Review

Sporothrix schenckii complex biology: environment and fungal pathogenicity

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Sporothrix schenckii is a complex of various species of fungus found in soils, plants, decaying vegetables and other outdoor environments. It is the aetiological agent of sporotrichosis in humans and several animals. Humans and animals can acquire the disease through traumatic inoculation of the fungus into subcutaneous tissue. Despite the importance of sporotrichosis, it being currently regarded as an emergent disease in several countries, the factors driving its increasing medical importance are still largely unknown. There have only been a few studies addressing the influence of the environment on the virulence of these pathogens. However, recent studies have demonstrated that adverse conditions in its natural habitats can trigger the expression of different virulence factors that confer survival advantages both in animal hosts and in the environment. In this review, we provide updates on the important advances in the understanding of the biology of Spor. schenckii and the modification of its virulence linked to demonstrated or putative environmental factors.

Introduction

Sporotrichosis is an acute or chronic granulomatous mycosis of humans and mammals that has a worldwide distribution. Initially the causal agent of this disease was thought to be a unique thermally dimorphic fungus, Sporothrix schenckii; however, it has been recently proposed, based on physiological and molecular aspects, that Spor. schenckii is a complex of various species (Lopes-Bezerra et al., 2006; Marimon et al., 2007, 2008a, b; Oliveira et al., 2014). Sporotrichosis was originally described in 1898 by Schenck at the Johns Hopkins Hospital in Baltimore, who recognized that the infection was most likely due to a previously undescribed fungal pathogen (Schenck, 1898). Two years later, Hektoen & Perkins (1900) confirmed these observations in a report of a second case and a detailed morphological description of the pathogen, which included studies involving laboratory animals. Other cases in the United States and Western Europe were recognized, especially in France, where large numbers of cases were reported in the early 20th century (de Beurmann & Gougerot, 1912).

Recently, Spor. schenckii has received particular attention due to the increased number of infections caused worldwide. The presence of the Spor. schenckii complex and cases of sporotrichosis have been reported from all of the continents (Madrid et al., 2009; Barros et al., 2010) and in different geographical regions, such as tropical and subtropical regions. Some areas have declared sporotrichosis an emerging health problem, with a growing interest for sanitary authorities (Feeney et al., 2007; Hay & Morris-Jones, 2008; López-Romero et al., 2011; Rodrigues et al., 2013a). Sporotrichosis is now the most common subcutaneous mycosis in the Americas (especially Brazil, Mexico, Peru, Colombia and Uruguay) and also in Japan, India and South Africa (Carrada-Bravo & Olvera-Maciás, 2013). A particular geographical area called Abancay, in the south central Peruvian Highlands, is considered a hyperendemic area, with an estimated incidence of approximately 50–60 cases per 100 000 inhabitants per year (Bustamante & Campos, 2001). Meanwhile in Brazil there are increasing reports of sporotrichosis in areas where the disease was rare decades ago, with an intriguing zoonotic transmission by cats (Borges et al., 2013; Rodrigues et al., 2013a, b). In Europe, sporotrichosis is less frequent, although it was a common disease in France in 1900 but declined after two decades. Currently, sporotrichosis is intermittently reported.

Abbreviations: MAPK, mitogen-activated protein kinase; ROS, reactive oxygen species.
in several other countries, such as Italy, Spain, Portugal, the UK and Turkey (Gürçan et al., 2007; Criseo et al., 2008; Dias et al., 2011; Ojeda et al., 2011). Another interesting observation is that from the beginning of the AIDS era, new clinical forms of disseminated sporotrichosis have been reported, including cerebral, endocardial and ocular involvement (Galhardo et al., 2010; Ramos-e-Silva et al., 2012; Silva-Vergara et al., 2012).

The presence or even an abundance of the fungus in nature is not enough to explain the development of the disease. Although the fungus is frequently isolated in environmental and commercial samples, it is unclear why sporotrichosis has a low incidence. However, the different locally emerging outbreaks reaching sometimes epidemic proportions lead to questions regarding the relevance of the host in the development of the disease (López-Romero et al., 2011), the virulence associated with genetic polymorphisms in the Spor. schenckii complex (Sasaki et al., 2014), and other environmentally associated factors not yet sufficiently studied.

The aim of this work is to provide an update on the recent advances in the understanding of the biology of the Spor. schenckii complex, addressing different ways through which environmental factors may modify the virulence of the fungus, as a possible contribution to the sporotrichosis outbreaks.

The Spor. schenckii complex and sporotrichosis

The genus Sporothrix is a species-rich taxon of Ascomycetes, which varies according to the ecological niche, frequency, distribution and virulence. Some of these fungi have the potential to survive in mammalian hosts and are able to cause damage in multiple species of animals, including humans (de Hoog, 1974; Fernandes et al., 2013). Spor. schenckii is recognized as a cryptic species complex including Sporothrix brasiliensis, Sporothrix globosa, Sporothrix mexicana, Sporothrix luriei, Sporothrix pallida (formerly Sporothrix albicans) and Spor. schenckii sensu stricto (Oliveira et al., 2014). Sporothrix species, with the exception of Spor. pallida, have been reported to cause sporotrichosis in humans and animals (Lopes-Bezerra et al., 2006; Marimon et al., 2007, 2008a, b, Romeo & Criseo, 2013; Oliveira et al., 2014).

The fungi belonging to the Spor. schenckii complex are ascomycetous dimorphic organisms (division Ascomycota, class Pyrenomycetes, order Ophiostomatales, family Ophiostomataceae), naturally found in substrates such as living and dead vegetation, animal excreta, and soils plentiful in cellulose, with a pH range of 3.5 to 9.4, a mean temperature of 31 °C, and a relative humidity above 92 %. This fungus is phenotypically characterized by the ability to produce conidias in its filamentous form, and cigar-shaped yeast-like cells when cultured at 35–37 °C or as the infectious form in animals and humans (Lopes-Bezerra et al., 2006).

Sporotrichosis affects humans and other mammals, such as cats, dogs, rats, armadillos and horses. As the fungus is abundant in soil, wood and moss, most infections occur following minor skin trauma in people with outdoor occupations or hobbies, such as gardening, farming, hunting or other activities involving close contact with vegetation and soil. The zoonotic transition from sick or carrier animals (mainly cats) to man is gaining a growing importance. Similarly, inoculation may also occur after motor vehicle accidents and in laboratory personnel handling Sporothrix-infected specimens (Hay & Morris-Jones, 2008; Romeo & Criseo, 2013).

In humans, the disease has different clinical manifestations and can be classified into fixed cutaneous, lymphocutaneous, disseminated cutaneous, and extracutaneous or systemic sporotrichosis. Fixed cutaneous sporotrichosis is located in the skin and is restricted to the injury site without lymphatic involvement. Lymphocutaneous sporotrichosis is the most common manifestation and is characterized by papular and nodular superficial ulcers and/or vegetative plaque lesions in the skin and along the trajectory of the draining lymph vessels. The disseminated cutaneous form of sporotrichosis consists of multiple and distant lesions in the skin due to haematogenous dissemination or multiple inoculations of the fungus. In extracutaneous or systemic sporotrichosis, multiple cutaneous and visceral lesions involving the liver, joints, spleen, bones, bone marrow, lungs, eyes, testis and central nervous system, associated with cellular immunodeficiency have been described (Edwards et al., 2000; Rocha et al., 2001; Ramos-e-Silva et al., 2012). Primary pulmonary sporotrichosis is another clinical type of this infection, caused by the inhalation of infectious conidia in contaminated environments (Aung et al., 2013).

The response of the Spor. schenckii complex to changing environmental conditions

The origin and maintenance of virulence in dimorphic fungi is enigmatic because an interaction with a mammalian host is not a requisite for fungal survival and virulence (Steinbergen et al., 2004). Casadevall et al. (2003) called this phenomenon ‘ready-made’ virulence. The influence of different environmental contaminants on pathogenic or non-pathogenic fungi has been studied (Gadd, 1993; Valix et al., 2001; Valix & Loon, 2003; Zafar et al., 2007; Anahid et al., 2011). Spor. schenckii complexes are present in nature in environments with diverse conditions, including polluted environments (Cooke & Foter, 1958; Dixon et al., 1991; Ulfig, 1994; Ulfig et al., 1996; Kacprzak & Malina, 2005; Pečiulytė, 2010; Chao et al., 2012; Yazdanparast et al., 2013), environments with a wide pH range from 2.2 to 12.5 (Noriega et al., 1993; Ferreira et al., 2009), the floor of swimming pools (Staib & Grosse, 1983), desiccated storage rooms (Kazanas, 1987), fleas, ants and horse hair (Carrada-Bravo & Olvera Macías, 2013). Moreover, specific indoor environments also select for certain stress-tolerant fungi and can drive their evolution towards acquiring medically important traits (Gostincar et al., 2011).
Physical factors

*Spor. schenckii* is able to resist extreme conditions, such as very low temperatures for several years (Pasarell & McGinnis, 1992; Mendoza et al., 2005) and extreme osmotic pressure (Castellani, 1967; de Moraes Borba et al., 1992; de Capriles et al., 1993; Mendoza et al., 2005; Ferreira et al., 2009). Some studies have revealed that different levels of UV light exposure by *Spor. schenckii* strains resulted in a conserved viability, although with a high frequency of morphological variants, depending on the strain and UV dose. The main morphological variants had smaller colonies or altered shape. Stable and non-stable morphological variants were found in the population, and the reversion of the mutant phenotype was always to the WT phenotype (Torres-Guerrero & Arenas-López, 1998).

In another study, the gamma radiation effects on the yeast cells of *Spor. schenckii* were analysed, showing that yeast cells remained viable up to 9.0 kGy, though synthetic protein metabolism was strongly affected, while at a dose of 7.0 kGy they retained viability, metabolic activity, and morphology but their capacity to produce infection was abolished (de Souza Lacerda et al., 2011).

Metals

Micro-organisms, including fungi, have been shown to possess an ability to survive by adapting or mutating at high concentrations of toxic heavy metals. In general, two mechanisms have been proposed for heavy metal tolerance in fungi: (1) extracellular sequestration with chelation and cell-wall binding, mainly employed to avoid metals entering cells, and (2) intracellular physical sequestration of metals by binding to metallothioneins, sequestration as insoluble phosphates or removal from the cell via transporters such as CadA, ZntA or PbrA, preventing the damage caused by metals to sensitive cellular targets and reducing the metal burden in the cytosol (Anahid et al., 2011; Jarosławiecka & Piotrowska-Seget, 2014). Most fungi synthesize siderophores, which chelate iron, which is ultimately taken up as a siderophore–iron complex (Kosman, 2003). The capacity to accumulate iron is critical for the survival of fungal pathogens in different conditions (Schaible & Kaufmann, 2004). Unlike other fungi, such as *Saccharomyces cerevisiae* (Kaplan et al., 2006), *Spor. schenckii* is capable of producing its own siderophores in response to low iron availability (Pérez-Sánchez et al., 2010). This mechanism can be involved in the pathogenicity and survival under conditions of environmental stress or inside the host.

Chemical contaminants

Environmental fungi can biodegrade aromatic hydrocarbons in their habitat (Cerniglia, 1997). An interesting report revealed that several fungi, including *Spor. schenckii*, were isolated from air biofilters exposed to hydrocarbon-polluted gas streams and assimilated volatile aromatic hydrocarbons as the sole source of carbon and energy. The data in this report show that many volatile-hydrocarbon-degrading strains are closely related to, or in some cases clearly conspecific with, the very restricted number of human-pathogenic fungal species causing severe mycoses, especially neurological infections, in immunocompetent individuals (Prenafeta-Boldu et al., 2006). In addition, the effect of fungicides against several environmental pathogenic fungi was evaluated, and *Spor. schenckii* had greater resistance to these products than the other fungi (Morehart & Larsh, 1967).

Other micro-organisms

A phenomenon insufficiently studied is the interaction of pathogenic fungi with other micro-organisms in the neighbourhood. Chaturvedi et al. (1988) evaluated the in vitro interactions between colonies of *Blastomyces dermatitidis* and six other zoopathogenic fungi. The interactions were found to range from neutral with *Histoplasma capsulatum* and *Candida albicans* to strongly antagonistic with *Microsporum gypseum*, *Pseudallescheria boydii* and *Spor. schenckii*, and included lysis by *Cryptococcus neoformans*. In another study, *Cryp. neoformans* was shown to interact with macrophages, slime moulds and amoebae in a similar manner, suggesting that fungal pathogenic strategies may arise from environmental interactions with phagocytic micro-organisms (Steenbergen et al., 2003).

In another interesting report, Steenbergen et al. (2004) examined the interactions of three dimorphic fungi, *B. dermatitidis*, *H. capsulatum* and *Spor. schenckii*, with the soil amoeba *Acanthamoeba castellanii*. The ingestion of the yeast by this amoeba resulted in amoeba death and fungal growth. For each fungal species, the exposure of yeast cells to amoebae resulted in an increase in hyphal cells. The biochemical events during phagocytosis by either *A. castellanii* or immune phagocytes appear similar, suggesting that the ‘respiratory burst’ enzyme(s) responsible for oxy-radical generation in these two cell types is structurally related (Davies et al., 1991). Thus, soil amoebae may contribute to the selection and maintenance of pathogenic dimorphic fungi in the environment, conferring these microbes with the capacity for virulence in mammals.

Despite the necessity for more studies evaluating the influence of different environmental factors on the physiology and pathogenicity of the *Spor. schenckii* complex, all the available data suggest that the strategies that pathogenic fungi acquire to survive this environmental competition may, in turn, provide the ability to infect animals and may further allow the emergence of opportunistic pathogens from these microenvironments (Baumgardner, 2012) (Fig. 1).

Fungal elements involved in environmental resistance and pathogenicity

Although it is known that many external influences can affect the pathogenicity of the *Spor. schenckii* complex as environmental pathogenic fungi, these influences and mechanisms have not been sufficiently studied for this
complex. However, the presence of the same molecules interacting with environmental toxins as described in other fungi (Casadevall et al., 2003) is a good reason to hypothesize that similar mechanisms may be acting in order to face these extreme conditions. As Casadevall et al. (2003) reported for Cryptococcus neoformans, several virulence factors in Sporothrix schenckii also appear to have ‘dual use’ capabilities for the survival in both animal hosts and the environment (Table 1).

**Dimorphism**

The dimorphic fungi comprise a group of important human pathogens and represent a family of seven phylogenetically related ascomycetes that include *B. dermatitidis, Coccioidioides immitis, Coccidioides posadasii, H. capsulatum, Paracoccidioides brasiliensis, Penicillium marneffei* and *Sporothrix schenckii*. These fungi possess the unique ability to switch between mould and yeast cells in response to thermal stimuli and other environmental conditions. In the environment, they grow as mould that produces conidia or infectious spores, which, when transmitted to humans or other susceptible mammalian hosts, are capable of converting to pathogenic yeasts that cause serious infection (Klein & Tebbets, 2007).

The dimorphism in the *Sporothrix schenckii* complex has also been associated with the ability to adapt to environmental changes and to yield an increased virulence (Nemecek et al., 2006; Gauthier & Klein, 2008). This fungus exhibits mycelium morphology in its saprophytic phase at 25 °C in laboratory conditions and yeast morphology in host tissues at 35–37 °C. On the other hand, in an environment with varying temperature they are able to keep the mycelial form. The formation of yeast cells was thought to be a requisite for the pathogenicity of *Sporothrix schenckii*, however, the mechanisms that regulate the dimorphic switch remain unclear. Some recent findings have begun to clarify these mechanisms.

The principal intracellular receptors of environmental signals are the heterotrimeric G proteins, and they are involved in fungal dimorphism and pathogenicity. Valentin-Berrios et al. (2009) described a new G protein α-subunit gene in *Sporothrix schenckii* ssg-2, and demonstrated that the SSG-2 G α-subunit of 40.90 kDa interacts with the cytosolic phospholipase A2, participating in the control of the dimorphism in this fungus.

The yeast-to-mycelium transition is dependent on calcium uptake (Serrano & Rodríguez-del Valle, 1990; Rivera-Rodriguez & Rodríguez-del Valle, 1992). In *Sporothrix schenckii*, a Ca\(^{2+}\)/calmodulin-dependent protein kinase (CaMK) encoded by the calcium/calmodulin kinase I (sscmk1) gene (GenBank accession no. AT823266) was described (Valle-Aviles et al., 2007). Experiments using different inhibitors of the CaMK pathway showed that they inhibited the transition from yeast cells to hyphae, which suggests a calcium/calmodulin pathway is involved in the regulation of the dimorphism in *Sporothrix schenckii* (Valle-Aviles et al., 2007). Similarly, RNAi technology was used to silence the expression of the sscmk1 gene. The RNAi transformants were unable to grow as yeast cells at 35 °C and showed a decreased tolerance to this temperature (Rodriguez-Caban et al., 2011).

The mitogen-activated protein kinase (MAPK) cascade and cAMP signalling pathways are known to be involved in fungal morphogenesis and pathogenic development. The MAPK and cAMP pathways are both activated by an upstream branch, two-component histidine protein kinase.
Table 1. Some attributes of Spor. schenckii complex with demonstrated or putative effects in the protection against environmental stressors and as virulence factor in the host (dual use)

<table>
<thead>
<tr>
<th>Attribute</th>
<th>In the environment</th>
<th>Function</th>
<th>In the host</th>
<th>Selected references</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell wall</td>
<td>Protects cell from drastic changes in external environment</td>
<td>Protects cell from aggressive conditions in host tissue</td>
<td>Oda et al. (1983), Carlos et al. (2003), Madrid et al. (2010), López-Esparza et al. (2013)</td>
<td></td>
</tr>
<tr>
<td>Melanin</td>
<td>UV shielding, extreme temperature protection, reduced susceptibility to enzymic degradation</td>
<td>Resistance to phagocytosis and oxidative killing by phagocytic cells; antifungal drug resistance</td>
<td>Almeida-Paes et al. (2009), Romero-Martinez et al. (2000), Morris-Jones et al. (2003)</td>
<td></td>
</tr>
<tr>
<td>Ergosterol</td>
<td>Protection against oxidative killing by soil amoebae?</td>
<td>Protection against oxidative killing by phagocytic cells</td>
<td>Sgarbi et al. (1997)</td>
<td></td>
</tr>
<tr>
<td>Dimorphism</td>
<td>Mycelium morphology in its saprophytic phase</td>
<td>Yeast morphology in host tissues at 35–37°C</td>
<td>Nemecek et al. (2006), Gauthier &amp; Klein (2008)</td>
<td></td>
</tr>
<tr>
<td>Adhesins</td>
<td>In other fungi, adhesion genes are activated by diverse environmental triggers like carbon and/or nitrogen starvation, changes in pH or ethanol levels</td>
<td>Adhesion to the dermal and subendothelial matrix; migration across the endothelial barrier; immunomodulators</td>
<td>Verstrepen et al. (2003), Sampermans et al. (2005), Figueiredo et al. (2007), Lima et al., (1999, 2001, 2004), Ruiz-Baca et al. (2009), Nascimento et al. (2008), Teixeira et al., 2009</td>
<td></td>
</tr>
<tr>
<td>Proteinase</td>
<td>Nutritional function</td>
<td>Tissue damage; degradation of antibodies</td>
<td>Davies et al. (1991), Wang et al. (2008)</td>
<td></td>
</tr>
<tr>
<td>Catalase</td>
<td>Protection against ROS of soil amoebae?</td>
<td>Protection against ROS of host phagocytes</td>
<td>Pérez-Sánchez et al. (2010)</td>
<td></td>
</tr>
<tr>
<td>Superoxide dismutase</td>
<td>Protection against oxygen-derived oxidants?</td>
<td>Intracellular growth</td>
<td>Pérez-Sánchez et al. (2010)</td>
<td></td>
</tr>
<tr>
<td>Nitroreductase</td>
<td>Tolerance of environmental contaminants?</td>
<td>Resistance to NO in phagocytes?</td>
<td>Stopiglia et al. (2013), Aviv et al. (2014)</td>
<td></td>
</tr>
<tr>
<td>Siderophores</td>
<td>Iron uptake in the environment</td>
<td>Iron uptake inside host</td>
<td>Pérez-Sánchez et al. (2010)</td>
<td></td>
</tr>
<tr>
<td>SSG-1</td>
<td>Survival under conditions of stress and nutrient limitation</td>
<td></td>
<td>Pérez-Sánchez et al. (2010)</td>
<td></td>
</tr>
</tbody>
</table>

(HPK) phospho-relay system (Hou et al., 2013). Nemecek et al. (2006) reported that a long-sought regulator controls the switch from a non-pathogenic mould form to a pathogenic yeast form in dimorphic fungi. They found that DRK1, a hybrid dimorphism-regulating histidine kinase, functions as a global regulator of the dimorphism and virulence in B. dermatitidis and H. capsulatum and is required for the phase transition from mould to yeast, expression of virulence genes, and pathogenicity in vivo. More recently, Hou et al. (2013) developed molecular cloning, characterization and differential expression of DRK1 in Spor. schenckii. In this report, quantitative real-time reverse transcriptase PCR revealed that SsDRK1 was more highly expressed in the yeast stage than in the mycelial stage, which indicated that SsDRK1 may be involved in the dimorphic switch in Spor. schenckii.

Zhang et al. (2013) reported other proteins involved in the dimorphism process. The Ste20-related kinases are involved in signalling through the MAPK pathways and in morphogenesis through the regulation of cytokinesis and actin-dependent polarized growth. The expression of other proteins that may affect biosynthetic and metabolic processes, such as Cullin-3, CdcH and 4-coumarate-CoA ligase, was found by Zhang’s group. The expression of the genes for these proteins was detected predominantly in the yeast form because this is the form of Spor. schenckii found in the host; the expression of these genes could reflect the ability of the yeast phase to adapt to growth-limiting environments. This group also demonstrated an upregulation of the Hsp70 chaperone Hsp88 in the development of the yeast phase of Spor. schenckii at 37 ºC; however, the precise role the hsp88 gene remains unclear (Zhang et al., 2012).

Lipids are thought to play important roles in the regulation of the dimorphism and virulence in pathogenic fungi. Generally, the ratio of phospholipid/ergosterol is less than 1 in the yeast form and 2–20 in the mycelial form cells in Cand. albicans and Spor. schenckii. During the transition from the yeast to mycelial form, phosphatidylinositol and phosphatidylserine are reduced in amount, whereas phosphatidylcholine increases. Phospholipase D is activated during this transition (Kitajma, 2000).

Phorbol-12-myristate-13-acetate (PMA), a tumour-promoting agent and protein kinase C (PKC) activator, was
found to stimulate, in a concentration-dependent manner, germ tube formation and germ tube growth by yeast cells induced to undergo a transition to the mycelium form. PMA also had a stimulatory effect on DNA and RNA synthesis in cells induced to undergo the yeast-to-mycelium transition and was found to inhibit cell duplication and bud formation in yeast cells induced to re-enter the budding cycle. In contrast, polymyxin B, an inhibitor of PKC, inhibited germ tube formation by yeast cells. This inhibition could be overcome if PMA or calcium was added to the medium, suggesting that the inhibition obtained in the presence of this antibiotic was due to the inhibition of PKC. These results support the involvement of PKC in the control of the dimorphic expression in Spor. schenckii (Colon-Colon & Rodriguez-del Valle, 1993).

Genetic polymorphism

Recently, there has been an increasing interest in studying the biology of the Spor. schenckii complex, and particular attention has been focused on its molecular phylogeny, which has improved our knowledge of the taxonomy, pathogenic characteristics and the epidemiology of this pathogenic fungus. Recent studies have shown important differences in the virulence and drug resistance profiles among different species in the Spor. schenckii complex. Spor. brasiliensis is the most virulent species in comparison with Spor. globosa and Spor. mexicana, which show little or no virulence in murine models (Fernández-Silva et al., 2012; Fernandes et al., 2013). In addition, the test for susceptibility to antifungal drugs showed that Spor. schenckii sensu stricto and Spor. brasiliensis were highly susceptible to most of the antifungals tested in vitro in comparison with the more resistant Spor. globosa and Spor. mexicana species (Marimon et al., 2008a, b). These and other observations suggest that possible genetic differences may be involved.

The genomic organization and chromosome number in different species in the Spor. schenckii complex remain unknown. However, sequencing studies in the calmodulin-encoding gene in the different members of the Spor. schenckii complex have offered valuable information for elucidating the relationships and differences among these species and their role in pathogenic manifestations (Romeo et al., 2011). A recent study revealed a high diversity in the calmodulin gene (regulated by Ca\(^{2+}\)) of Spor. schenckii. In contrast, Spor. brasiliensis and Spor. globosa appeared to be more homogeneous, with a low genetic diversity. The electrophoretic karyotype profiles of Spor. brasiliensis isolates showed less variability than those observed in Spor. schenckii sensu strictu isolates (Sasaki et al., 2014). These results were consistent with the phylogenetic data, which showed the variability among isolates within the species was less frequent in Spor. brasiliensis than in Spor. schenckii sensu strictu (Marimon et al., 2006, 2007; Rodrigues et al., 2013a, b).

Future studies are necessary to determine the role of different genes from the species of the Spor. schenckii complex in virulence, drug resistance and environmental resistance. Changes in the expression of such genes might be beneficial to Spor. schenckii survival in the natural environment and also within a host during infection. Comparative genomics and transcriptomics analysis between highly pathogenic Sporothrix species and species with reduced or absent pathogenicity certainly will accelerate the discovery of new proteins for diagnostics, drug targets and vaccines (Romeo & Criseo, 2013).

Cell wall

The fungal wall protects the cell from drastic changes in the external environment; it is the first point of contact with the host (Mora-Montes et al., 2009). The Spor. schenckii cell wall is composed of glucans, galactomannans, rhamnmannans, chitin, glycoproteins, glycolipids and melanin (Travassos et al., 1977; Travassos & Lloyd, 1980; Lopes-Bezerra et al., 2006; López-Romero et al., 2011). Despite the need for more studies in order to know the detailed structure, it seems that a proper cell wall composition is required for supporting external stress and for virulence (Madrid et al., 2010; López-Esparza et al., 2013).

Melanin

An interesting mechanism to resist adverse environmental conditions is the production of melanin, also implicated in the pathogenesis of several important human fungal pathogens. Several types of melanin have been described in the fungal kingdom but the majority are derived from 1,8-dihydroxynaphthalene (DHN) and known as DHN-melanin (Eisenman & Casadevall, 2012). Melanin has been referred to as ‘fungal armour’ owing to the ability of the polymer to protect micro-organisms against a broad range of toxic insults, warranting the survival of fungi in the environment and during infection (Gómez & Nosanchuk, 2003). Melanization reduces the susceptibility to enzymic degradation, the toxicity of heavy metals, UV and nuclear radiation, extremes of temperature, and oxygen and nitrogen free radicals, which may afford the fungus protection against similar insults in the environment (Wang & Casadevall, 1994a, b; Rosas & Casadevall, 1997, 2001; Taborda et al., 2008; Gessler et al., 2014). Melanin also reduces the susceptibilities of pathogenic fungi to different antifungal drugs (van Duin et al., 2002; Nosanchuk & Casadevall, 2006) and to different immune mechanisms in the host, especially phagocytosis (Steenbergen et al., 2004).

Spor. schenckii produces melanin or melanin-like compounds in vitro. While melanin is an important virulence factor in other pathogenic fungi, this pigment also has a similar role in the pathogenesis of sporotrichosis (Morris-Jones et al., 2003). Melanized cells of WT Spor. schenckii and the albino grown on scytalone-amended medium were less susceptible to killing by chemically generated oxygen-and nitrogen-derived radicals and by UV light than were the conidia of mutant strains. WT melanized conidia and the scytalone-treated albino were also more resistant to antioxidative insults, warranting the survival of fungi in the environment (Gómez & Nosanchuk, 2003). Melanization reduces the susceptibility to enzymic degradation, the toxicity of heavy metals, UV and nuclear radiation, extremes of temperature, and oxygen and nitrogen free radicals, which may afford the fungus protection against similar insults in the environment (Wang & Casadevall, 1994a, b; Rosas & Casadevall, 1997, 2001; Taborda et al., 2008; Gessler et al., 2014). Melanin also reduces the susceptibilities of pathogenic fungi to different antifungal drugs (van Duin et al., 2002; Nosanchuk & Casadevall, 2006) and to different immune mechanisms in the host, especially phagocytosis (Steenbergen et al., 2004).
phagocytosis and killing by human monocytes and murine macrophages than were the un melanized conidia of two mutants (Romero-Martínez et al., 2000). Similar to Cryp.
neoforms and Para. brasiliensis, Spor. schenckii can utilize phenolic compounds to augment melain production, which may be associated with a concomitant increase in the protec-
tion against unfavourable conditions both in the environ-
ment and during infection (Almeida-Paes et al., 2009).

The expression of adhesins

The adhesion ability of a fungus is important in the coloni-
zation of different environmental extracts, and in the
host it is essential for colonization and the establish-
ment of infection. Specific microbial adhesins mediate adherence to host tissues by participating in sophisticated interactions with some of the host proteins that compose the extracellular matrix (Teixeira et al., 2009). The adhesin-
encoding genes are not constitutively expressed. Instead, adhesin is under tight transcriptional control by several interacting regulatory pathways. The switch from non-
adhherence to adherence may allow yeasts to adapt to stress (Verstrepen & Klis, 2006). The adhesin genes are activated by diverse environmental triggers such as carbon and/or nitrogen starvation, changes in pH or ethanol levels (Verstrepen et al., 2003; Sampermans et al., 2005). Fungi need to adhere to the appropriate host tissues to establish an infection site. Apart from being a stress-defence mechanism, adhesion is also crucial for fungal pathogenesis (Verstrepen & Klis, 2006).

The outermost layer of the cell wall of Spor. schenckii has molecules involved in the adhesion of the fungus to host tissues and has a central role in host–pathogen interactions, mediating several interactions associated with the patho-
genic processes of this micro-organism. It has been reported that Spor. schenckii adheres to fibronectin and laminin in soluble and immobilized forms (Lima et al., 1999, 2001, 2004) and that this is a key step for migration across the endothelial barrier (Figueiredo et al., 2007). Independently, Ruiz-Baca et al. (2009) and Nascimento
et al. (2008) characterized a 70 kDa glycoprotein (gp70) on the cell wall of Spor. schenckii that mediates the adhesion of the fungus to the dermal and subendothelial matrix. This gp70 and another protein with a slightly lower molecular mass (67 kDa) were detected from different isolates. This variation might be related to differences in glycosylation (Teixeira et al., 2009). Interestingly, sera obtained from patients with sporotrichosis reacted mainly with the 40 and 70 kDa antigens (Scott & Muchmore, 1989; Alves et al., 1994). In addition, hyperimmune sera of mice infected with Spor. schenckii reacted with gp 70 (Nascimento & Almeida, 2005), and monoclonal antibodies specific for gp70 were protective against experimental infection (Nascimento et al., 2008). Moreover, recently Lopes-Bezerra’s group reported that a reduced level of gp70 expression was found in virulent Spor. brasiliensis and Spor. schenckii strains, while high expression of this molecule is associated with a lower virulence profile of the strains. This is further evidence that this antigen induces a protective host response (Castro et al., 2013).

In another pathogenic micro-organism, Staphylococcus aureus, many molecules involved in adhesion are also involved in different immune evasion mechanisms, not related to the specific process of adhesion (Zecconi & Scali, 2013). However, in the case of the Spor. schenckii complex, the role of adhesins as immune evasion factors is unknown.

Enzyme production

The Pires de Camargo group studied the different enzymic activities related to fungal virulence of 151 Brazilian Spor.
schenckii isolates from five different geographical regions of Brazil. All (100 %) of the Spor. schenckii isolates presented urease and DNase activities. Only three (15.78 %) isolates (one from the north and two from the south-east region) showed gelatinase activity, and five (26.31 %) isolates (one from the north, three from the north-east, and one from the south-east region) showed proteinase activities, of 0.68, 0.88, 0.85, 0.55 and 0.78, respectively. Additionally, only four (21.05 %) isolates (one from the north, one from the central west, and two from the south-east region) showed caseinase activities, of 0.75, 0.87, 0.89 and 0.87, respectively (Ferreira et al., 2009). Another report of isolates from Venezuela confirmed the urease activity (Mendoza et al., 2005), while another study in India reported that all of the mycelial forms of Spor. schenckii could split urea (Ghosh et al., 2002).

However, Fernandes et al., from the same Pires de Camargo group, also found that while the ‘highly virulent’ Spor. schenckii isolates show a profile of secreted enzymes (proteinase, caseinase, gelatinase, DNase and urease), most of these enzymes were not observed in the hypervirulent species Spor. brasiliensis (Fernandes et al., 2009). This observation indicates that the mechanisms promoting pathogenesis are much more complex, and perhaps not conserved among closely related Sporothrix species, and they most likely involve different virulence factors to evade the host immune system (Romeo & Criseo, 2013).

An interesting enzyme present in the Spor. schenckii complex is the nitroreductase, a member of a group of enzymes that reduces the wide range of nitroaromatic compounds, which has potential industrial applications (Stopiglia et al., 2013). Nitroreductase activity has been detected in a diverse range of bacteria and in yeast (Xu et al., 2007; Lee et al., 2008; Aviv et al., 2014). A recent study in an emergent Salmonella enterica serovar Infantis strain demonstrated that the fixation of adaptive mutations in the DNA gyrase (gyrA) and nitroreductase (nfsA) genes confers resistance to quinolones and nitrofurans, and contributes to stress tolerance and pathogenicity of this bacterium (Aviv et al., 2014). The possible role of the nitroreductase in Spor. schenckii tolerance of adverse conditions in the environment and in the host, in the resistance to oxidative
stress and to antifungal drugs, and in other aspects involved in the pathogenesis needs to be studied.

Additionally, the recent finding in *Spor. schenckii* of intracellular molecules and signals associated with the heterotrimeric G protein z-subunit SSG-1, involved in the response to different external adverse conditions, helps to explain how this fungus is able to survive under external stress (environmental and inside the host) (Valentín-Berrios et al., 2009; Pérez-Sánchez et al., 2010).

**Infections of an alternative host**

*Sporotrichosis* is a human mycosis; however, there is an alarming increase in the frequency of infections in domestic animals, principally in cats, which has led to an increase in the relevant importance from an epidemiological perspective (Reed et al., 1993; Smilack, 1993; De Lima et al., 2001; Barros et al., 2008; Lloret et al., 2013). Its ease of transmission to cohabitant humans and other animals, especially by bites and scratches, makes it a significant zoonosis (Read & Sperling, 1982; Nusbaum et al., 1983; Dunstan et al., 1986). Cats develop disseminated skin ulcers, with often fatal infection (Lloret et al., 2013). The fungus is spread from an ulcer or from bites and scratches. The propensity of cats to transmit the infection to humans may be attributable to the large number of organisms associated with the lesions in most infected cats (Yegneswaran et al., 2009).

However, there are reports of the presence of *Spor. schenckii* in samples from the nails of apparently healthy domestic cats (Schubach et al., 2001, 2002). The cat’s habits of digging holes and covering its excrement with soil and sand, and sharpening its nails on wood including tree trunks, are most likely responsible for the carriage of the fungus on its nails and claws, even as healthy carrier (Carrada-Bravo & Olvera Macías, 2013). Healthy carriers can be important in the dissemination of the fungus and the occurrence of sporotrichosis cases when the conditions are favourable. Human infections have also been associated with insect stings, fish handling, and bites or scratches from birds, dogs, squirrels, horses, reptiles, parrots and rodents, even though clinical symptoms may not be present in these animals (Werner & Werner, 1994; Kauffman, 1999; Liu & Lin, 2001; Ranjana et al., 2001; Haddad et al., 2002a b; Barros et al., 2011; Carrada-Bravo & Olvera-Macías, 2013).

**The ability to escape from host immune mechanisms**

Members of the *Spor. schenckii* complex are able to produce localized infections in immunocompetent hosts. They have developed different mechanisms permitting the evasion of host immunological mechanisms.

**Phagocytosis inhibition**

It has long been reported that phagocytosis is an important defence mechanism against *Spor. schenckii* (Cunningham et al., 1979; Oda et al., 1983). The recognition of the fungus is mediated by molecules such as Toll-like receptors (TLRs): TLR2 (Negrini et al., 2013, 2014), TLR4 (Sassa et al., 2012) and carbohydrate receptors (Guzman-Beltran et al., 2012). Sialic acid residues are expressed at the cell surface of *Spor. schenckii* (Alviano et al., 1982). These residues protect unopsonized fungal cells from phagocytosis by resident mouse peritoneal macrophages. The enzymic removal of sialic acids from the external layers of *Spor. schenckii* envelopes renders the yeast cells more susceptible to phagocytosis (Oda et al., 1983; Alviano et al., 1999). Other *in vitro* studies have shown that galactomannan, ramnomannan and a lipid extract purified from the fungal cell wall are able to inhibit the phagocytosis of *Spor. schenckii* yeast by peritoneal macrophages (Carlos et al., 2003).

Several processes associated with phagocytosis, such as the cytotoxicity mediated by reactive oxygen and nitrogen species (ROS and NO), are involved in the destruction of invading organisms, and their virulence may be related to a differential susceptibility to these mediators (Carlos et al., 2009; Sassa et al., 2012; Wang et al., 2008). A study demonstrated that the *Spor. schenckii* catalase is involved in the defence against the ROS induced by environmental changes *in vitro*, and the authors proposed that catalase, as a main component of antioxidant defence, may contribute to the survival of *Spor. schenckii* during infection (Wang et al., 2008). Moreover, it is known that ergosterol is the major sterol observed in fungal membranes (Weete, 1989), while ergosterol peroxide, the putative product of the \( \text{H}_2\text{O}_2 \)-dependent enzymic oxidation of ergosterol (Bates et al., 1976), is a constituent of the membrane of *Spor. schenckii*, where it could inactivate or scavenge toxic oxygen products that are formed in phagocytic cells (Basaga, 1990; da Graça Sgarbi et al., 1997). It is conceivable that in *Spor. schenckii* ergosterol peroxide is formed as a protective mechanism to evade ROS during phagocytosis (Sgarbi et al., 1997).

Carlos et al. (2003) studied the effect of three different components of the *Spor. schenckii* wall cell (a lipid extract, exo-antigens and the alkali-insoluble fraction) and observed a drastic inhibition of the phagocytosis of yeast in murine peritoneal macrophages previously treated with a lipid extract. This inhibitory effect was associated with a high production of TNF-\( \alpha \) and nitric oxide (NO). Studies performed in our laboratory demonstrated a high fungal burden in a murine model of *Spor. schenckii* infection during the second and fourth week after a previous high production of NO (unpublished data). Similarly, Niedbala et al. (2007, 2011) reported that NO induces a population of CD4\(^+\) CD25\(^+\)Foxp3\(^+\) regulatory T-cells (NO-Tregs) that suppress the functions of CD4\(^+\) CD25\(^+\) effector T-cells *in vitro* and *in vivo*. The helper 17 (Th17) cells are important in antifungal mechanisms (Hernández-Santos & Gaffen, 2012). Interestingly, recently it was discovered that NO-Tregs suppressed Th17 but not Th1 cell differentiation and function, suggesting a differential suppressive function between NO-Tregs and natural Tregs (nTregs) (Niedbala et al., 2007, 2011). Other studies (Niedbala et al., 2007, 2011) have observed a drastic inhibition of the phagocytosis of yeast in murine peritoneal macrophages previously treated with a lipid extract. This inhibitory effect was associated with a high production of TNF-\( \alpha \) and nitric oxide (NO). Studies performed in our laboratory demonstrated a high fungal burden in a murine model of *Spor. schenckii* infection during the second and fourth week after a previous high production of NO (unpublished data). Similarly, Niedbala et al. (2007, 2011) reported that NO induces a population of CD4\(^+\) CD25\(^+\)Foxp3\(^+\) regulatory T-cells (NO-Tregs) that suppress the functions of CD4\(^+\) CD25\(^+\) effector T-cells *in vitro* and *in vivo*. The helper 17 (Th17) cells are important in antifungal mechanisms (Hernández-Santos & Gaffen, 2012). Interestingly, recently it was discovered that NO-Tregs suppressed Th17 but not Th1 cell differentiation and function, suggesting a differential suppressive function between NO-Tregs and natural Tregs (nTregs) (Niedbala et al., 2007, 2011).
et al., 2013). In addition, severe infection promotes the release of pro-inflammatory mediators, including TNF-α and NO, with the subsequent induction of molecules, such as IL-10, Fasl and CTLA-4, which lead to the suppression of the T-cell response (Fernandes et al., 2008).

Production of proteases
Many species of human pathogenic fungi secrete proteases. Fungal proteases have been intensively investigated as potential virulence factors. It is evident that secreted proteases are important for the virulence of dermatophytes because these fungi grow exclusively in the stratum corneum, nails and hair, which constitute their sole nitrogen and carbon sources (Monod et al., 2002). The proteases secreted by Candida albicans are involved in the adherence process and penetration of tissues and in interactions with the immune system, thereby stimulating inflammatory process in the infected host (Pietrella et al., 2013; Wu et al., 2013). Spor. schenckii produces proteases that are able to cleave different subclasses of human IgG, suggesting a sequential production of antigens and molecules that could interact and interfere with the immune response of the host (Da Rosa et al., 2009). Other effects of proteases secreted by Spor. schenckii are being investigated.

Transitory early immunosuppression
Different reports derived from mouse models of infections with Spor. schenckii reveal a transitory early immunosuppression, favouring a fungal burden. Studies performed by Carlos et al. demonstrated the production of interleukin-1 (IL-1) and TNF by adherent peritoneal cells from BALB/c mice, which was measured at weeks 2, 4, 6, 8 and 10 after intravenous inoculation with Spor. schenckii yeast. Compared with age-matched controls, IL-1 and TNF production by adherent peritoneal cells from Spor. schenckii-infected mice was reduced severely at weeks 4 and 6 of infection and was greater than normal at weeks 8 and 10. Moreover, between weeks 4 and 6 of infection, there was a depression of the delayed-type hypersensitivity response to a specific whole soluble antigen and an increase in fungal multiplication in the livers and spleens of the infected mice. Thus, the deficits of cell-mediated immunity in mice with systemic Spor. schenckii infection may derive, in part, from an impaired amplification of the immune response due to the abnormal generation of IL-1 and TNF (Carlos et al., 1994). Other reports have demonstrated that, although NO is an essential mediator in the in vitro killing of Spor. schenckii by macrophages, the activation of the NO system in vivo contributes to the immunosuppression and cytokine balance during the early phases of infection with Spor. schenckii (Fernandes et al., 2008).

The liberation of exo-antigen
A peptide-polysaccharide released from the fungal cell wall, called exo-antigen (ExoAg), has been identified as an important virulence factor for sporotrichosis (Nascimento et al., 2008; Teixeira et al., 2009). The main immunogenic proteins of the cell wall (gp70 and gp40) are present in the ExoAg, and it is thought that the secretion of this outer component can distract several immune mechanisms and serve as a fungal evasion mechanism (Carlos et al., 2009). This hypothesis needs to be confirmed.

Opportunistic infections in immunodeficient hosts
Infection with Spor. schenckii causes a localized lymphocutaneous disease in the immunocompetent host, while it frequently results in disseminated disease in the immunocompromised patient. Disseminated sporotrichosis occurs in individuals with impaired cellular immunity, such as in cases of neoplasmia, transplantation, diabetes, and especially AIDS (Wroblewska et al., 2005; Freitas et al., 2012; Silva-Vergara et al., 2012). The extracutaneous forms of sporotrichosis without skin manifestations and with no previous history of traumatic injuries have been described in renal transplant recipients not treated with antifungal prophylaxis (Geweehr et al., 2013). Additionally, this disease has been described in a patient with X-linked chronic granulomatous disease (Trotter et al., 2014). Interestingly, more than 20 cases of sporotrichosis meningitis have been reported, several of which were associated with immunosuppression (Silva-Vergara et al., 2005; Vilela et al., 2007; Galhardo et al., 2010).

Concluding remarks and future perspectives
The vast majority of human pathogenic fungi are environmental fungi, which normally live outside the human body and can cause infections in certain susceptible hosts. These fungi have most likely gained their pathogenic potential in environmental niches, which in certain aspects resemble the host body. In these environmental niches, fungi acquire the capacity to adhere to surfaces, form biofilms, compete with bacteria, acquire all necessary nutrients and deal with changes in temperature, UV radiation, pH, osmolarity, environmental chemical contaminants, meteorological factors and other physical, chemical and biological stress factors. In addition, these fungi may be challenged by amoebae, which share many characteristics with human phagocytes (Davies et al., 1991). Thus, in nature or in animal hosts, fungal cells must respond efficiently to changing environmental conditions in order to survive (Pérez-Sánchez et al., 2010).

The Spor. schenckii complex uses signal transduction pathways to sense the environment and to adapt quickly to changing conditions (Rodriguez-Caban et al., 2011). Despite these findings, little is known about the genetic mechanisms of virulence and drug resistance in the Spor. schenckii complex. The ecological niches occupied by the Spor. schenckii complex are extraordinarily complex and variable and most likely include biological elements, such as other fungi, viruses, bacteria, animals, protists, algae and
plants, together with physical (e.g., temperature, humidity, radiation) and chemical (pH, metals, hydrocarbons, pesticides, etc.) components.

The chronic effect of some of these components, including heavy metals, UV radiation and pesticides, with immunotoxic effects in potential hosts such as cats and other animals and outdoor workers such as farmers and gardeners, can contribute to the occurrence of fungal infection. The understanding of the interactions between fungi and their potential hosts in the environment is in its infancy; however, initial observations suggest that this will be an extremely rich area of investigation for exploring the fundamental questions of fungal pathogenesis (Casadevall et al., 2003; Prenafeta-Boldú et al., 2006). This knowledge will contribute to the design of new strategies for the control of sporotrichosis.

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