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Minireview

MINIREVIEW

Current progress in the biology of members of the *Sporothrix schenckii* complex following the genomic era

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One sentence summary: This review article will discuss the development of molecular tools and the recent advances brought by the genome sequencing of *Sporothrix* spp. to study the virulence of emerging pathogenic species.
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ABSTRACT

Sporotrichosis has been attributed for more than a century to one single etiological agent, *Sporothrix schenckii*. Only eight years ago, it was described that, in fact, the disease is caused by several pathogenic cryptic species. The present review will focus on recent advances to understand the biology and virulence of epidemiologically relevant pathogenic species of the *S. schenckii* complex. The main subjects covered are the new clinical and epidemiological aspects including diagnostic and therapeutic challenges, the development of molecular tools, the genome database and the perspectives for study of virulence of emerging *Sporothrix* species.

Keywords: *Sporothrix schenckii*; *Sporothrix brasiliensis*; *Sporothrix globosa*; genome; molecular tools; pathogenesis

INTRODUCTION

Sporotrichosis is an acute or chronic granulomatous subcutaneous mycosis of humans and mammals that has a worldwide distribution and several clinical manifestations (Lopes-Bezerra, Schubach and Costa 2006). Disseminated sporotrichosis is currently associated with a high probability of HIV infection (Lopez-Romero *et al.* 2011). Schenck (1898), at Johns Hopkins Hospital in Baltimore, published the first clinical case of sporotrichosis. Two years later, the etiologic agent was isolated, identified and classified (Hektoen and Perkins 1900), followed by the description of the yeast parasitic phase in 1907 (Lutz and Splendore 1907). For just over a century, this subcutaneous mycosis was assigned to a single etiologic agent, *Sporothrix schenckii*. In 2007, a new benchmark was established in the study of this disease with the description of the so-called *S. schenckii* complex (Marimon *et al.* 2007). The authors reported the existence of cryptic pathogenic species within this complex, among which we highlight *S. schenckii sensu stricto*, *S. brasiliensis* and *S. globosa*. According to recent epidemiological data, these are the species of greatest clinical and global epidemiological importance (Chakrabarti *et al.* 2015).

In Latin America, sporotrichosis is the most prevalent subcutaneous mycosis; however, changes in the epidemiological

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pattern of the disease have been reported. Classically, sporotrichosis is acquired through traumatic inoculation of the fungus into a host's subcutaneous tissue with contaminated plant debris, thorns or soil. This process initiates as an occupational disease that is associated with gardening and rural work. However, the zoonotic transmission by domestic cats (*Felix catus*) is of clinical and epidemiological importance and is changing the paradigm of this disease. Transmission through contact with sick and apparently healthy cats may derive from bites and scratches, or by contact with nasal secretions (Schubah, Barros and Wanke 2008). Although feline sporotrichosis has been reported in several countries, in Brazil, a major zoonotic outbreak has registered more than 4000 cases of feline sporotrichosis in Rio de Janeiro State (Schubach, Barros and Wanke 2008; Gremiao *et al*. 2015) and is spreading to other regions (Montenegro *et al*. 2014). In parallel, the number of human cases is also increasing exponentially and severe clinical manifestations are being reported, also in immunocompetent hosts (Lopes-Bezerra and Nascimento 2012). Therefore, up to now the number of human cases registered in Rio de Janeiro State is far beyond 4000 (personal communication).

Interestingly, feline sporotrichosis was associated with *S. brasiliensis* (Rodrigues *et al*. 2013), an emerging species that appears to be geographically restricted to Brazil (Chakrabarti *et al*. 2015). However, cases of feline sporotrichosis were recently described in Malaysia caused by clade D *S. schenckii sensu stricto* (Kano *et al*. 2015). The clinical and epidemiological paradigm is changing because domestic cats play an important role in hosting and spreading species of the *S. schenckii* complex. Furthermore, *S. globosa* has been associated with isolated human cases in Europe (de Oliveira *et al*. 2014) and with endemic areas in Northeast China, where the infection is due to contact with reeds or cornstalks (Yu *et al*. 2013; Liu, Zhang and Zhou 2014). Therefore, identifying the factors in the adaptation of these fungal pathogens to other environments, thus redefining the route of transmission of sporotrichosis, is of key importance.

The species belonging to the *S. schenckii* complex are ascomycetous and thermodimorphic fungi with a mycelial saprophytic phase and a yeast parasitic phase (Howard 1961), which can be easily obtained in the laboratory by growing the fungus at 25–28°C and 35–37°C, respectively. In general, the mycelial saprophytic phase is characterized by hyaline, septate and branched hyphae containing thin conidiophores whose apex forms a small vesicle with sympodially arranged conidia (2–4 μm) in a flower-like arrangement. The conidia become detached from the conidiophores, sometimes being arranged side by side in a row bilaterally to the hyphae. The yeast parasitic phase is pleomorphic, with spindle shaped and/or oval cells measuring 2.5–5 μm in diameter and resembling a 'cigar' (Travassos and Lloyd 1980). Morphological variations have been reported among cryptic species of the *S. schenckii* complex (Marimon *et al*. 2007). Although little is known about the factors that can induce the mycelium to yeast transition, some groups are studying possible regulators of this process in *S. schenckii*. Experimental evidence points to a calcium/calmodulin kinase-encoding gene in *S. schenckii* and its possible involvement as an effector of dimorphism in this fungus (Valle-Aviles *et al*. 2007). Additionally, a homolog of a hybrid histidine kinase, *DRK1*, known as a global regulator of dimorphism and virulence in dimorphic fungi, was recently reported in *S. schenckii* (Hou *et al*. 2013). The SsDrk1 was predicted to be a soluble histidine kinase and to contain three domains: a sensor, a linker and a functional domain. The authors showed by quantitative real-time RT-PCR that *SsDRK1* was more highly expressed in the yeast phase compared with the mycelial phase, suggesting its involvement in the dimorphic transition. Furthermore, with the genomes of *Sporotrhix* spp. already available (Teixeira *et al*. 2014), other putative regulators of morphogenesis, such as the Efg1 transcription factor, can be further studied within the *S. schenckii* complex.

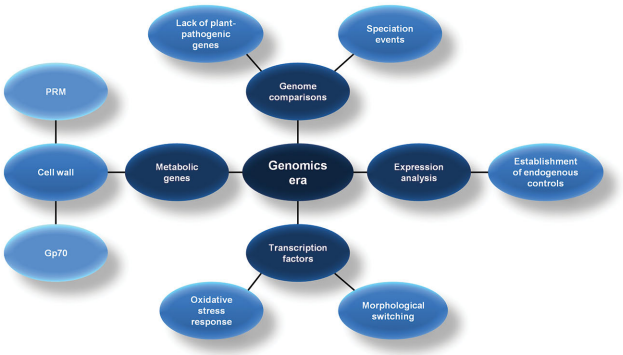

Figure 1. General overview of the main subjects covered by this minireview article about the emerging cryptic species of the *S. schenckii* complex.

In the last decade, studies focused on understanding the biology of these fungal pathogens have shed some light on the attributes that allow them to cause sporotrichosis. Furthermore, systematic studies of the clinical and environmental isolates of these fungi have investigated the genetic and phenotypic variability of some pathogenic species, an important topic that will be discussed in this review. The genomic data and the development of genetic tools impact the study of other pathogenic fungi. Therefore, the recent publication of the complete genomes of two pathogenic species of epidemiological relevance, *S. schenckii sensu stricto* and *S. brasiliensis* (Teixeira *et al*. 2014), will leverage other genomic studies and impact basic research into this disease.

In addition, special consideration must be given to the relationship between this pathogen and its human host. In consequence, understanding the biology of the fungus and the factors that contribute to the survival of *Sporothrix* spp. in the diverse environments they can be found, mapping proteins or other factors that are unique to these lower eukaryotes can lead to targets for the development of more selective antifungal drugs. Furthermore, based on recent epidemiological data, molecular methods for clinical diagnosis at the species level are also necessary.

Considering the latest research advances in human and animal sporotrichosis, this minireview will focus on new findings related to genetic variation, genomics and protein expression, as well as the development of genetic tools that will improve our understanding of the biology of these fungal species and contribute to the discovery of molecules related to pathogenesis (Fig. 1).

THE GENOME IMPACT AND THE DEVELOPMENT OF MOLECULAR TOOLS TO STUDY *SPOROTHRIX* AND RELATED PATHOGENIC SPECIES

Sporotrichosis is an important mycological disease that is frequently covered in pathophysiology/medical microbiology textbooks for undergraduate healthcare education programs. As mentioned above, *S. schenckii*, long believed to be the single causative agent of sporotrichosis, is in fact a group of

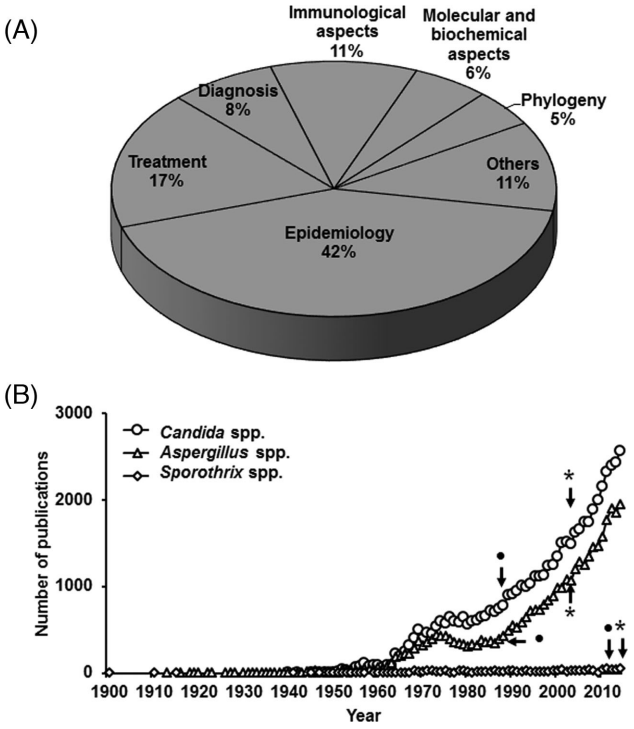

Figure 2. *Sporothrix* as a publication subject during 1900–2014. An analysis within the PubMed database (http://www.ncbi.nlm.nih.gov/pubmed) searching for the term *Sporothrix* retrieved 1124 published papers that were classified according the main subject addressed (panel **A**). In panel **B**, the number of published papers per year addressing either *Sporothrix* (open diamonds), *Aspergillus* (open triangle) or *Candida* (open circle) is shown. The arrow and closed circle indicate the year where the first report about molecular tools for genetic manipulation was published, while the arrow and asterisk show the year in which the first genome sequence was published.

at least four phylogenetically related species (Marimon *et al.* 2007). Thus, this infectious disease is now recognized as being caused by several species, some of them present in the same geographical area (Chakrabarti *et al.* 2015). Despite this advance, we have limited information about the basic biology of these organisms, particularly in fundamental areas such as their ability to survive in the environment without a mammalian host, their virulence factors and the molecular requirements for the immune recognition by the human and feline hosts are poorly understood (Lopez-Romero *et al.* 2011; Martinez-Alvarez *et al.* 2014; Tellez *et al.* 2014). A recent analysis showed that if the keyword '*Sporothrix*' is used to search for scientific literature in the PubMed database (http://www.ncbi.nlm.nih.gov/pubmed), 1124 papers are retrieved. This list comprises works published until 2014; of these, 67% covered clinical aspects of these fungi and/or the disease (epidemiology, treatment and diagnosis), 11% of them covered immunological topics, 11% discussed molecular and biochemical aspects, and the remaining 11% studied other subjects related to either *Sporothrix* or sporotrichosis (Fig. 2A). This number represents the accumulated literature from 1900 to 2014 about this disease and its causative agent, giving an average of only 9.8 publications/year, which is low compared to the amount of literature published within the same period about *Candida* spp. and *Aspergillus* spp., the classic models of human fungal pathogens—465.2 publication/year and 340.3 publications/year, respectively (Fig. 2B). Therefore, more efforts are required to understand the clinical and basic biology of members of the *S. schenckii* complex.

Among the possible explanations for this difference, beside any epidemiological impact, landmark events increased the interest in *Candida* and *Aspergillus* species. The first of them is the development of molecular tools for their genetic manipulation (Durrens *et al.* 1986; Spencer, Spencer and Reynolds 1988), which allowed an exponential publication of papers (Fig. 2B); secondly, the availability of their genomic sequences (Jones *et al.* 2004; Galagan *et al.* 2005) sustained this increased interest (Fig. 2B).

Genomics is the assembly and analysis of the genes and their biological functions encoded within an organism, using bioinformatics, DNA sequencing techniques and recombinant DNA technology (Kotra, Vakulenko and Mobashery 2000). Thus, during the second half of the 1990s, the main aim of some research groups was to sequence the genome of their favorite organism with the aim to reveal fundamental aspects of its evolution, niche adaptation, and in the case of pathogens, the genetic basis of its ability to harm the common host. Although the information we can obtain from a genome sequence is highly valuable and essential for understanding key differences between organisms, such as pathogenic vs. non-pathogenic fungi, this techniques is limited if we compare it with the information generated by other -omics techniques, such as transcriptomics, proteomics and metabolomics (Henry *et al.* 2014). The combination of all these powerful strategies, based on the information generated by genomics, provides a robust and complete functional analysis of the response of an organism to changes in the surrounding environment, including interactions with members of the same species or the host (Ohashi *et al.* 2015). Thus, the generation of genomic information is often comparable with the tip of an iceberg: there is a large amount of information about the genome sequence of an organism, but not enough to understand all its biological processes in detail. We recently published the genome sequences of *S. schenckii sensu stricto* and *S. brasiliensis*, two causative agents of sporotrichosis in both humans and animals (Teixeira *et al.* 2014). This has allowed us to understand some basic aspects of their life style and probably boosted the interest of the community and, thus, the number of reports about these pathogens. However, as mentioned, the genome sequences are just the beginning of a thorough study of *Sporothrix* spp.

The fungal cell wall is often an important topic of study in fungal pathogen research, as the cell wall protects the cell from external insults, displays proteins and molecules associated with host cell damage, and is composed of polysaccharides not synthesized by the human host, allowing the immune system to establish antifungal responses (Netea *et al.* 2008; Lopes-Bezerra 2011; Diaz-Jimenez *et al.* 2012; Martinez-Alvarez *et al.* 2014). Thus far, limited information about the detailed structure, biosynthesis and functional relevance of the cell wall of *S. schenckii* is available. Early works by Travassos and Lloyd (1980) had identified the main glycoconjugates present in the cell wall of *S. schenckii* composed by mannose, rhamnose and galactose: a peptidorhamnomanan (PRM), and a peptidorhamnogalactan. The PRM has been of special interest because of its unusual structure and relevance during the immune sensing of *S. schenckii* cells. The main oligosaccharide found in this glycoconjugate is an O-linked glycan composed of two mannose residues, one glucuronic acid and one or two rhamnose units (Lopes-Alves *et al.* 1992). The PRM of *S. schenckii* has the ability to stimulate the production of circulating antibodies in both laboratory animals and infected patients, but immunosuppressive activities have also been related with this molecule (Carlos, Sgarbi and Placeres 1999). The availability of a *Sporothrix* genome sequence has allowed us to identify candidate genes to

participate not only in the elaboration of PRM but also in the synthesis of different cell wall components (Teixeira *et al*. 2014), an area that has progressed slowly in the last 10 years. For instance, a significant amount of time and effort have been devoted to isolating a full open reading frame from *S. schenckii*, using reverse genetics, and this was translated in a handful of studies published between 2001 and 2015 (Kano *et al*. 2001; Valentin-Berrios *et al*. 2009; Rodriguez-Caban *et al*. 2011; Hernandez-Cervantes *et al*. 2012; Robledo-Ortiz *et al*. 2012; Lopez-Esparza *et al*. 2013; Sanchez-Lopez *et al*. 2015). Now, in the *S. schenckii* genomics era, this type of project has been simplified to a search in a database, accelerating the generation of information aiming to understand the function of *S. schenckii* genes (Lopes-Bezerra *et al*. 2015). Another example of benefits generated by the *Sporothrix* genome sequence is the study of the antigen Gp70. After PRM, Gp70 is the main cell wall component characterized in *S. schenckii* (Lopes-Bezerra 2011), and it has received special attention as a potent antigen (Nascimento and Almeida 2005; Ruiz-Baca *et al*. 2011). Antibodies against Gp70 protect mice from the infection caused by both *S. schenckii* and *S. brasiliensis* (Nascimento *et al*. 2008; de Almeida *et al*. 2015). Early efforts to identify the polypeptide were unsuccessful (Teixeira *et al*. 2009), but recent proteomic and genomics approaches identified the gene encoding Gp70 (Castro *et al*. 2013) and, more importantly, the versatility of this protein in terms of post-translational modifications (Rodrigues *et al*. 2015).

The *Sporothrix* genome lacks obvious polysaccharide lyases and polygalacturonase genes, which offers an attractive hypothesis to explain its inability to establish a pathogenic cycle in a plant host (Teixeira *et al*. 2014). However, no biochemical, proteomic or transcriptomic studies have been conducted to discard alternative, non-classical genes that could be activated upon interaction with plant tissues (Couturier *et al*. 2013; Blackman, Cullerne and Hardham 2014).

Traditionally, rRNA has been used as an endogenous control in gene expression analysis techniques like RT-PCR and northern blotting (Rodriguez-Caban *et al*. 2011; Hou *et al*. 2014). However, rRNA is useless when a technique requires mRNA isolation and enrichment, such as microarray analysis or RNA-sequencing techniques, where housekeeping genes with constitutive and constant expression are used as endogenous expression controls to normalize and validate the data (Mantione *et al*. 2014). The *Sporothrix* genome sequences are allowing us to identify such genes, and we currently have a short list of three candidate genes with constitutive and constant expression that are validated in different growing conditions to use as controls in expression analysis (unpublished results).

The importance of a specific gene in the biology of an organism is usually assessed either by knocking out/down the gene or by its overexpression. Until now, limited attempts of genetic manipulation have been published for *Sporothrix* (Rodriguez-Caban *et al*. 2011), perhaps in part because few gene sequences were available (limiting the number of possible target genes) but also because a reproducible strategy had not been discovered to transform these organisms (Zhang *et al*. 2011). We are currently developing stable transformants that allow the expression of foreign genes, gene silencing and targeted gene disruption (unpublished results). As a proof of concept, we have generated a *S. schenckii* transformant able to express the green fluorescent protein (GPF; Fig. 3). This mutant will allow GFP labeling of specific proteins to localize them within the fungal cell, as in other

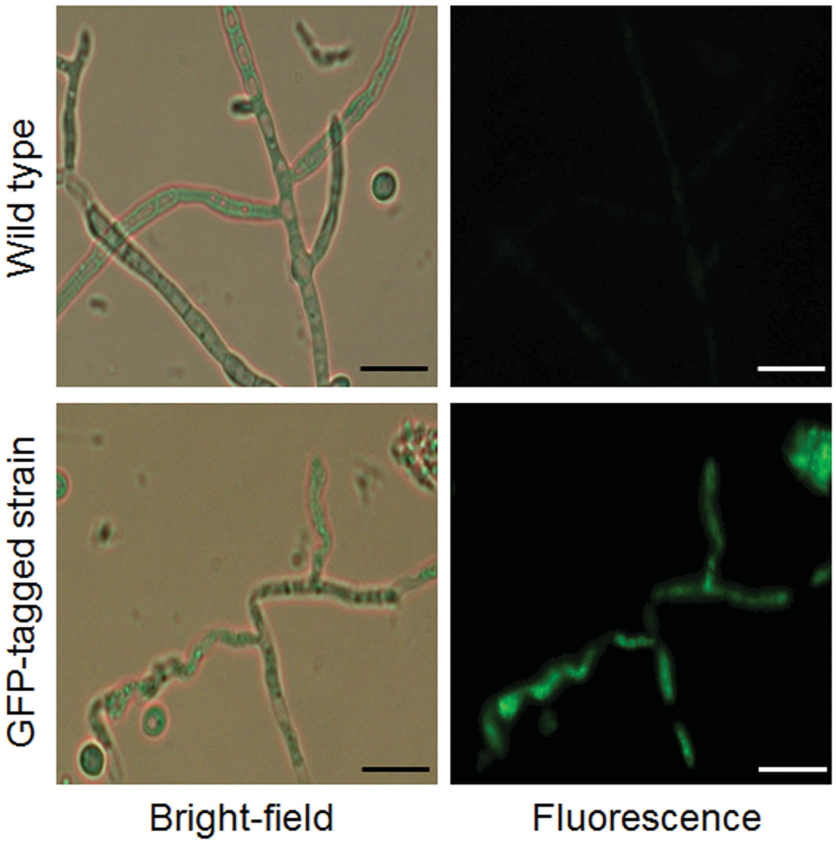

Figure 3. Expression of GFP in *S. schenckii*. Brightfield and fluorescence microscopy of wild-type and GFP-tagged *S. schenckii* strains. Scale bar = 10 μM.

fungal models (Huh et al. 2003), which is particularly relevant for the large numbers of proteins in the genomes of both S. schenckii and S. brasiliensis with unknown functions (Teixeira et al. 2014). In addition, the expression of GFP will allow us to generate bioluminescent fungal cells and track them within host tissues, as recently reported in Candida albicans (Brothers, Newman and Wheeler 2011; Jacobsen et al. 2014), which will provide useful information about the tropisms and dissemination of Sporothrix spp.

DIAGNOSIS, GENETIC VARIABILITY AND DNA-BASED LABORATORY REMARKS

In addition to the classical mycological diagnosis, some authors sought to establish serological methods with different antigens for research purposes and for application in sporotrichosis diagnosis and seroepidemiological surveys, in both humans and cats (Bernardes-Engemann et al. 2005; Almeida-Paes et al. 2007; Fernandes et al. 2011). Serological methods are more accurate for sporotrichosis diagnosis in human clinical specimens (Bernardes-Engemann et al. 2005) from special groups of patients, such as pediatric or HIV-positive patients (Bernardes-Engemann et al. 2015) or those whose infections display diverse clinical presentations such as arthritis (Orofino-Costa et al. 2010) and dacryocystitis (Marques de Macedo et al. 2015).

Molecular research is another important tool for the epidemiological characterization of Sporothrix spp. genetic variability and for describing isolates that differ in their interactions with the host. This research has been carried out by studying variations in the Sporothrix mtDNA sequence (Ishizaki et al. 2009), by sequencing highly conserved regions such as the CAL (calmodulin) (Marimon et al. 2007; Oliveira et al. 2011; Rodrigues et al. 2013) and β-tubulin genes (Liu, Zhang and Zhou 2014) and the ITS genomic regions (Zhou et al. 2013; Estrada-Barcenas et al. 2014; Liu, Zhang and Zhou 2014) and by the RAPD technique (Random Amplified Polymorphic DNA; Reis et al. 2009). All of these molecular approaches can contribute to our understanding of the genetic diversity and geographic distribution of the S. schenckii complex species. Another important outcome from genome-based methods is the capacity to compare the molecular profiles of the fungi isolated from environmental niches and healthy (colonized) animals to those from animal and human lesions. These comparisons can trace Sporothrix spp. sources and dissemination routes and the pathways to infection within different hosts.

By using molecular investigative tools, in 2009, Ishizaki et al. proposed the existence of more than one pathogenic species in the S. schenckii complex, based on significant variations detected in mtDNA among isolates from India, Thailand, Brazil, Colombia, Guatemala and Mexico. In a previous study, Marimon et al. (2007), using other molecular markers of genetic variability such as variations in the CAL gene sequence, phylogenetic data and both physiological and morphological information, demonstrated the existence of three new species that had previously identified as S. schenckii. Thus, isolates of the genus were subdivided into five major clades according to their genetic characteristics and associated morpho-physiological data such as differences in conidia, growth rate and assimilation of carbohydrates. The authors further suggested that the observed clades I, III, IV and V do represent different species of this genus. The first three clades were presented as the new species S. brasiliensis, S. globosa and S. mexicana, respectively. Class V was verified to be S. albicans (formerly Sporothrix pallida). The latest understanding of the S. schenckii complex is that it is composed of six distinct species: S. brasiliensis, S. mexicana, S. globosa, S. schenckii sensu stricto, S. luriei and S. albicans. All these species, except S. albicans, have been described in Brazil and the differences in the species' isolation frequency from various environments, including human hosts, and their diverse pathogenicity have been investigated (Rodrigues, de Hoog and de Camargo 2013). Recently, Rodrigues et al. (2013) investigated the CAL and elongation factor 1 alpha-encoding (EF1α) genes, and determined that S. brasiliensis is the most prevalent species in the Brazilian epidemics and has at least two different geographical origins. The group was able to reach these conclusions by comparing Sporothrix isolates from five Brazilian states, covering a large area of the South and Southeast Brazilian regions. These authors also verified that S. brasiliensis reveals low genetic diversity compared to S. schenckii sensu stricto. On the other hand, S. globosa and S. mexicana identified as human pathogens in a large collection of Brazilian clinical cases (Rodrigues, de Hoog and de Camargo 2013) were not frequently implicated, appearing in a low number that seems to have remained constant. Another useful method for investigating Sporothrix isolates obtained from different Brazilian areas is the evaluation of polymorphisms at the chromosomal level (Sasaki et al. 2014). This method revealed that closely related species show similar genetic organization through PFGE (pulsed field gel electrophoresis), as previously identified by molecular sequencing. Recently, S. schenckii complex genotyping was greatly simplified by the description of PCR-RFLP (restriction fragment length polymorphism), which allows the quick and relatively low-cost determination of medically significant species (Rodrigues, de Hoog and de Camargo 2014). Finally, a major comparative genome study, including transposable elements, the mitochondrial genome and diverse protein families, was designed to phylogenetically investigate the two major pathogenic species, S. brasiliensis and S. schenckii sensu stricto, leading to the suggestion of a recent event of speciation. The authors concluded that for the Sporothrix lineage to evolve from a plant-associated fungus to a mammalian parasite, a unique ecological shift took place (Teixeira et al. 2014).

Despite these advances, whether the existence of different Sporothrix species or genetically diverse isolates within each species influences the sources of contamination, the dynamics of the pathogens' interactions with the host, the magnitude of the clinical presentations and the host response to treatment are questions yet to be answered. Overall, these studies will have a strong impact on the development of efficient measures of sporotrichosis prevention and control.

PERSPECTIVES IN THE STUDY OF VIRULENCE AND THE HOST–PATHOGEN INTERPLAY DURING SPOROTRICHOSIS: WHAT HAVE WE LEARNED SO FAR AND WHAT IS TO COME

In the battle between a fungal pathogen and its host, the molecular mechanisms of the host immune response and how this response affects fungal biology and either fungal survival or death are critical areas of interest to researchers. Recent advances in large-scale molecular techniques have demonstrably improved our understanding of the molecular mechanisms employed by both sides during host–pathogen interactions. A better understanding of the mechanisms used by the host to eliminate the pathogen and win this battle against fungi will lead the way to improved diagnosis and treatment of mycosis.

The host's perception

When studying an infectious disease such as sporotrichosis, identifying which host factors are required to eliminate the pathogen and prevent the establishment of the disease is vital. Studies that focused on understanding the host's immune response to the cutaneous form of sporotrichosis suggested that cell-mediated immunity to *S. schenckii* is the key to controlling the infection (Carlos *et al.* 1992), with participation of macrophages, dendritic cells and mast cells (Uenotsuchi *et al.* 2006; Romo-Lozano, Hernandez-Hernandez and Salinas 2012; Kusuhara, Qian and Li 2014.)

Although less common, reports show that disseminated sporotrichosis also occurs, especially in alcoholics and immunosuppressed patients, strongly suggesting that defects in the cell-mediated immune response make patients more susceptible to the systemic form of the infection (Shiraishi, Nakagaki and Arai 1992; Al-Tawfiq and Wools 1998; López-Romero *et al.* 2011; Trotter *et al.* 2014). The same observation seems to be true in mouse models, because immunocompromised animals are more susceptible to *S. schenckii* than immunocompetent mice (Shiraishi, Nakagaki and Arai 1992). Furthermore, studies using mice with chronic granulomatous disease (CGD), which exhibit defects in the function of NADPH oxidase and the production of superoxide, have shown that CGD mice were unable to eliminate the fungi from the site of inoculation, allowing the fungus to disseminate from the local site of infection to cause fatal systemic mycoses (Kajiwara, Saito and Ohga 2004). These results might also indicate that the production of microbicidal agents, such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), by phagocytic cells plays a key role in eliminating *S. schenckii* (Fernandes *et al.* 2000; Kajiwara, Saito and Ohga 2004). Interestingly, this ROS/RNS-mediated killing also seems to be important for the clearance of other fungal pathogens. For example, studies have shown that macrophages, neutrophils and other phagocytic cells can eliminate *C. albicans* by using oxidative mechanisms, playing a pivotal role in protecting the host against systemic candidiasis (Qian *et al.* 1994; Jensen, Warner and Balish 1994; Fulurija, Ashman and Papadimitriou 1996; Bogdan, Rollinghoff and Diefenbach 2000; Nathan and Shiloh 2000; Vonk *et al.* 2006).

Although several studies have focused on the host's immune response to *Sporothrix* spp. infection, very little is known about the main players and molecular mechanisms involved in innate immune recognition. Host innate immune cells' pattern-recognition receptors (PRRs) can recognize fungal organisms mainly through components of the fungal cell wall, also known as pathogen-associated molecular patterns (PAMPs). Once the phagocytic cells recognize the fungal pathogen, these host components of the immune system can activate an effector response, which consists of the production of ROS/RNS and the further induction of adaptive T helper (Th) responses by antigen-presenting cells (Bourgeois and Kuchler 2012; Grimm *et al.* 2013).

In the case of sporotrichosis, some PRRs have been identified as participants in the recognition/activation process necessary to mount an efficient immune response. Toll-like receptor 2 (TLR2), for example, is a key PRR involved in recognizing *S. schenckii*, because macrophages from TLR2-deficient mice displayed impaired phagocytosis of *S. schenckii* cells and lower levels of proinflammatory cytokines (Negrini *et al.* 2013). Another PRR involved in the recognition/activation process is Toll-like receptor 4 (TLR4), which recognizes components of the *S. schenckii* yeast phase cell wall, resulting in the production of proinflammatory cytokines and effector molecules, such as nitric oxide (NO) (Sassá *et al.* 2009). Consistent with this observation,

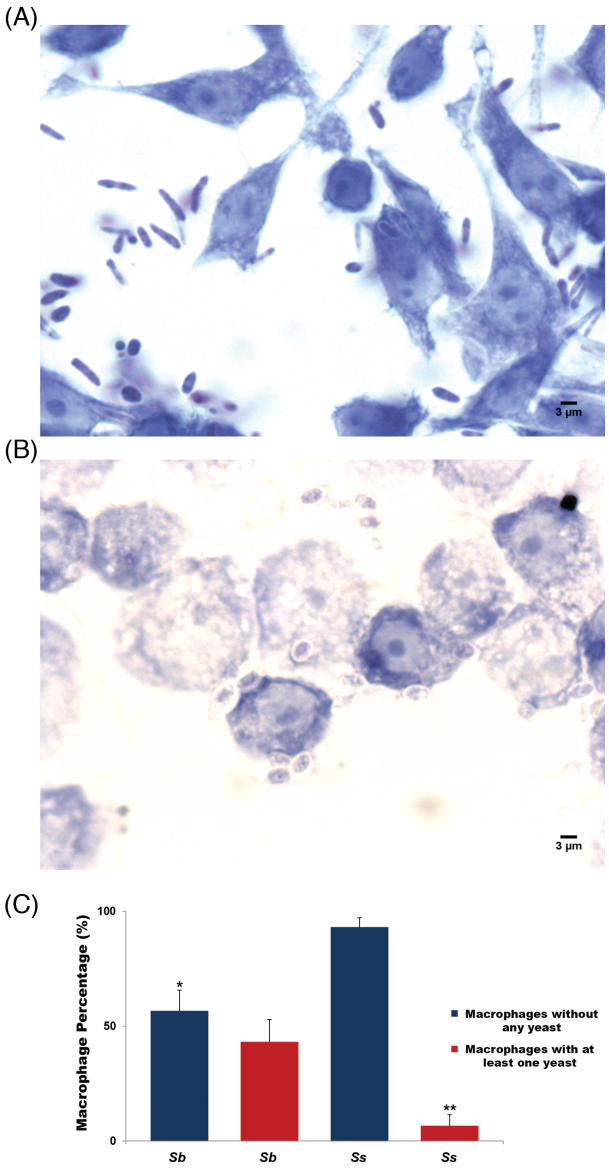

Figure 4. *Sporothrix brasiliensis* is internalized more effectively by J774 macrophages than *S. schenckii*. Micrograph showing J774 cells cocultured with *S. schenckii* (**A**) or *S. brasiliensis* (**B**). (**C**) Graph displaying the percentage of macrophages that have phagocytized *S. schenckii* (*Ss*) or *S. brasiliensis* (*Sb*). $^{*}P < 0.05$ Macrophages without *Sb* yeasts when compared to macrophages without *Ss* yeasts. $^{**}P < 0.05$ Macrophages with *Ss* yeasts when compared to macrophages without *Ss* yeasts.

TLR4-deficient mice infected with *S. schenckii* had difficulty mounting a proinflammatory response (Sassá *et al.* 2012). In addition to TLRs involved in the recognition of *S. schenckii*, mannose receptors also seem to play a role in this proinflammatory response (Guzman-Beltran *et al.* 2012). Interestingly, our recent unpublished results suggest that murine macrophages poorly phagocytize *S. schenckii* compared to *S. brasiliensis* (Fig. 4).

The pathogen's perception

Many intrinsic characteristics of these fungal pathogens allow them to survive inside the host; among them, we can highlight the ability to adhere to host cells, the secretion of extracellular hydrolytic enzymes, morphogenetic switching and robust fungal stress responses.

For instance, fungal pathogens are protected by a highly complex and flexible structure of carbohydrate polymers and proteins (Gow and Hube 2012), which plays a pivotal role in both protecting these pathogens against environmental stresses and mediating the recognition process by immune cells (Levitz 2010). Similar to other pathogenic fungi, *S. schenckii* is mainly immunorecognized by its cell wall. Although the *Sporothrix* spp. cell wall is a rich source of antigens recognized by antibodies raised against this organism, the specific contribution of its components during fungal sensing by immune cells is not known, and PAMPs have not been identified (Carlos, Sgarbi and Placeres 1999; Lima *et al.* 2001, 2004; Carlos *et al.* 2003; Nascimento and Almeida 2005; Lopes-Bezerra 2011; Ruiz-Baca *et al.* 2011).

Among the components of the *S. schenckii* cell wall that are recognized by the host's immune system, we can highlight the cell wall PRM which is recognized by IgG antibodies present in patient sera (Lloyd and Bitoon 1971; Penha and Lopes-Bezerra 2000) and also by receptors and matrix proteins of host cells (Lima *et al.* 2001; Figueiredo *et al.* 2004). Another cell wall component that has been thoroughly studied is the 70 kDa antigen, Gp70 (Lima and Lopes-Bezerra 1997; Nascimento and Almeida 2005; Nascimento *et al.* 2008), which seems to act also as an adhesin that mediates fungus adhesion to host tissues (Ruiz-Baca *et al.* 2009; Teixeira *et al.* 2009). Interestingly, a recent study suggested that the cell surface Gp70 antigen is directly associated with the pathogenicity of *Sporothrix* spp., because there was a correlation between higher levels of Gp70 expression on the fungal surface and reduced infectivity (Castro *et al.* 2013). Substantial work has been performed to characterize the *Sporothrix* spp. cell wall; however, its structure and biochemical composition in the pathogenic species of the *S. schenckii* complex (Marimon *et al.* 2007) remain unknown.

As discussed above, cells of the host immune system use ROS as a microbicide to eliminate fungal pathogens (Chauhan and Latge 2006; Brown, Haynes and Quinn 2009); hence, we can hypothesize that the *Sporothrix* species ability to survive host-generated ROS plays an important role in this organism's pathogenicity. Antioxidants such as melanin and catalase contribute to the prevention of cell death following peroxide treatment (Romero-Martinez *et al.* 2000; Wang *et al.* 2008), and a recent study has shown that *S. brasiliensis* is more resistant to peroxide stress than *S. schenckii sensu stricto* (Ortega *et al.* 2015), suggesting that oxidative stress responses allow *Sporothrix* spp. to survive in environments where this fungal pathogen is exposed to ROS, such as inside the phagosome of a phagocytic cell.

The recent sequencing of *S. schenckii sensu stricto* and *S. brasiliensis* genomes allows *in silico* analyses that can identify differences and similarities between proteins identified as virulence determinants in other pathogenic organisms and *Sporothrix* spp. (Teixeira *et al.* 2014; Ortega *et al.* 2015). For example, bioinformatics analyses have generated the hypothesis that the *Sporothrix* spp. AP1-like transcription factor, which is a key modulator of oxidative stress, is more similar to that of fungal plant pathogens such as Magnaporth*e oryzae*, *Ophiostoma piceae* and *Grosmannia clavigera* than to that of the fungal pathogen *C. albicans* (Ortega *et al.* 2015). Once this type of analysis is made, new hypotheses can then be proposed and tested.

CONCLUSIONS AND PERSPECTIVES ON THE HOST-*SPOROTHRIX* SPP. STUDIES

Even though significant progress has been made in the study of the immune response during the course of sporotrichosis, much remains to be discovered. Significant insight into the exact nature of the various microenvironments that this fungal pathogen occupies in mammalian hosts can be provided by high-throughput technologies that enable the quantification of fluctuations in the abundance of RNA transcripts (transcriptomic), proteins (proteomic) and other biomolecular components (metabolomic) (Fradin *et al.* 2003; Tierney *et al.* 2012; Muszkieta *et al.* 2013; Reales-Calderon *et al.* 2014). These technologies will aid in painting 'the big picture' of the battle happening between *Sporothrix* spp. and its hosts and in the identification of potential targets that regulate key responses that will give the advantage to either host or pathogen.

In addition, as discussed above, effective molecular tools for the study of *Sporothrix* spp., such as genetic manipulation, are still limited; the development of new or improved techniques will be required in order to study the hypotheses that will arise from high-throughput studies. In this context, the recent sequencing of the *Sporothrix* spp. genome is an important component in developing such tools (Teixeira *et al.* 2014).

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