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Molecular identification of the *Sporothrix schenckii* complex



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ABSTRACT

Sporothrix schenckii, an ascomycetous dimorphic organism that for over a century was recognized as the sole agent of sporotrichosis, a subcutaneous mycosis with a worldwide distribution. However, it has been proposed, based on physiologic and molecular aspects, that *S. schenckii* is a complex of distinct species: *Sporothrix brasiliensis*, *Sporothrix mexicana*, *Sporothrix globosa*, *S. schenckii sensu stricto*, *Sporothrix luriei*, and *Sporothrix albicans* (formerly *Sporothrix pallida*). Human disease has a broad range of clinical manifestations and can be classified into fixed cutaneous, lymphocutaneous, disseminated cutaneous, and extracutaneous sporotrichosis. The gold standard for the diagnosis of sporotrichosis is the culture; however, serologic, histopathologic and molecular approaches have been recently adopted for the diagnosis of this mycosis. Few molecular methods have been applied to the diagnosis of sporotrichosis to detect *S. schenckii* DNA from clinical specimens, and to identify *Sporothrix* spp. in culture. Until now, *Sporothrix* is the unique clinically relevant dimorphic fungus without an elucidated genome sequence, thus limiting molecular knowledge about the cryptic species of this complex, and the sexual form of all *S. schenckii* complex species. In this review we shall focus on the current diagnosis of the sporotrichosis, and discuss the current molecular tools applied to the diagnosis and identification of the *Sporothrix* complex species.

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Identificación molecular del complejo *Sporothrix schenckii*

RESUMEN

Sporothrix schenckii, un organismo ascomiceto dimorfo que durante más de un siglo fue reconocido como el único agente de esporotricosis, una micosis subcutánea con una amplia distribución mundial. Sin embargo, se ha propuesto, con base en los aspectos fisiológicos y moleculares, que *S. schenckii* es un complejo de especies distintas: *Sporothrix brasiliensis*, *Sporothrix mexicana*, *Sporothrix globosa*, *S. schenckii sensu stricto*, *Sporothrix luriei* y *Sporothrix albicans* (antes *Sporothrix pallida*). La enfermedad humana tiene una gama de manifestaciones clínicas y puede clasificar e cutánea fija, linfocutánea, cutánea diseminada y esporotricosis extracutánea. El estándar de oro para el diagnóstico de esporotricosis es el cultivo del patógeno, sin embargo, los métodos serológicos, histopatológicos y, recientemente, los moleculares se han usado para el diagnóstico de esta micosis. Pocos métodos moleculares han sido aplicados para el diagnóstico de la esporotricosis para detectar ADN de *S. schenckii* a partir de muestras clínicas y para identificar *Sporothrix* spp. en cultivo. Hasta ahora, *Sporothrix* es el único hongo dimorfo clínicamente relevante sin una secuencia genómica elucidada, limitando el conocimiento molecular de las especies crípticas de este complejo y la forma sexual de todas las especies del complejo *S. schenckii*. Esta revisión se centrará en el diagnóstico actual de la esporotricosis con énfasis en las herramientas moleculares vigentes aplicadas tanto al diagnóstico como en la identificación de las especies del complejo *Sporothrix*. Este artículo científico es parte de la serie de trabajos presentados en el «V International Workshop: Molecular genetic approaches to the study of human pathogenic fungi» (Oaxaca, México, 2012).

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Sporothrix schenckii is an ascomycetous dimorphic organism (Ascomycota, Pyrenomycetes, Ophiostomatales, Ophiostomataceae) that is found in substrates like living and decayed vegetation, animal excreta, and soil.^{5,19} This fungus is phenotypically characterized by the ability to produce sessile dematiaceous conidia along with hyaline sympodial conidia in its filamentous form, and cigar-shaped yeast-like cells in parasitism or when cultured at 35–37 °C on specific culture media.^{5,37}

For over a century, this species was recognized as the sole agent of sporotrichosis, a subcutaneous mycosis with a worldwide distribution.³⁴ However, it has been proposed, based on physiologic and molecular aspects that *S. schenckii* is a complex of four distinct species: *Sporothrix brasiliensis*, *Sporothrix mexicana*, *Sporothrix globosa*, and *S. schenckii sensu strictu*.³⁷ More recently, *S. schenckii* var. *luriei* was considered to be another species belonging to the *S. schenckii* complex, and was therefore named *Sporothrix luriei*.³⁸ Additionally, in other phylogenetic analysis from *Sporothrix albicans*, *Sporothrix pallida*, and *Sporothrix nivea* revealed a significant similarity among them. Therefore, it has been proposed that all these three non-pathogenic species closely related to *S. schenckii* were called *S. pallida*.^{12,54} So, *S. schenckii sensu strictu*, *S. brasiliensis*, *S. globosa*, *S. mexicana*, and *S. luriei* are now recognized as agents of sporotrichosis.

Sporotrichosis affects humans and other mammals, such as dogs, cats, rats, armadillos, and horses.^{5,34} Human disease has a broad range of clinical manifestations and can be classified into fixed cutaneous, lymphocutaneous, disseminated cutaneous, and extracutaneous sporotrichosis.⁵ Another clinical type of this infection is the primary pulmonary sporotrichosis, which results from inhalation of conidia.⁴⁹ Lymphocutaneous sporotrichosis is the most common manifestation and is characterized by lesions in the site of injury and along the lymph vessels path. These lesions can be papular, nodular, gummy (ulcerated or not), and/or vegetative plaque. In fixed cutaneous sporotrichosis, the lesion is restricted to the injury site, with no lymphatic involvement. Disseminated cutaneous form consists in several lesions in the body after hematogenous dissemination or multiple inoculation of the fungus into the skin. In extracutaneous disease, several organs or tissues can be affected, such as bones, lungs, eyes, and central nervous system.⁶² Along with these manifestations, other clinical aspects have been recently reported such as erythema nodosum,²¹ erythema multiforme,²⁰ and Sweet's syndrome.¹⁶

Definitive diagnosis of sporotrichosis is based on fungal detection in culture.⁶² Microscopic methods for yeast detection in clinical samples are of low sensitivity⁵ and asteroid body observation varies between different works.^{17,50} Cultivation of clinical specimens in mycological media such as Sabouraud dextrose agar or mycobiotic agar yields white filamentous colonies that become brown to black after a few days. Subculturing these colonies in brain heart infusion at 35–37 °C results in white to creamy yeast-like colonies.^{5,62} *S. schenckii* identification is based on its macro- and micromorphologies of the mycelial and yeast forms. These characteristics, however, do not differentiate the new described species on the *Sporothrix* complex. In order to physiologically differentiate the species within this complex, other tests such as carbohydrate assimilation (especially sucrose and raffinose), growth rates at 30 °C and 37 °C as well as production of dematiaceous conidia are necessary.^{37,38} However, discrepancies between physiological and molecular methods of identification have been described.⁴⁷

Several techniques have been described for the establishment of immunological diagnosis of sporotrichosis based on antibody detection in sera from infected patients. Precipitation and agglutination methods were first employed in the diagnosis¹ and more recently immunoenzymatic assays are used in sporotrichosis serodiagnosis.^{2,8} However, there is no immunological method described up to now that permits differentiation of the species

of the *Sporothrix* complex. Recent publications showed that the species of the *S. schenckii* complex differ in antifungal susceptibility and virulence.^{4,22,40} These publications have suggested that even lesion mechanisms could be related to these different species. Therefore, molecular methods which allow rapid identification and differentiation of these close-related fungi are very necessary to the mycology laboratory.

Molecular methods for sporotrichosis

PCR diagnosis based on the amplification of the fungal gene sequences is a powerful tool for identifying invasive mycoses. The first PCR for identification of *S. schenckii sensu lato* was described by Kano et al.²⁹ Specific oligonucleotide primers based on the chitin synthase 1 gene were designed, and PCR was able to detect a 10 pg genomic DNA fragment of *S. schenckii*. After, this methodology was employed for the molecular diagnosis of sporotrichosis.²⁸ A nested PCR assay for the detection of *S. schenckii* was evaluated in clinical samples using the 18S rRNA gene sequence as the target. The nested PCR could detect *S. schenckii* DNA in tissue samples from infected animals or from clinical specimens from patients with sporotrichosis confirmed by culture or histochemical staining. The test showed high sensitivity and specificity, indicating that the assay could provide rapid diagnosis with sufficient accuracy to be clinically useful for patients with sporotrichosis.²⁵ More recently, the same assay was used to detect *S. schenckii* DNA from 38 strains (including all 24 mitochondrial DNA types) collected from different areas of the world, and of tissues of experimentally infected mice, skin biopsies of nine patients with sporotrichosis, two strains of *Ceratomyces minor*, and 10 different species of other pathogenic fungi.⁶¹ The authors demonstrated that nested PCR could correctly identify all *S. schenckii* strains and clinical samples, corroborating the data obtained by Hu et al.²⁵ that the nested PCR assay is highly sensitive and specific and is a rapid method for diagnosis of sporotrichosis under contamination free conditions.⁶¹ Other studies also reported PCR methods applied in the diagnosis of sporotrichosis. Mendoza et al.⁴¹ showed an evolution in the previously described nested PCR.²⁵ However, this study demonstrated that this molecular methodology presented lower efficacy than conventional diagnostic methods, such as the gold standard culture. Liu et al.³³ reported a PCR in biopsy tissue using the primer S2-R2 that showed positive bands in twenty-five out of the thirty cases (83.3%). These primers were also successfully used for the diagnosis of feline sporotrichosis, with no cross-reactions with normal cat skin cells or other dimorphic fungi.³⁰ These studies did not permit classification at species level of the *Sporothrix* complex.

Molecular identification of *Sporothrix* species

In the last few years, the development of DNA-based methods to identify fungal isolates has led to a decrease in the time-consuming step of morphological identification, while maintaining or improving the specificity, accuracy and sensitivity. Until now, few molecular methods have been applied in the detection of *S. schenckii* DNA from clinical specimens, and in the identification of *Sporothrix* spp. in culture (Table 1). Diagnosis using PCR is based on the amplification of fungal gene sequences and is a powerful tool for identifying mycoses. One of the pioneering DNA-based methodologies used for the diagnosis of fungal infections was reported by Sandhu et al.⁵⁵ who developed 21 specific nucleic acid probes targeting the large subunit of the rRNA gene from several fungi, including *S. schenckii*. The results show a high level of specificity.⁵⁵ But previous studies^{56,57} using mitochondrial DNA analysis have reported heterogeneity profiles in *S. schenckii* strains. In fact, several molecular taxonomic studies using different methodologies,

Table 1
Molecular studies on the *Sporothrix schenckii* complex.

Method	Target gene	Species differentiation	Reference
RFLP ^a	mt DNA ^e	No	3,26,31,32,43,56,57
PCR sequencing	28S rDNA	No	55
PCR ^b	CHS ^f	No	28,33
RAPD ^c	10mer sequences	No	42
PCR sequencing	ITS ^g	No	6
Nested PCR	18SrRNA	No	25
PCR	Topoisomerase II	No	27
RFLP	ITS, 5.8S rDNA	No	60
AFLP ^d	ITS	No	44
RFLP	ITS, 18S	No	64
PCR sequencing	CAL ^h ; β tub ⁱ ; CHS	No	39
PCR sequencing	CAL; β tub; CHS	Yes	37
PCR fingerprinting and PCR sequencing	M13, ITS	No	22
PCR sequencing	ITS, LSU ^j , β tub	Yes	12
PCR sequencing	CAL	Yes	35,47,54
PCR fingerprinting	M13	No	51
Nested PCR	18SrRNA	No	41,61
PCR sequencing	ITS, β tub, CHS	Yes	63
PCR fingerprinting	T3B	Yes	48

^a Restriction fragment length polymorphism.

^b polymerase chain reaction.

^c random amplification of polymorphic DNA.

^d amplified fragment length polymorphism.

^e mitochondrial DNA.

^f chitin synthase.

^g internal transcriber space.

^h calmodulin.

ⁱ β -tubulin.

^j large subunit.

such as restriction fragment length polymorphism (RFLP) from different gene targets, random amplified polymorphic DNA (RAPD), DNA sequencing of internal transcriber spacer (ITS) regions of the ribosomal RNA gene (rRNA), PCR targeting the DNA topoisomerase II gene, amplified fragment length polymorphism (AFLP), and M13 PCR fingerprinting have demonstrated that *S. schenckii* isolates present different genetic characteristics, suggesting that they do not belong to the same species.^{3,26,27,32,39,43,44,56,57,60,64}

Hence, Marimon et al.³⁷ supported these findings describing through a combination of phenotypic and genetic features four *S. schenckii*-related species: *S. brasiliensis*, *S. globosa*, *S. luriei*, and *S. mexicana*. *S. globosa* has a worldwide distribution,^{11,35,46} whereas *S. mexicana* is restricted to Mexico and *S. brasiliensis* to Brazil.³⁷ However, a case of human sporotrichosis due to *S. mexicana* was reported in 2011 by Dias et al. in Portugal.¹³ The molecular tool proposed to differentiate these closely related species was partial calmodulin (CAL) gene sequencing.³⁷ Therefore, an identification key for the *S. schenckii* complex that includes morphologic analysis of conidia, auxonogram, genotyping via PCR amplification, and sequencing of the CAL gene. Based on this last analysis, Romeo et al.,⁵⁴ who were studying the molecular phylogeny and epidemiology of *Sporothrix* species isolated in Italy, demonstrated that 26 environmental strains co-clustered with *S. albicans*, and two clinical isolates grouped with *S. schenckii sensu stricto*.

Additionally, phylogenetic analysis based on the rDNA and the β -tubulin regions from *S. albicans*, *S. pallida* and *S. nivea* revealed a significant similarity among them. Therefore, it has been proposed that all the three species were called *S. pallida*. Moreover, other three environmental *Sporothrix* species were described using this molecular approach: *Sporothrix stylites*, *Sporothrix humicola* and *Sporothrix lignivora*. The two first species differ from *S. schenckii* by production of only hyaline conidia and consequent non darkening of colonies with age. *S. lignivora* has distinctive conidia that do not match in size and shape with other *Sporothrix* or *Ophiostoma* species. Isolates classified as *S. humicola* in this study were referred before as environmental isolates of *S. schenckii*. In their study,

the authors conclude that β -tubulin sequence analysis is strongly recommended in taxonomic studies from *Sporothrix* species isolated from the environment.¹² In fact, β -tubulin analysis, together with ITS sequencing enable the further description of other two environmental *Sporothrix* species: *Sporothrix brunneoviolacea* and *Sporothrix dimorphospora*.³⁶

After the description of *Sporothrix* complex, one important question is the search for rapid methods for species identification and typing. Oliveira et al.⁴⁸ reported a PCR fingerprinting using the universal primer T3B to distinguish among species of the *Sporothrix* complex. The T3B fingerprinting generated clearly distinct banding patterns, allowing the correct identification of all 35 clinical isolates at the species level, which was confirmed by partial CAL gene sequence analyses. Overall, there was a 100% agreement between the species identification using both genotypic methodologies. These profiles were also able to accurately distinguish the species misidentified by phenotypic analysis. The proposed identification technique is simple, reliable, more rapid, less expensive, and requires less technical expertise than sequencing. The computer scanned PCR profiles generated can form the basis of a computer database, which can be used for future identifications of atypical or unidentifiable *Sporothrix* isolates. This methodology is supposed to be an ideal routine identification system for clinical mycology laboratories, particularly those with limited facilities or technical expertise. There are only a few molecular tools for *Sporothrix* typing,^{31,51} and the development of new typing methods are necessary.

Other molecular aspects of *Sporothrix*

The sexual form of all *S. schenckii* complex species is unknown nowadays; however, there are strong molecular evidences that this fungus undergoes recombination in nature.⁴² Molecular analyses from 18S region of ribosomal DNA have shown indirect evidence that the sexual form of *S. schenckii* was *Ophiostoma stenoceras*.⁷ On the other hand, morphological and physiological studies

show consistent differences between these two species.^{14,45} These observations lead to consider as different species the *O. stenoceras* anamorph and *S. schenckii*. Meanwhile, other molecular studies^{6,12,24} reinforce that the *S. schenckii* teleomorph belongs to the genus *Ophiostoma*, but it is different from *O. stenoceras*.

Molecular analysis of *S. schenckii* can also be linked with fungal virulence. A study about the ribosomal conserved region (i.e., ITS) and the nontranscribed spacer (NTS) demonstrated that a *S. schenckii sensu strictu* strain from disseminated cutaneous sporotrichosis presents a 10 bp deletion in the ribosomal NTS region, when compared to isolates controls obtained from patients with fixed cutaneous sporotrichosis.⁶³ Nucleotide polymorphisms are also able to separate environmental and clinical *S. schenckii* strains. Two single-base transitions in the D1–D2 domain of rDNA differentiate strains from these groups.¹⁰

Rapid amplification of cDNA ends technique was recently applied to characterize the gene sequence of a physiology-related protein in *S. schenckii*. A progesterone adiponectinQ receptor was found to be responsible for an inhibition of, especially, the yeast form of the fungus.¹⁸ However, it is necessary a large investigation about this inhibition, since there are several cases of sporotrichosis in woman, pregnant or not,^{5,9,15} particularly in the endemic area of sporotrichosis where *S. brasiliensis* predominates.⁴⁷

S. schenckii is the only clinically relevant dimorphic fungus without an elucidated genome sequence, thus limiting molecular knowledge about the cryptic species of this complex.⁵³ However, some genes have been recently described, such as the α -subunit of the endoplasmic reticulum glucosidase II,⁵² the α 1,2-mannosyltransferase,²³ a cytosolic phospholipase A2,⁵⁸ and a calcium/calmodulin kinase gene.⁵⁹ These sequences are potential targets for the development of new identification and typing methods to be applied within the *Sporothrix* complex species.

Conclusion

Nevertheless, nucleotide probes, specific for the *Sporothrix* species complex, and DNA amplification procedures, such as PCR, allow more rapid and precise diagnosis, which can lead to earlier treatment. The gold standard in diagnosis continues to be using the culture with the correlation of phenotypic characteristics and molecular data.

Conflict of interest

The authors declare no conflict of interest.

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References

- Albornoz MB, Villanueva E, Torres ED. Application of immunoprecipitation techniques to the diagnosis of cutaneous and extracutaneous forms of sporotrichosis. *Mycopathologia*. 1984;85:177–83.
- Almeida-Paes R, Pimenta MA, Monteiro PCF, Nosanchuk JD, Zancopé-Oliveira RM, Immunoglobulins G, M, and A against *Sporothrix schenckii* exoantigens in patients with sporotrichosis before and during treatment with itraconazole. *Clin Vaccine Immunol*. 2007;14:1149–57.
- Arenas R, Miller D, Campos-Macias P. Epidemiological data and molecular characterization (mtDNA) of *Sporothrix schenckii* in 13 cases from Mexico. *Int J Dermatol*. 2007;46:177–9.
- Arrillaga-Moncrieff I, Capilla J, Mayayo E, Marimón R, Marine M, Gene J, et al. Different virulence levels of the species of *Sporothrix* in a murine model. *Clin Microbiol Infect*. 2009;15:651–5.
- Barros MBL, Almeida-Paes R, Schubach AO. *Sporothrix schenckii* and sporotrichosis. *Clin Microbiol Rev*. 2011;24:633–54.
- Beer ZW, Harrington TC, Vismer HF, Wingfield BD, Wingfield MJ. Phylogeny of the *Ophiostoma stenoceras*–*Sporothrix schenckii* complex. *Mycologia*. 2003;95:434–41.
- Berbee ML, Taylor JW. 18S ribosomal RNA gene sequence characters place the human pathogen *Sporothrix schenckii* in the genus *Ophiostoma*. *Exp Mycol*. 1992;16:87–91.
- Bernardes-Engemann AR, Costa RC, Miguens BR, Penha CV, Neves E, Pereira BA, et al. Development of an enzyme-linked immunosorbent assay for the serodiagnosis of several clinical forms of sporotrichosis. *Med Mycol*. 2005;43:487–93.
- Costa RO, Bernardes-Engemann AR, Azulay-Abulafia L, Benvenuto F, Neves ML, Lopes-Bezerra LM. Sporotrichosis in pregnancy: case reports of 5 patients in a zoonotic epidemic in Rio de Janeiro, Brazil. *An Bras Dermatol*. 2011;86:995–8.
- Criseo G, Romeo O. Ribosomal DNA sequencing and phylogenetic analysis of environmental *Sporothrix schenckii* strains: comparison with clinical isolates. *Mycopathologia*. 2011;169:351–8.
- Cruz R, Vieille P, Oschilewski D. Aislamiento ambiental de *Sporothrix globosa* en relación a un caso de esporotricosis linfo-cutánea. *Rev Chil Infectol*. 2012;29:401–5.
- de Meyer EM, de Beer ZW, Summerbell RC, Moharram AM, de Hoog GS, Vismer HF, et al. Taxonomy and phylogeny of new wood-and soil-inhabiting *Sporothrix* species in the *Ophiostoma stenoceras*–*Sporothrix schenckii* complex. *Mycologia*. 2008;100:647–61.
- Dias NM, Oliveira MME, Santos C, Zancopé-Oliveira RM, Lima N. Sporotrichosis caused by *Sporothrix mexicana*, Portugal. *Emerg Infect Dis*. 2011;17:1975–6.
- Dixon DM, Salkin IF, Duncan RA, Hurd NJ, Haines JH, Kemna ME, et al. Isolation and characterization of *Sporothrix schenckii* from clinical and environmental sources associated with the largest U.S. epidemic of sporotrichosis. *J Clin Microbiol*. 1991;29:1106–13.
- Ferreira CP, Valle ACF, Freitas DFS, Reis RS, Gutierrez-Galhardo MC. Pregnancy during a sporotrichosis epidemic in Rio de Janeiro, Brazil. *Int J Gynaecol Obstet*. 2012;117:294–5.
- Freitas DFS, Valle ACF, Cuzzi T, Brandão LG, Zancopé-Oliveira RM, Gutierrez-Galhardo MC. Sweet syndrome associated with sporotrichosis. *Br J Dermatol*. 2012;166:212–3.
- Gezuele E, Rosa D. Relevancia del cuerpo asteroide asporotricósico en el diagnóstico rápido de la esporotricosis. *Rev Iberoam Micol*. 2005;22:147–50.
- Gonzalez-Velazquez W, Gonzalez-Mendez R, Rodriguez-del Valle N. Characterization and ligand identification of a new membrane progesterone receptor in fungi: existence of a novel PAQR in *Sporothrix schenckii*. *BMC Microbiol*. 2012;12:194.
- Guarro J. Taxonomía y biología de los hongos causantes de infección en humanos. *Enferm Infecc Microbiol Clin*. 2012;30:33–9.
- Gutierrez-Galhardo MC, Barros MBL, Schubach AO, Cuzzi T, Schubach TMP, Lazera MS, et al. Erythema multiforme associated with sporotrichosis. *J Eur Acad Dermatol Venereol*. 2005;19:507–9.
- Gutierrez-Galhardo MC, Schubach AO, Barros MBL, Moita Blanco TC, Cuzzi-Maya T, Schubach TMP, et al. Erythema nodosum associated with sporotrichosis. *Int J Dermatol*. 2002;41:114–6.
- Gutierrez-Galhardo MC, Zancopé-Oliveira RM, Valle ACF, Almeida-Paes R, Tavares PMS, Monzon A, et al. Molecular epidemiology and antifungal susceptibility patterns of *Sporothrix schenckii* isolates from a cat-transmitted epidemic of sporotrichosis in Rio de Janeiro, Brazil. *Med Mycol*. 2008;46:141–51.
- Hernandez-Cervantes A, Mora-Montes HM, Alvarez-Vargas A, Jimenez DF, Robledo-Ortiz CI, Flores-Carreón A. Isolation of *Sporothrix schenckii* MNT1 and the biochemical and functional characterization of the encoded α 1,2-mannosyltransferase activity. *Microbiology*. 2012;158:2419–27.
- Hintz WE. Sequence analysis of the chitin synthase A gene of the Dutch elm pathogen *Ophiostoma novo-ulmi* indicates a close association with the human pathogen *Sporothrix schenckii*. *Gene*. 1999;237:215–21.
- Hu S, Chung WH, Hung SI, Ho HC, Wang ZW, Chen CH, et al. Detection of *Sporothrix schenckii* in clinical samples by a nested PCR assay. *J Clin Microbiol*. 2003;41:1414–8.
- Ishizaki H, Kawasaki M, Aoki M, Vismer H, Muir D. Mitochondrial DNA analysis of *Sporothrix schenckii* in South Africa and Australia. *Med Mycol*. 2000;38:433–6.
- Kanbe T, Natsume L, Goto I, Kawasaki M, Mochizuki T, Ishizaki H, et al. Rapid and specific identification of *Sporothrix schenckii* by PCR targeting the DNA topoisomerase II gene. *J Dermatol Sci*. 2005;38:99–106.
- Kano R, Matsuoka A, Kashima M, Nakamura Y, Watanabe S, Mizoguchi M, et al. Detection of *Sporothrix schenckii* chitin synthase 1 (CHS1) gene in biopsy specimens from human patients with sporotrichosis. *J Dermatol Sci*. 2003;33:73–4.
- Kano R, Nakamura Y, Watanabe S, Tsujimoto H, Hasegawa A. Identification of *Sporothrix schenckii* based on sequences of the chitin synthase 1 gene. *Mycoses*. 2001;44:261–5.
- Kano R, Watanabe K, Murakami M, Yanai T, Hasegawa A. Molecular diagnosis of feline sporotrichosis. *Vet Rec*. 2005;156:484–5.
- Kawasaki M, Anzawa K, Mochizuki T, Ishizaki H. New strain typing method with *Sporothrix schenckii* using mitochondrial DNA and polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) technique. *J Dermatol*. 2012;39:362–5.
- Lin J, Kawasaki M, Aoki M, Ishizaki H, You G, Li R. Mitochondrial DNA analysis of *Sporothrix schenckii* clinical isolates from China. *Mycopathologia*. 1999;148:69–72.

33. Liu X, Zhang Z, Hou B, Wang D, Sun T, Li F, et al. Rapid identification of *Sporothrix schenckii* in biopsy tissue by PCR. *J Eur Acad Dermatol Venereol*. 2013;27:1491–7.
34. Lopes-Bezerra LM, Schubach AO, Costa RO. *Sporothrix schenckii* and sporotrichosis. *An Acad Bras Cienc*. 2006;78:293–308.
35. Madrid H, Cano J, Gene J, Bonifaz A, Toriello C, Guarro J. *Sporothrix globosa*, a pathogenic fungus with widespread geographical distribution. *Rev Iberoam Micol*. 2009;26:218–22.
36. Madrid H, Gene J, Cano J, Silvera C, Guarro J. *Sporothrix brunneoviolacea* and *Sporothrix dimorphospora*, two new members of the *Ophiostoma stenoceras-Sporothrix schenckii* complex. *Mycologia*. 2010;102:1193–203.
37. Marimon R, Cano J, Gene J, Sutton DA, Kawasaki M, Guarro J. *Sporothrix brasiliensis*, *S. globosa*, and *S. mexicana*, three new *Sporothrix* species of clinical interest. *J Clin Microbiol*. 2007;45:3198–206.
38. Marimon R, Gene J, Cano J, Guarro J. *Sporothrix luriei*: a rare fungus from clinical origin. *Med Mycol*. 2008;46:621–5.
39. Marimon R, Gene J, Cano J, Trilles L, Lazera MS, Guarro J. Molecular phylogeny of *Sporothrix schenckii*. *J Clin Microbiol*. 2006;44:3251–6.
40. Marimon R, Serena C, Gene J, Cano J, Guarro J. In vitro antifungal susceptibilities of five species of *Sporothrix*. *Antimicrob Agents Chemother*. 2008;52:732–4.
41. Mendoza M, Brito A, Schaper DA, Spooner VA, Alvarado P, Castro A, et al. Evaluación de la técnica PCR anidada para el diagnóstico de la esporotricosis experimental. *Rev Iberoam Micol*. 2012;29:120–5.
42. Mesa-Arango AC, Reyes-Montes MR, Perez-Mejia A, Navarro-Barranco H, Souza V, Zuniga G, et al. Phenotyping and genotyping of *Sporothrix schenckii* isolates according to geographic origin and clinical form of Sporotrichosis. *J Clin Microbiol*. 2002;40:3004–11.
43. Mora-Cabrera M, Alonso RA, Ulloa-Arvizu R, Torres-Guerrero H. Analysis of restriction profiles of mitochondrial DNA from *Sporothrix schenckii*. *Med Mycol*. 2001;39:439–44.
44. Neyra E, Fonteyne PA, Swinne D, Fauche F, Bustamante E, Noland N. Epidemiology of human sporotrichosis investigated by amplified fragment length polymorphism. *J Clin Microbiol*. 2005;43:1348–52.
45. O'Reilly LC, Altman SA. Macrorestriction analysis of clinical and environmental isolates of *Sporothrix schenckii*. *J Clin Microbiol*. 2006;44:2547–52.
46. Oliveira MME, Almeida-Paes R, Muniz MM, Barros MBL, Gutierrez-Galhardo MC, Zancopé-Oliveira RM. Sporotrichosis caused by *Sporothrix globosa* in Rio de Janeiro, Brazil: case report. *Mycopathologia*. 2010;169:359–63.
47. Oliveira MME, Almeida-Paes R, Muniz MM, Gutierrez-Galhardo MC, Zancopé-Oliveira RM. Phenotypic and molecular identification of *Sporothrix* isolates from an epidemic area of sporotrichosis in Brazil. *Mycopathologia*. 2011;172:257–67.
48. Oliveira MME, Sampaio P, Almeida-Paes R, Pais C, Gutierrez-Galhardo MC, Zancopé-Oliveira RM. Rapid identification of *Sporothrix* species by T3B fingerprinting. *J Clin Microbiol*. 2012;50:2159–62.
49. Pluss J, Opal S. Pulmonary sporotrichosis: review of treatment and outcome. *Medicine (Baltimore)*. 1986;65:143–53.
50. Quintella LP, Passos SRL, Valle ACF, Gutierrez-Galhardo MC, Barros MBL, Cuzzi T, et al. Histopathology of cutaneous sporotrichosis in Rio de Janeiro: a series of 119 consecutive cases. *J Cutan Pathol*. 2011;38:25–32.
51. Reis RS, Almeida-Paes R, Muniz MM, Tavares PMS, Monteiro PCF, Schubach TMP, et al. Molecular characterisation of *Sporothrix schenckii* isolates from humans and cats involved in the sporotrichosis epidemic in Rio de Janeiro, Brazil. *Mem Inst Oswaldo Cruz*. 2009;104:769–74.
52. Robledo-Ortiz CI, Flores-Carreón A, Hernández-Cervantes A, Álvarez-Vargas A, Lee KK, Díaz-Jiménez DF, et al. Isolation and functional characterization of *Sporothrix schenckii* ROT2, the encoding gene for the endoplasmic reticulum glucosylase II. *Fungal Biol*. 2012;116:910–8.
53. Romeo O, Criseo G. 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. *Virulence*. 2013;4:1–4.
54. Romeo O, Scordino F, Criseo G. New insight into molecular phylogeny and epidemiology of *Sporothrix schenckii* species complex based on calmodulin-encoding gene analysis of Italian isolates. *Mycopathologia*. 2011;172:179–86.
55. Sandhu GS, Kline BC, Stockman L, Roberts GD. Molecular probes for diagnosis of fungal infections. *J Clin Microbiol*. 1995;33:2913–9.
56. Suzuki K, Kawasaki M, Ishizaki H. Analysis of restriction profiles of mitochondrial DNA from *Sporothrix schenckii* and related fungi. *Mycopathologia*. 1988;103:147–51.
57. Takeda Y, Kawasaki M, Ishizaki H. Phylogeny and molecular epidemiology of *Sporothrix schenckii* in Japan. *Mycopathologia*. 1991;116:9–14.
58. Valentin-Berrios S, Gonzalez-Velazquez W, Perez-Sanchez L, Gonzalez-Mendez R, Rodriguez-Del Valle N. Cytosolic phospholipase A2: a member of the signalling pathway of a new G protein alpha subunit in *Sporothrix schenckii*. *BMC Microbiol*. 2009;9:100.
59. Valle-Aviles L, Valentin-Berrios S, Gonzalez-Mendez RR, Rodriguez-Del Valle N. Functional, genetic and bioinformatic characterization of a calcium/calmodulin kinase gene in *Sporothrix schenckii*. *BMC Microbiol*. 2007;7:107.
60. Watanabe S, Kawasaki M, Mochizuki T, Ishizaki H. RFLP analysis of the internal transcribed spacer regions of *Sporothrix schenckii*. *Nihon Ishinkin Gakkai Zasshi*. 2004;45:165–75.
61. Xu TH, Lin JP, Gao XH, Wei H, Liao W, Chen HD. Identification of *Sporothrix schenckii* of various mtDNA types by nested PCR assay. *Med Mycol*. 2010;48:161–5.
62. Zancopé-Oliveira RM, Almeida-Paes R, Oliveira MME, Freitas DFS, Gutierrez-Galhardo MC. New diagnostic applications in sporotrichosis. In: Khopkar U, editor. *Skin biopsy-perspectives*. Rijeka: InTech Europe; 2011. p. 53–72.
63. Zhang Z, Liu X, Lv X, Lin J. Variation in genotype and higher virulence of a strain of *Sporothrix schenckii* causing disseminated cutaneous sporotrichosis. *Mycopathologia*. 2011;172:439–46.
64. Zhang Z, Liu X, Yang G, Gao X, Jin L, An L. Genotyping of *Sporothrix schenckii* by analysis of ribosomal DNA regions. *Mycoses*. 2006;49:305–10.