in the first group developed larger sporotrichosis lesions than mice of the second group.<sup>87</sup> These observations are related to MCs proinflammatory cytokines IL-6 and TNF- $\alpha$  pronounced production due to *S. schenckii* stimulus.<sup>87</sup> An earlier study also showed a correlation between MCs depletion and the decrease of fungal load in organs<sup>88</sup> In addition, both

groups showed decreased numbers of basophils, thus a contribution from these cells in the infection development may be suggested.<sup>87</sup> According to this, there might be a dissimilar interaction between mast cells and *S. globosa*, as it is less virulent, and the host is more capable of controlling the infection. However, further investigation is needed.

## Adaptative Immune Response

When activated, dendritic cells (DCs) PRRs recognize PAMPs and a signaling pathway gets triggered, causing the pathogen phagocytosis for subsequently MHC antigen presentation to naïve T-cells in secondary lymphoid tissues; thus, dendritic cells have been extensively studied in fungal infections and are considered to be a bridge between the innate and adaptative immune responses.<sup>89</sup> Thus far, three signals are needed for T-cell activation: 1) DCs' MHC presenting pathogen-derived peptides, 2) co-stimulatory molecules CD80 and CD86 expressed by DCs, and 3) DCs release of interleukins that promote T-cell differentiation.<sup>79</sup>

It has been demonstrated that DCs stimulation with *S. schenckii* yeast-like cells or a peptide-polysaccharide (Exoantigen or ExoAg) extracted from the supernatant of the fungal culture triggers the co-stimulatory molecules CD80 and CD86, and pro-inflammatory cytokines secretion, such as IL-12 and IL-6, that induce Th1 and Th17 T-cell differentiation, respectively.<sup>90,91</sup> Th1 response has been considered critical to host defense against *S. schenckii* and *S. brasiliensis* infection since produces an increase in cytokines levels such as IFN- $\gamma$ , which activates immune effector cells,<sup>83</sup> while Th17 response is found crucial for optimal fungal clearance.<sup>92</sup> Additionally, an enhanced Th1 response is reported when the fungal stimulus comes from a cutaneous infection and not from the visceral disease, being the latter responsible to induce a Th2 response.<sup>93</sup> In contrast, recent studies have demonstrated a predominant Th2 response against *S. globosa*, while in systemic *S. schenckii* infected mice it was reported to begin in the later phase of infection.<sup>92</sup> Moreover, studies in other pathogenic fungi, such as *Cryptococcus neoformans*, have shown that Th2 cells may inhibit Th1 and Th17 responses, prolonging fungal survival and inducing alternatively activated macrophages, which results in a worsened fungal infection.<sup>92</sup> On the other hand, regulatory T-cells ratio is reported to be increased in *S. schenckii* infected mice at the early phase of infection, while in *S. brasiliensis*-infected mice, a long-lasting elevated regulatory T cells (Tregs) response is reported.<sup>92</sup> *S. globosa*-infected patients have shown decreased Tregs cytokines, thus suggesting a dysfunction in the response of these immune cells.<sup>92</sup> Furthermore, previous reports indicate that humoral immunity also participates in the antifungal response against *S. schenckii* and *S. brasiliensis* infection. In contrast, in the *S. globosa* infection, a positive correlation between Th2/Tregs and IgD-CD27-double-negative B cells (DN B) was found, whose role is still unclear, but it is believed that DN B cells might be exhausted memory B cells or a memory B cell precursor.<sup>92</sup>

## Humoral Immunity

Although very little is known about humoral immunity when it comes to *S. globosa* infections in humans, its importance has been proven for the host defense against some other fungi, including *C. neoformans*, *C. albicans*, and *Paracoccidioides brasiliensis*.<sup>94</sup> The antibodies role in fungal infections may involve mechanisms of protection such as fungal cells agglutination, interference with fungal attachment, phagocytosis enhancement by host effector cells, immunoregulatory molecules neutralization, and complement activation.<sup>63</sup>

Serum from patients with sporotrichosis infection have been positive for the immunoglobulin isotypes IgA, IgG, and IgM analyzed by ELISA against mycelial-phase exoantigens. In addition, cross-reactions with serum from healthy patients and patients with other infectious diseases such as histoplasmosis, tuberculosis, leishmaniasis, aspergillosis, paracoccidioidomycosis, and even cryptococcosis have been observed.<sup>95</sup> IgM antibodies have had a higher number of cross-reactions, which may be due to their role in complement activation by the classical pathway in several mycotic diseases.<sup>95,96</sup> On the other hand, IgA might be an important mechanism of defense as this immunoglobulin is predominantly present in mucosa.<sup>95</sup> When the immune profile in circulation against *S. globosa* was investigated, a decreased level of IgG2 in patients of short and long-duration infection was observed, as well as in lymphocutaneous and fixed cutaneous forms.<sup>92</sup> IgG2 antibodies are induced in the absence of T-cell help and in the presence of polysaccharide antigens,<sup>97</sup> and their decreased levels found in *S. globosa* infection is thought to be associated with a decrease in B cells subsets, which are responsible for IgM and IgG secretion.<sup>92</sup>

## Vaccine Against *Sporothrix globosa*

Antifungal treatments can lead to the rising of drug resistance, even when an intermittent dosage is given. Moreover, the use of these treatments may cause liver injury in risk patients such as children, pregnant women, and patients with liver disorders.<sup>33</sup> Furthermore, in the case of *S. globosa*, it has been determined that this specie is less susceptible to most of the antifungals tested, such as amphotericin B, ketoconazole, posaconazole, and itraconazole;<sup>16</sup> therefore, the generation of an effective prophylactic or therapeutical vaccine has gained great interest in the sporotrichosis treatment.

Although mycelial components have been tested for vaccine design, Gp70 has been the most studied antigen for a long time, since Rodrigues et al, argued that the 70 kDa glycoprotein is the same molecule reported as exoantigen, cell-free antigen, and cell wall virulence factor for *Sporothrix* species, now identified as a 3-carboxymuconate cyclase.<sup>58,60</sup> The importance of this antigen comes not only from its high immunoreactivity but also from its presence in species of the *Sporothrix* pathogenic clade, which may lead to a broad-spectrum vaccine against sporotrichosis.<sup>68</sup> In 2017, Chen et al developed a successful Th1 and Th17 inducing vaccine against *S. globosa*, based on a Gp70 displaying recombinant phage (phage KR), whose safety has been approved by the Federal Drug Administration, USA.<sup>64</sup> Furthermore, IgG antibodies against Gp70 produced in mice immunized with the recombinant phage were proved to protect the host during a lethal challenge of *S. globosa*, with an 80% of survival and a decreased fungal burden, demonstrating the antibodies efficiency as a treatment.<sup>98</sup> These previous results show that the Gp70 displaying phage vaccine may function as an improvement in both protective cell-mediated and humoral immune responses, representing a new and potentially safe alternative for the treatment of the infection.

Monoclonal antibodies (mAb) such as IgG1 against *S. schenckii* Gp70, also known as mAbP6E7, have also shown an important role in decreasing fungal organ burden in infected mice. However, due to its origin, mAbP6E7 cannot be tested for human treatment. Humanized P6E7 (hP6E7) developed by genetic engineering technics also showed a similar antigen-binding capacity that promotes phagocytosis, which could be use for sporotrichosis treatment.<sup>63</sup>

## Sporotrichosis Caused by *Sporothrix globosa*

Sporotrichosis is usually found as a subcutaneous mycosis, but other clinical forms have also been reported, such as mucosal, extracutaneous, or systemic and disseminated disease. The development of one clinical form or the other depends on diverse factors, such as the inoculum size, the infection route, strain virulence and thermal tolerance, and the host immunological status.<sup>45</sup> When different *S. schenckii* isolates from different clinical forms were inoculated into healthy mice, to compare their virulence, it was observed that the strains isolated from disseminated sporotrichosis caused a severe disease, killing all of the subjects within 10 days, while the strains isolated from lymphocutaneous and fixed cutaneous forms, had mild and low virulence, respectively, both failing to cause death.<sup>99</sup> Thus, proving that the same species can have different virulence profiles.

In terms of mortality, tissue damage, and fungal burden, *S. brasiliensis* has been reported as the most virulent species. When different inoculum concentrations and two different strains of *S. brasiliensis*, *S. schenckii*, *S. globosa*, *Sporothrix mexicana*, and *Sporothrix albicans* were intravenously inoculated in an immunocompetent murine model, high mortality was only observed for *S. brasiliensis* and *S. schenckii*, being *S. brasiliensis* the only species capable of killing mice with the lowest inoculum size.<sup>45</sup> On the contrary, *S. globosa* presented low virulence in this model.<sup>45</sup> These data have been confirmed in various studies using other models, such as *Tenebrio molitor*, where larvae were inoculated with different fungal doses to evaluate animal mortality, cytotoxicity and immunological parameters. These results, along with what has been observed in human clinical cases, have shown that *S. globosa* is associated with lesser host damage upon inoculation, with different clinical manifestations mainly limited to the skin (Table 3).<sup>23,62,100–103</sup> Despite not being as virulent as *S. brasiliensis* and *S. schenckii*, infections caused by *S. globosa* have a broader distribution, as they have been found in Europe, America, and Asia, increasing its clinical significance.<sup>1</sup>

The first report of *S. globosa* isolation in Brazil suggests that transmission of the fungus usually occurs by traumatic inoculation while working with soil.<sup>23</sup> Nevertheless, the source of infection differs between species. The mycosis caused by *S. schenckii* presents a higher prevalence among patients who commonly interact with animals, particularly cats, and it is not gender-related,<sup>23</sup> while a phylogeographic study showed that cats have never been associated as sources of



**Table 3** Clinical Manifestations and Treatments of Sporotrichosis Caused by *S. globosa*

Patient	Clinical Manifestations	Risk Factor	Treatment	Outcome	Reference
<b>77-year-old woman (Brazil)</b>	Verrucous lesion on the right dorsal hand with 12 ascending subcutaneous nodules on the arm, some of them fistulized	The patient handled plants, vegetable garden and soil, but did not remember local trauma	Itraconazole 100 mg/day for 5 months	Regression of all the lesions	de Oliveira et al 2010 <sup>23</sup>
<b>70-year-old man (China)</b>	Solitary, well-defined, painless, red plaque on the right forearm, with a central ulcer and yellow crusting	The patient was a greenhouse farmer with iatrogenic topical immunosuppression, and described a prior trauma with a wooden thorn	Itraconazole 200 mg/day for 4 months	Regression of the lesion	Gu et al 2020 <sup>101</sup>
<b>51-year-old man (China)</b>	Suppurative wound and progressive subcutaneous nodules at the right arm, that became a verruca-like nodule	The patient was bitten by a stray cat	Itraconazole 200 mg/twice per day and terbinafine 250 mg/day for 1 month	Regression of the lesion	Liu et al 2020 <sup>102</sup>
<b>50-year-old woman (China)</b>	Small mass on the right upper eyelid with rough keratinization, that often discharged purulent secretion	-	Resistant to amphotericin B, voriconazole, fluconazole, terbinafine and caspofungin Treated with itraconazole 0.2 g/twice per day for 3 months	Regression of the lesion	Liu et al 2021 <sup>103</sup>
<b>66-year-old man (Brazil)</b>	Scaly erythematous lesion that mimicked a sarcoid lesion with a well-demarcated ulcer in the middle of the left arm, without suppuration nor subcutaneous nodules or fistulization.	The patient was a farmer and was involved in rural activities, and described a prior trauma	Resistant to itraconazole Treated with a saturated solution of KI 1 g/day increased weekly by 1 g up to a maximum of 4 g/day for 6 months	Regression of the lesion	Gompertz et al 2016 <sup>100</sup>
<b>Mice with cutaneous infection</b>	Soft subcutaneous lumps	-	Combination of itraconazole 60 mg/kg/day for 3 weeks, PDT at an irradiation energy density of 40 J/cm <sup>2</sup> once every week for 3 weeks and methylene blue 2 mg/mL in the feet until they became dark blue	Reduction of the lesion and regression of the lesion in some mice at day 20	Li et al 2019 <sup>113</sup>
<b>Mice with disseminated infection</b>	-	-	One day after infection, mice were treated once every 3 days with purified antibody against phage-KR	Increased in the survival rate (80%)	Chen et al 2019 <sup>98</sup>

sporotrichosis in *S. globosa* endemic areas.<sup>15</sup> However, a cat-associated sporotrichosis case caused by *S. globosa* was found in China. A healthy 51-year-old man was bitten by a cat in his right hand, and developed an unhealing wound that progressed into multiple ascending subcutaneous nodules.<sup>102</sup> Sporotrichosis diagnosis was confirmed by culture, and the species identification was performed by amplification and sequencing of the chitin synthase 1 gene, *chs*; the DNA topoisomerase II gene *top2*, the DNA polymerase  $\alpha$  subunit B gene, and the DNA-directed RNA polymerase I and II subunits genes, suggesting that *S. globosa* can also cause cat bite-associated sporotrichosis.<sup>102</sup>

Moreover, a dominance of female patients with confirmed *S. globosa* infection has been reported, although this gender difference might be related to higher exposure.<sup>11</sup> In addition, systemic sporotrichosis is often associated with

immunocompromised patients, although it is not the most common fungal form for this species,<sup>3</sup> due to *S. globosa* inadequate growth at 37°C.<sup>1</sup>

## ***S. globosa* Identification**

The importance of an efficient identification of the sporotrichosis etiological agent is due to the necessity of accurate and well-timed treatment. Clinical samples culture is the typical method for sporotrichosis diagnosis, but it is not enough to identify the causative agent at the species level.<sup>104</sup> *S. globosa* is characterized by the production of both hyaline and sessile brown to dark brown conidia, as it has been reported on colonies grown on cornmeal agar at 37°C.<sup>11</sup> The fungus on potato dextrose agar at 25°C grows as septate hyphae with terminal clavate obovoid structures.<sup>11</sup> *S. globosa* growth on potato dextrose agar at 21 days shows a better development when the temperature rises from 20°C to 30°C but does not display an efficient growth when the temperature goes from 30°C to 37°C, which is related to the inability of the fungus to cause disseminated or systemic infections.<sup>21</sup> Despite this, studies have reported *S. globosa* isolation and growth at 37°C,<sup>11,23</sup> and it is hypothesized that this inconsistency may be related to the fungal genomic plasticity.

Molecular techniques are required to discern among clade members. Sequencing the calmodulin (*CAL*), beta-tubulin (*BT*) and translational elongation factor 1-alpha (*TEF-1α*) genes lead to *S. globosa* identification,<sup>20</sup> and nowadays numerous methods for faster identification have been developed. Zhang et al established a novel real-time PCR method using the ITS (internal transcribed spacer) sequence for *S. globosa* identification, and its detection limit is the same as that of the species-specific PCR,<sup>105</sup> in which primers are designed using representative orthologous genes. In addition, de Oliveira and collaborators evaluated T3B PCR fingerprinting to differentiate clinical *Sporothrix* strains at the species level. They obtained profiles that showed DNA fragments with different banding patterns, allowing a distinction between species, proposing a simpler, faster, and cheaper technique.<sup>106</sup> Also, Rodrigues et al determined that rolling circle amplification and species-specific primers (*CAL-Fw* and *CAL-Rv*) may perform well for detecting *Sporothrix* DNA directly in complex environmental samples, expanding research on the distribution in nature, especially in endemic regions.<sup>107</sup>

For the diagnosis of sporotrichosis caused by *S. globosa*, a combination of microbiologic and molecular methods has been used, including pus or tissue culture; direct microscopic examination of tissue fragments with KOH; histopathological and microbiological examination of biopsy samples; hematoxylin and eosin, and periodic acid–Schiff (PAS) and Grocott stainings; partial sequencing of the nuclear *CAL* gene, the *chs* gene, the *top2* gene, the DNA polymerase α subunit B, and the DNA-directed RNA polymerase I and II subunits.<sup>23,100,101</sup>

## **Therapy**

Sporotrichosis treatment hinges on the clinical form, infection persistence, the patient's health status, and the etiological agent.<sup>108</sup> In addition, it has been reported that the development of the infection also depends on the strain virulence, as it was demonstrated using isolates from lymphocutaneous, fixed, and disseminated sporotrichosis, where mice inoculated with strains from the disseminated infection had an early onset of the mycosis, more severe lesions and died within the first 10 days of the infection.<sup>99,109</sup> All of these factors should be taken into consideration to choose an accurate treatment.

Here are enlisted some antifungal drugs frequently used for treatment, according to the infection clinical form. For lymphocutaneous and fixed cases, potassium iodide (KI) is the treatment of choice in many developing countries, due to its high efficacy and low cost. However, it is not recommended for disseminated, extra-cutaneous cases, for immunocompromised patients, or during pregnancy.<sup>110</sup>

The Clinical Practice Guidelines for the Management of Sporotrichosis consider itraconazole as the drug of choice for sporotrichosis therapy, its use is recommended in lymphocutaneous, fixed, and disseminated infections.<sup>111</sup> It presents fewer side effects than KI, a better cost–benefit relationship, and an easier administration.<sup>110</sup> Yet, itraconazole presents multiple-drug interactions and is forbidden during pregnancy.<sup>112</sup>

Fluconazole can be used in lymphocutaneous cases, it is considered as the second line of therapy, and although it presents a modest efficacy, it is recommended when itraconazole cannot be tolerated. Similarly, terbinafine is highly recommended when itraconazole cannot be used, it presents low efficacy and can be used in patients with comorbidities due to its low drug interaction rate, which is lower than fluconazole and itraconazole.<sup>110</sup>

There are scarce reports for the treatment of sporotrichosis caused by *S. globosa*, but some cases have reported successful regression of the lesions with different treatment schemes in human patients, including itraconazole<sup>23,101,103</sup> and KI<sup>100</sup>; and in murine models, such as treatment with photodynamic therapy and methylene blue,<sup>113</sup> and phage-driven immunotherapy (Table 3).<sup>98</sup>

Nevertheless, reports that evaluate the correlation of treatment and the clinical form, are usually focused on infections caused by *S. schenckii*. Ottonelli-Stopiglia et al studied antifungal susceptibilities of the *Sporothrix* clinical clade and reported that there were no significant differences among species,<sup>114</sup> which is not in agreement with other studies,<sup>44,45</sup> that reported several differences in the susceptibility between species. Further work in this area is required to address this subject. However, here we described the antifungal susceptibility for *S. globosa*, regardless of the clinical form from where the isolates were obtained.

There is disagreement about whether the drug sensitivity tests for *S. globosa* should be performed using yeast or mycelial phase.<sup>115</sup> Various reports used the yeast-like cells<sup>116</sup> according to the statement that these cells are the infective form, but still, the results obtained from using different growth phases of this morphology are considerably different.<sup>115</sup>

Bao and collaborators compared the antifungal sensitivity of *S. globosa* yeast and mycelial phases. They found that all the studied clinical isolates were highly sensitive to itraconazole and insensitive to fluconazole. However, they also reported differences in the minimum inhibitory concentration (MIC) between the mycelial and yeast phases, which were significant for itraconazole, voriconazole, posaconazole, 5-fluorouracil, and echinocandins. The reasons for this variation in antifungal susceptibility in vitro are still unclear.<sup>115</sup>

A study that tested in vitro antifungal susceptibility of 80 *S. globosa* isolates showed that the sensitivity is dependent on the incubation temperature and growth phase. The in vitro susceptibility profile of the nine drugs tested demonstrated that as the incubation temperature of the microplates increased, MICs were significantly decreased, except for amphotericin B and terbinafine.<sup>117</sup> Additionally, terbinafine was the most active agent, regardless of incubation temperature or growth phase; however, it is not recommended as a first-line treatment for cutaneous sporotrichosis. All isolates tested were resistant to voriconazole.<sup>117</sup>

Amphotericin B is suggested for life-threatening cases.<sup>104</sup> High MIC values are reported against *S. globosa* mycelial and yeast phases, and there were no significant differences between them.<sup>115</sup>

Drug resistance and complications during antifungal therapy are major concerns, especially in patients with liver disorders, children, and pregnant women. Different alternatives are being developed, such as photodynamic therapy (PDT). Li et al evaluated the in vitro and in vivo *S. globosa* susceptibility to photodynamic inactivation using methylene blue as a photosensitizer and LED as the light source. PDT-methylene blue could effectively inhibit sporotrichosis in vivo, and this method also shows the advantage of avoiding the adverse side effects caused by drugs (Table 3).<sup>113</sup>

## Concluding Remarks

Although the first sporotrichosis case was reported more than a century ago, it was until 2007 that the etiological agents of this mycosis were identified, and even more recently their genomes were sequenced and made available.<sup>24,28</sup> Therefore, the current vision we have about the clinically relevant *Sporothrix* species can be considered recent and limited.

Many efforts to understand the *Sporothrix*-host interaction have been made, but these have focused on *S. schenckii* and *S. brasiliensis*. However, to fully understand sporotrichosis, all the causative agents should be studied at the same level. So far, *S. globosa* genetic modification has not been reported, like in *S. schenckii*, and the availability of the genome sequences opens the possibility to generate mutant strains and to produce recombinant proteins to start characterizing the virulence factors of this fungal species. Although the genome is not annotated, the information that we already know about *S. schenckii* and *S. brasiliensis* paves the way to start analyzing the mechanisms behind *S. globosa* virulence and antifungal sensibility. For example, the contribution of some virulence factors already confirmed in *S. schenckii* and *S. brasiliensis*, such as SsCmk1, SsDrk1, Gp70, and proteins of the PRM, can be evaluated in *S. globosa* to establish whether they have similar functions to those reported in other *Sporothrix* species, or perform species-specific tasks, adding evidence that helps us to better understand the phenotypes displayed by these organisms.

Also, a deeper insight into the immune response against *S. globosa* could contribute to the knowledge of the host–fungus interaction. For this, the composition of the *S. globosa* cell wall needs to be fully evaluated, to explain the differences observed in the recognition by the host immune response of the three *Sporothrix* species. Moreover, it is important to determine the immune receptors that recognize this species and the role of different immune cells when interacting with *S. globosa* cells.

The antifungal drug resistance observed in *S. globosa* highlights the importance of prompt identification of the sporotrichosis etiological agent, to provide a suitable treatment to control the infection.

In conclusion, *S. globosa* is a well-known etiological agent of sporotrichosis, but there is an opportunity area in the study of its biological and clinical aspects. Therefore, further molecular, genetic, immunological, epidemiological, and clinical studies of this organism will positively impact the diagnosis and treatment of this infection.

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## Author Contributions

All authors made a significant contribution to the work reported, in the conception, design, execution, acquisition of data, analysis, and interpretation, critically reviewed the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agreed to be accountable for all aspects of the work.

## Disclosure

The authors declare no conflict of interest.

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