Virulence 4:3, 203–206; April 1, 2013; © 2013 Landes Bioscience

What lies beyond genetic diversity in *Sporothrix schenckii* species complex?

New insights into virulence profiles, immunogenicity and protein secretion in *S. schenckii sensu stricto* isolates

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Keywords: *Sporothrix schenckii*, sporotrichosis, *Sporothrix* complex, phylogenetics, virulence, immunogenicity, exoantigens

In the last few years there has been an increasing interest in studying the biology of the dimorphic fungus *Sporothrix schenckii,* and particular attention has been focused on its molecular phylogeny which has undoubtedly improved our knowledge of the taxonomy, and most importantly, of the epidemiology of this pathogenic fungus.

Currently, *S. schenckii* is recognized as a cryptic species complex that includes six phylogenetically closely related species, most of which are capable of causing serious life-threatening infections in humans and other animals.

For decades, researchers around the world have reported differences in virulence among *S. schenckii* isolates but in light of recent taxonomic changes that have affected this species, the question of whether the observed striking variability is really due to different isolates of the same species or whether it is attributable to different species of the cryptic complex remains speculative and unresolved. A compelling response to this question comes from a recent research, published in this issue of *Virulence*, which reports new insights into virulence profiles, immunogenicity and protein secretion in *S. schenckii* sensu stricto isolates.

The dimorphic fungi comprise a group of important human pathogens and represent a family of seven phylogenetically related ascomycetes that include *Blastomyces dermatitidis*, *Coccidioides immitis*, *Coccidioides posadasii*, *Histoplasma capsulatum*, *Paracoccidioides brasiliensis*, *Penicillium marneffei* and *Sporothrix schenckii*.[1] These primary pathogens possess the unique ability to switch between two different morphologies (mold and yeast) in response to thermal stimuli. In the environment, they grow as mold that produces conidia or infectious spores which, when transmitted to humans or other susceptible mammalian hosts, are capable to convert into pathogenic yeasts causing serious life-threatening infections.[1]

Over the last few years, *S. schenckii* has received particular attention due the increased number of infections caused by it worldwide. Taxonomically, this species belongs to the phylum Ascomycota, order Ophiostomatales, family Ophiostomataceae and it has largely been known as the etiological agent of sporotrichosis, a cutaneous, subcutaneous or a systemic mycosis that affects humans and other mammals.[2]

Despite the existence of the fungus worldwide, infections due to *S. schenckii* are more common in certain geographical areas such as tropical and subtropical regions.[2,3] Many South American countries (especially Brazil, Peru, Colombia and Uruguay) including Mexico, South Africa, India and Japan are considered endemic regions for sporotrichosis.[4] In addition, a particular geographical area called Abancay, in the south central Peruvian highlands, showed a relatively high prevalence of this disease and is considered to be a hyperendemic area with an estimated incidence of approximately 50–60 cases per 100,000 inhabitants per year.[4] However, although now it is considered a rare or sporadic mycosis, sporotrichosis was also a common disease in France in 1900;[2] it declined after two decades and today intermittently appears in some European countries. In 2009, an autochthonous case was reported in France,[5] and several others have been described in Italy,[6-8] Spain,[9] Portugal,[10] United Kingdom[11] and Turkey.[12] Such data indicate that sporotrichosis is more widespread in European countries than was previously thought and *S. schenckii* could represent a re-emerging pathogen in the future.

As the fungus is abundant in soil, wood and moss, most infections occur following minor skin trauma in people with occupations or hobbies involving the outdoors such as gardening, farming, hunting or other activities that entail contact with vegetation. Similarly, inoculation may also occurs after motor vehicle accidents and in laboratory personnel handling Sporothrix-infected specimens.[2,13]

Despite the increased incidence of sporotrichosis observed in the last few years and the worldwide distribution of the fungus,[2,14,15] the study of this microrganism is still in its infancy and very little is known about its basic biology including genetics, virulence, pathogenicity and immune modulation of the host. However, a particular attention has recently been focused

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Submitted: 12/28/12; Accepted: 01/02/13
http://dx.doi.org/10.4161/viru.23467
Comment on: Fernandes GF, et al. Virulence 2013; 4:241-9; PMID: 23324498; http://dx.doi.org/10.4161/viru.23112

on the study of its biology at molecular level and a special emphasis has been given to molecular phylogeny which has significantly improved our knowledge of the its taxonomy and biodiversity. Results gained from these studies have confirmed the initial assumption that more species could be present within *S. schenckii* population.[16] In fact currently, *S. schenckii* is recognized as a cryptic species complex including *Sporothrix brasiliensis*, *Sporothrix globosa*, *Sporothrix mexicana*, *Sporothrix luriei*, *Sporothrix pallida* (formerly *Sporothrix albicans*) and *S. schenckii sensu stricto*.[11,17,18] All these species share many genetic and phenotypic characteristics with *S. schenckii* and they are, therefore, easily misidentified as such by conventional methods of identification. This limits our understanding of their epidemiology and consequently their clinical significance is still little known. However, except for *S. pallida*, the other *Sporothrix* species have been reported to cause sporotrichosis in humans and other animals.[2,10,11,19,20] In particular it has been recently reported that *S. mexicana* and *S. globosa* were present in clinical collections since 1955.[15] In addition, the few data published so far have clearly shown that the geographic distribution of these fungi as well as their virulence, pathogenicity and trends in antifungal susceptibilities are variable[11,21,22] and some species, such as *S. brasiliensis* and *S. mexicana,* appear to be almost exclusively restricted to certain geographic areas.[11,15]

In past years, using mammalian animal models, several research groups have studied the effect of infection caused by *S. schenckii* without taking into account the occurrence of its phylogenetically closely related relatives with reduced or absent pathogenicity.[23-28] Such studies have demonstrated variation in virulence levels among different *S. schenckii* isolates although the lack of proper species identification could have led to erroneous conclusions regarding the pathogenicity of this species.[21,28] In fact more recent studies have reported that not all the members of *S. schenckii* complex are equally likely to cause infections in mice and some of them, especially clade I strains, today known as *S. brasiliensis*,[11] are particularly virulent, spread rapidly to other organs such as liver, kidneys, and brain and cause more severe infections than other *Sporothrix* species.[21] Therefore it is not yet well-known whether the striking variability in virulence, previously observed for *S. schenckii*, is really due to different isolates of the same species or whether it is attributable to different species of the cryptic complex. However, this question was initially addressed in 2009 when different levels of virulence were reported among the diverse species of the complex.[21] In that comparative study, *S. brasiliensis* was the most virulent and pathogenic species followed by *S. schenckii* sensu stricto, *S. globosa*, *S. mexicana* and avirulent species *S. pallida*.[21] Subsequently was evaluated the virulence of *S. luriei* in a murine model of disseminated infection and even though only one strain was tested, the obtained results indicated that the level of virulence displayed by this species is comparable to that of *S. brasiliensis* and or *S. schenckii*.[29] However, a comprehensive comparative study on virulence, using a number of *S. schenckii* sensu stricto isolates, has never been performed before and therefore, in light of recent taxonomic changes that have affected this species, the question of whether different isolates of *S. schenckii* sensu stricto have similar levels of virulence, or not, remains purely speculative.

Putting together all these observations it is easy to capture the originality of the study of Fernandes et al.[30] published in this issue of *Virulence*. This study, performed using a number of *S. schenckii* sensu stricto isolates including *S. brasiliensis* and *S. globosa*, reports new data on virulence of these *Sporothrix* species and also evaluates protein secretion and immunogenicity of these pathogenic fungi.

One important aspect of this study is that the fungal strains used were initially identified using phenotypic and molecular methods according to recent studies.[11,30] Interestingly, based on partial sequencing of the calmodulin-encoding gene, Fernandes et al.[30] described one intriguing isolate that although it was identified as *S. schenckii,* it was also unable to assimilate sucrose, a disaccharide that is, typically, not assimilated by *S. brasiliensis* and *S. luriei* and it is, therefore, often used as phenotypic marker to presumptively differentiate these fungi from all other sucrose-positive species of the cryptic complex.[11,18] This indicates that phenotypic methods should be used with caution in the identification of the *Sporothrix* species and that molecular methods should be always employed.

One other remarkable aspect of the work of Fernandes and colleagues[30] is that their results confirm the existence of different virulence profiles in *S. schenckii* sensu stricto isolates. In particular, based on survival times of infected BALB/c mice and fungal tissue burden, the authors observed a great deal of heterogeneity of virulence which allowed them to classify the fungal isolates in four discrete groups: highly, medium-, low- and non-virulent.[30] Nevertheless, although two *S. schenckii* isolates were considered as "highly virulent," they were less virulent than *S. brasiliensis* used as control thereby reinforcing the initial assumption that this latter species is the most pathogenic among all those belonging to the cryptic complex.[21] However, according to previous studies of Fernandes et al.[31] the authors also found that one of the "highly virulent" *S. schenckii* isolates shows a number of secreted enzymes (proteinase, caseinase, gelatinase, DNase and urease) most of which were not observed in the hypervirulent species *S. brasiliensis*.[30] This indicates that the mechanisms promoting pathogenesis are much more complex, perhaps not conserved among closely related *Sporothrix* species and probably involve different virulence factors to evade the host immune system.

The immunological mechanisms implicated in the prevention and control of sporotrichosis are still not fully understood, but it known that they include both cellular and humoral immune responses.[2,32,33] Studies in mice suggested that the cellular response appear to be triggered by surface cell antigens, especially some lipids, that inhibit the phagocytosis process and induce high liberation of tumor necrosis factor-α (TNF-α) and nitric oxide (NO) in macrophage cultures.[34] Conversely, the humoral response is induced by secreted proteins, the so-called exoantigens, which are not involved in cellular response.[2]

One of the major exoantigens detected in the cell wall of both morphologies of *S. schenckii* is a 70-kDa glycoprotein (gp70)

which also functions as an adhesin by mediating the interaction of the fungus with the extracellular matrix protein fibronectin and host tissues.[14,35] In the study of Fernandes et al.[30] the protein profiles of exoantigens obtained from *S. schenckii, S. brasiliensis,* and *S. globosa* isolates were rather heterogeneous with two principal molecules (60 and 46 kDa) secreted by all examined isolates. However in spite of being commonly produced these antigens were not always recognized by respective antisera obtained from infected mice. In particular the 60-kDa antigen was immunogenic only with antisera obtained using virulent isolates of *S. schenckii*, including *S. brasiliensis*, whereas it was not recognized by antisera from non-virulent isolates and *S. globosa*.[30] In addition, one *S. schenckii*, classified as "medium virulent" isolate, secreted the 60-kDa molecule but the antigen was not recognized by the corresponding antiserum indicating that most likely the antigenicity of this molecule varies in relation to the post-translational modifications which can produce isoforms that do not elicit a humoral immune response. On the other hand one other hypothesis that may explain the different behavior of the 60-kDa protein might be related to its expression in different cell types (mold and yeast) or to experimental methods used for producing the antigen and antiserum respectively. In fact a recent study, using 2D-immunoblotting with anti-*S. schenckii* antibodies, revealed that a 60-kDa antigenic glycoprotein was present only on the surface of yeast-like cells[36] and not in mycelial cells as reported instead by Fernandes et al.[30] Anyhow, being the immunodominant molecule in the *S. schenckii* complex, this protein represents a good candidate for further studies on the immunogenicity and pathogenesis of these species.

S. schenckii have always attracted considerable attention because of its intrinsic pathogenic nature, but, in recent years, even more because sporotrichosis has become a significant public global health problem.[14] Today we know that this disease is also caused by other recently described *Sporothrix* species but the study of their basic biology including virulence and pathogenicity is still too limited.

In our opinion, according to the great deal of data reported by Fernandes et al.[30] and the advent of high-throughput next generation sequencing technologies, the genome of *S. schenckii* and its closest relatives should be sequenced in the coming years. In fact it is singular that among seven clinically relevant dimorphic fungi only *S. schenckii* has not yet sequenced. Comparative genomics and transcriptomics analysis between highly pathogenic *Sporothrix* species and species with reduced or absent pathogenicity, certainly, will encourage the molecular study of virulence factors involved in pathogenesis and will accelerate the discovery of new proteins for diagnostics, drug targets and vaccines.

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