



Morphological and physiological comparison of taxa comprising the *Sporothrix schenckii* complex*

Ming-dan ZHAO¹, Xun ZHOU^{†‡1,2}, Ting-ting LIU¹, Zhi-bang YANG³

⁽¹⁾Department of Dermatology, the First Affiliated Hospital of Chongqing Medical University, Chongqing 400016, China)

⁽²⁾Department of Dermatology and Cosmetology, Chongqing Hospital of Traditional Chinese Medicine, Chongqing 400021, China)

⁽³⁾The Laboratory of Pathogen Biology and Immunology, Basic Medical Experiment Teaching Center, Chongqing Medical University, Chongqing 400016, China)

[†]E-mail: zhouxun123@sina.com

Received Mar. 8, 2015; Revision accepted Sept. 14, 2015; Crosschecked Oct. 21, 2015

Abstract: Based on recent molecular data, it has been suggested that *Sporothrix globosa* is the main causal agent of sporotrichosis in China. The objective of this study was to compare the morphology, growth characteristics, patterns of carbon source usage, and susceptibility to antifungal agents among *Sporothrix* strains. A total of 15 clinical strains confirmed to be *S. globosa*, from three different regions of China, and 11 ex-type strains from the CBS-KNAW biodiversity center were obtained. The elongated conidia of *S. pallida*, *S. variecibatus*, *S. schenckii*, and *S. schenckii luriei* were clearly different from the subglobose and globose conidia of *S. globosa* strains. *S. schenckii* is able to assimilate sucrose, raffinose, and ribitol. Susceptibility profiles of these *Sporothrix* species were evaluated by measuring minimum inhibitory concentrations (MICs). Fluconazole, itraconazole, terbinafine, and amphotericin B showed good activity against most *S. globosa* clinical isolates from China. Potassium iodide also showed a low MIC against *S. pallida*, while fluconazole showed a high MIC for *S. mexicana*, *S. humicola*, *S. globosa*, *S. schenckii*, and *S. inflata*; these strains might be considered tolerant. The species showed differences in susceptibility to antifungal drugs and should therefore be properly identified during diagnosis prior to designing therapeutic strategies.

Key words: *Sporothrix globosa*, Phenotypic characters, Antifungal susceptibility

doi:10.1631/jzus.B1500055

Document code: A

CLC number: R375

1 Introduction

Sporotrichosis is a chronic subcutaneous mycosis that can be acquired by contact with contaminated objects or organic material (O'Reilly and Altman, 2006). It also can be acquired through zoonotic transmission (Oliveira *et al.*, 2011; Silva-Vergara *et al.*, 2012). The incidence of sporotrichosis is increasing in China, particularly in the northeastern region of the country (Zhang and Lin, 2008). *Sporo-*

thrix globosa was identified as the causal agent of sporotrichosis in many of these cases (Tan *et al.*, 2013; Yu *et al.*, 2013; Liu *et al.*, 2014; Zhou *et al.*, 2014). Four species of the *Sporothrix* complex have been reported as causal agents of sporotrichosis: *S. brasiliensis*, *S. globosa*, *S. schenckii s. str.*, and *S. schenckii luriei* (Rodrigues *et al.*, 2014). *S. pallida* and *S. mexicana* are mainly environmental but may also be potentially pathogenic to humans and other mammals. It is not clear if these species have different physiological features that determine their pathogenicity or if they differ in their susceptibility to antifungals.

Species in the *S. schenckii* complex have been shown to differ in their calmodulin (CAL), internal transcribed spacer (ITS), and a fragment of the

[‡] Corresponding author

* Project supported by the National Natural Science Foundation of China (No. 31270062)

ORCID: Ming-dan ZHAO, <http://orcid.org/0000-0002-7578-4212>

© Zhejiang University and Springer-Verlag Berlin Heidelberg 2015

β -tubulin gene sequences (Marimon *et al.*, 2007). Few studies have focused on the morphological and physiological variation among strains that comprise the *S. schenckii* complex. Recent studies have shown that morphologically similar species in this group could differ in their physiological features (Marimon *et al.*, 2007; Rodrigues *et al.*, 2014). Colony characteristics, growth rates, and carbon assimilation tests have been shown to be useful for distinguishing species of *Sporothrix* (Marimon *et al.*, 2007; de Meyer *et al.*, 2008). Moreover, the distribution of sessile conidia and their morphological variation have been suggested to be the key features distinguishing species within the *Sporothrix* complex. In this study, using type strains of different species of *Sporothrix*, we compared colony and microscopic morphologies, assimilation of carbon sources, and antifungal susceptibilities. The variation in these characteristics among different *Sporothrix* species, as reported here, provides new insights into their pathogenicity mechanisms and suggests different susceptibilities to antifungals, thereby providing new avenues for clinical therapeutic strategies.

2 Materials and methods

2.1 Strains

Fifteen strains of *Sporothrix* from clinical sources in China were included in the study. Isolates consisted of 15 strains that were morphologically and genetically identified as *S. globosa* (Liu *et al.*, 2014) and 11 ex-type strains, including *S. brunneoviolacea* ($n=1$), *S. dimorphospora* ($n=1$), *S. globosa* ($n=1$), *S. humicola* ($n=1$), *S. inflata* ($n=1$), *S. lignivora* ($n=1$), *S. mexicana* ($n=1$), *S. pallida* ($n=1$), *S. variecibatus* ($n=1$), *S. schenckii* ($n=1$), and *S. schenckii luriei* ($n=1$) (Table 1). Isolates were stored at room temperature on slant cultures containing 2% (0.02 g/ml) potato dextrose agar. Cultures were stored at 4 °C in distilled water for long-term preservation.

2.2 Morphological characteristics

Mycelial discs (2 mm diameter) of each strain were transferred to three replicate plates containing 2% (0.02 g/ml) potato dextrose agar, and the plates were incubated at 25 °C. Colony size, shape, and pigment production were recorded after 7, 14, 21, and

Table 1 Sources of isolates used in this study and analysis

Strain	Name	Source	Country of origin
LC2454	<i>S. globosa</i>	Clinical strain	Beijing, China
LC2460	<i>S. globosa</i>	Clinical strain	Beijing, China
LC2466	<i>S. globosa</i>	Clinical strain	Beijing, China
LC2467	<i>S. globosa</i>	Clinical strain	Beijing, China
LC2469	<i>S. globosa</i>	Clinical strain	Beijing, China
LC2432	<i>S. globosa</i>	Clinical strain	Chongqing, China
LC2433	<i>S. globosa</i>	Clinical strain	Chongqing, China
LC2445	<i>S. globosa</i>	Clinical strain	Chongqing, China
LC2447	<i>S. globosa</i>	Clinical strain	Chongqing, China
LC2480	<i>S. globosa</i>	Clinical strain	Chongqing, China
LC2404	<i>S. globosa</i>	Clinical strain	Jilin, China
LC2405	<i>S. globosa</i>	Clinical strain	Jilin, China
LC2411	<i>S. globosa</i>	Clinical strain	Jilin, China
LC2413	<i>S. globosa</i>	Clinical strain	Jilin, China
LC2419	<i>S. globosa</i>	Clinical strain	Jilin, China
CBS 120341	<i>S. mexicana</i>	Environmental	Mexico
CBS 124561	<i>S. brunneoviolacea</i>	Soil	Spain
CBS 118129	<i>S. humicola</i>	Soil	South Africa
CBS 239.68	<i>S. inflata</i>	Wheat-field soil	Germany
CBS 131.56	<i>S. pallida</i>	<i>Stemonitis fusca</i>	Japan
CBS 121961	<i>S. variecibatus</i>	<i>Trichopoda</i> sp. from <i>Protea repens</i>	South Africa
CBS 119148	<i>S. lignivora</i>	Utility poles	South Africa
CBS 120340	<i>S. globosa</i>	Clinical strain	Spain
CBS 553.74	<i>S. dimorphospora</i>	Soil	Canada
CBS 359.36	<i>S. schenckii</i>	Clinical strain	Unknown
CBS 937.72	<i>S. schenckii luriei</i>	Clinical strain	South Africa

28 d of incubation. Colony diameter was measured daily for 7 d and growth rate was calculated as the 7-d average of mean weekly growth (mm/week). Growth rates were subjected to an analysis of variance ($P < 0.05$) with Duncan's multiple range test by using SPSS software (Version 16.0). After 7 d, the shape and size of the appressoria formed across the underside of the cover slip were studied (Marimon *et al.*, 2007).

2.3 Physiological studies

Carbon assimilation was tested in liquid medium according to Marimon *et al.* (2007). Variation in the ability to grow on sucrose, raffinose, and maltose was tested in all strains. The tests were conducted in 96-well microplates, containing either no carbon source (negative control) or glucose (positive control). The inocula were adjusted to a final concentration of from 2×10^5 to 2×10^6 colony-forming unit (CFU)/ml. Microplates were read (530 nm) after 5 d of incubation at 25 °C, as previously described (Marimon *et al.*, 2007).

2.4 Antifungal susceptibility testing

The following antifungals were tested for their activity against *Sporothrix* strains: fluconazole (FLC), itraconazole (ITC), terbinafine (TRB), amphotericin B (AMB), and potassium iodide (KI). Antifungal susceptibility tests were performed on the isolates using the methods recommended by the Clinical Laboratory and Standards Institute standard document M38-A2 (Kohler *et al.*, 2004; Trilles *et al.*, 2005; Marimon *et al.*, 2008; Oliveira *et al.*, 2011). Ninety-six-well microplates containing 0.01 ml of RPMI-1640 nutrient broth in each well were incubated at 25 °C. Nutrient broth RPMI-1640 was amended with antifungal drug concentrations to final concentrations that spanned 0.50 to 500.00 µg/ml (KI), 0.125 to 64.00 µg/ml (FLC), and 0.03 to 16.00 µg/ml (ITC, TRB, and AMB). The concentration of cells used to inoculate assays was adjusted to a final concentration that ranged from 1×10^4 to 5×10^4 CFU/ml. *Candida parapsilosis* (ATCC 22019) was included as a control for each antifungal test (Marimon *et al.*, 2008). Growth was monitored spectrophotometrically at 530 nm during the growth period and growth in the presence of antifungals was compared with growth in the absence of antifungals to establish minimum inhibitory concentrations (MICs) (Espinel-Ingroff *et al.*, 2002). The MICs for AMB, ITC, and KI were defined as the lowest concentration showing 100% growth

inhibition. MICs for TRB were defined as the lowest concentrations that showed 80% inhibition of growth, and for FLC as the lowest concentration that produced 50% growth inhibition (Trilles *et al.*, 2005; Marimon *et al.*, 2008; Oliveira *et al.*, 2011; Rodrigues *et al.*, 2014). The tests were carried out three times and an MIC based on these replicates was calculated. Carbon assimilation was tested in liquid medium according to Marimon *et al.* (2007). Variation in the ability to grow on sucrose, raffinose, and maltose was tested in all strains. The tests were conducted in 96-well microplates, containing either no carbon source (negative control) or glucose (positive control). The inocula were adjusted to a final concentration of from 2×10^5 to 2×10^6 CFU/ml. Microplates were read (530 nm) after 5 d of incubation at 25 °C, as previously described (Marimon *et al.*, 2007).

3 Results

3.1 Morphological characteristics

3.1.1 Colony characters

Images of *Sporothrix* isolate colonies are depicted in Fig. 1. All strains grew well on potato dextrose agar at 25 °C. Colonies of clinical isolates from China produced cream-colored, smooth or verrucous, moist colonies with occasional aerial mycelia. Over time, these colonies matured into black leathery colonies with a wrinkled and folded surface. Colonies of *S. schenckii*, *S. schenckii luriei*, *S. pallida*, and *S. humicola* produced white aerial mycelium, with white colonies in the center. These colonies remained white to cream-colored over time.

3.1.2 Conidial morphology

The morphology of conidia in *S. schenckii* isolates differed from that of the *S. globosa* isolates examined. The conidia of *S. globosa* isolates were septate hyphae with terminal clavate obovoid structures. The conidia of *S. schenckii* isolates were ovate, hyaline, and thin-walled conidia in sympodial conidiophores, with oval to oblong phialides. The type strain of *S. inflata* produced globose, sessile, brown conidia. The type strains of *S. mexicana*, *S. variacibatus*, *S. lignivora*, and *S. pallida* produced oval conidia and hyphae with terminal obovoid conidia. The type strain of *S. brunneoviolacea* showed hyaline, globose to smooth and thin-walled guttulate conidia.

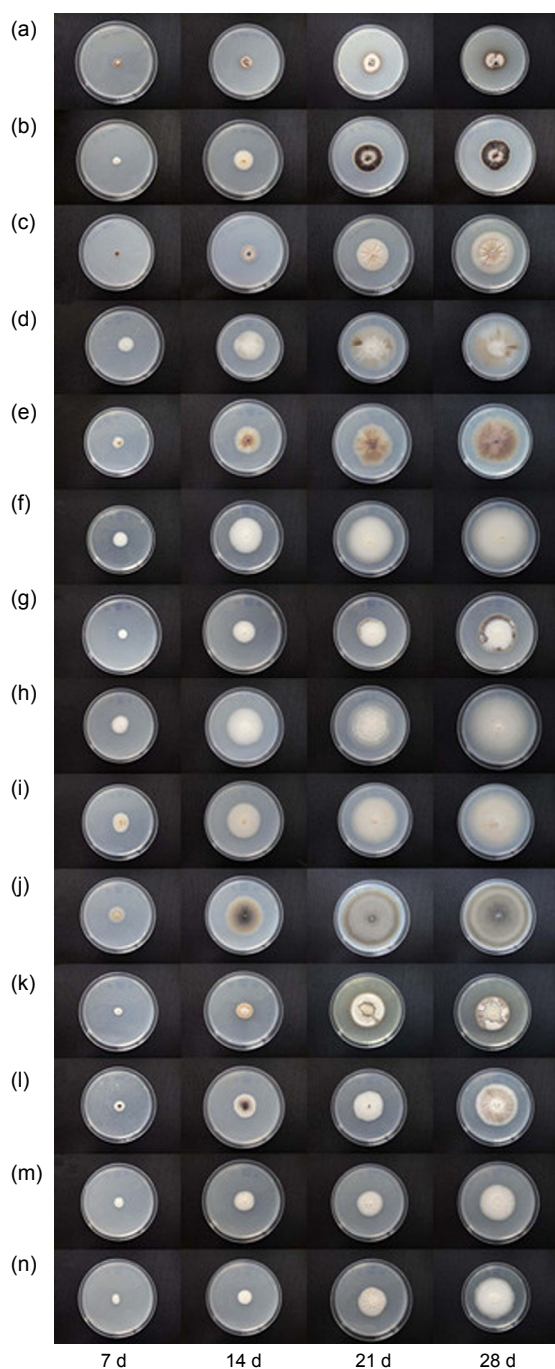


Fig. 1 Colony morphology of isolates grown on potato dextrose agar at 25 °C following incubation for 7, 14, 21, and 28 d

(a) *S. globosa* (Beijing, China); (b) *S. globosa* (Jilin, China); (c) *S. globosa* (Chongqing, China); (d) *S. mexicana* (CBS 120341); (e) *S. brunneoviolacea* (CBS 124561); (f) *S. humicola* (CBS 118129); (g) *S. inflata* (CBS 239.68); (h) *S. pallida* (CBS 131.56); (i) *S. variecibatus* (CBS 121961); (j) *S. lignivora* (CBS 119148); (k) *S. globosa* (CBS 120340); (l) *S. dimorphospora* (CBS 553.74); (m) *S. schenckii* (CBS 359.36); (n) *S. schenckii luriei* (CBS 937.72)

The conidia of ex-type *S. humicola* were solitary, straight, and hyaline. The type strain of *S. dimorphosporas* showed subglobose to obovoid sessile conidia in a conidiogenous terminal structure. The type strain of *S. schenckii luriei* produced septate, oblong, and hyaline conidia that were slightly irregular to irregular in shape (Fig. 2).

3.1.3 Growth rate

Duncan's multiple range test showed no significant differences in the growth rates among 15 *S. globosa* clinical strains from China ($P=0.45$). However, there was a statistical difference in growth rates among the different species that comprise the complex. Moreover, statistical analysis revealed a faster growth rate of strains isolated from the natural environment compared with those obtained from clinical specimens ($P<0.05$). The maximum growth rate of *S. globosa* strains was (12.33 ± 5.29) mm/week and the minimum growth rate was (8.41 ± 3.02) mm/week; these were not significantly different ($P>0.05$).

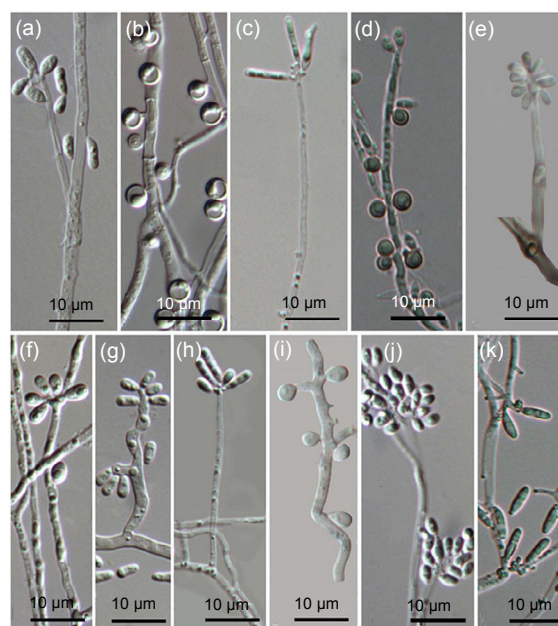


Fig. 2 Morphology of the conidia of the species in *Sporothrix schenckii sensu lato* when grown on potato dextrose agar at 25 °C for 7 d

(a) *S. mexicana* (CBS 120341); (b) *S. brunneoviolacea* (CBS 124561); (c) *S. humicola* (CBS 118129); (d) *S. inflata* (CBS 239.68); (e) *S. pallida* (CBS 131.56); (f) *S. variecibatus* (CBS 121961); (g) *S. lignivora* (CBS 119148); (h) *S. globosa* (CBS 120340); (i) *S. dimorphospora* (CBS 553.74); (j) *S. schenckii* (CBS 359.36); (k) *S. schenckii luriei* (CBS 937.72)

Table 2 Summary of morphological data obtained from *Sporothrix* isolates

Fungus isolate	Diameter (mm)				Growth rate (mm/week)
	7 d	14 d	21 d	28 d	
LC2454	10.15±4.83	21.33±1.36	26.83±1.72	38.33±3.20	8.41±3.02
LC2460	10.65±4.84	20.50±1.51	32.00±3.03	39.83±3.31	9.17±4.05
LC2466	11.66±1.21	21.16±1.47	30.33±1.36	40.66±2.94	9.05±1.84
LC2467	11.66±1.03	21.00±1.54	33.16±2.02	42.66±3.66	10.44±3.68
LC2469	12.16±1.16	21.16±1.72	32.66±2.33	44.33±3.93	10.72±2.63
LC2432	9.98±4.48	21.16±2.31	31.83±2.99	46.10±1.16	11.83±4.34
LC2433	8.16±6.26	22.63±2.16	34.33±3.20	44.33±1.50	11.11±3.06
LC2445	8.28±5.76	22.66±3.93	35.50±2.07	43.00±3.57	11.38±4.52
LC2447	9.98±4.57	21.50±1.37	30.33±2.58	44.66±2.16	11.11±3.37
LC2480	9.63±4.35	21.50±2.73	31.66±2.92	44.50±2.25	11.22±4.09
LC2404	9.96±4.63	20.16±1.16	31.00±3.09	39.50±3.01	9.55±2.70
LC2405	10.15±4.74	20.50±1.08	31.50±2.66	39.33±2.42	9.50±4.24
LC2411	9.80±4.86	20.33±2.33	31.33±3.38	47.66±1.36	12.33±5.29
LC2415	9.96±4.55	20.00±2.28	31.50±2.25	46.00±3.46	11.05±2.97
LC2419	9.46±6.67	21.00±0.89	32.16±2.31	47.83±3.18	11.88±4.01
<i>S. mexicana</i>	25.70±2.98	46.00±2.13	63.63±2.13	85.50±0.83	19.93±2.15
<i>S. brunneoviolacea</i>	16.83±2.50	33.16±1.72	52.66±2.25	64.50±0.57	15.85±3.65
<i>S. humicola</i>	27.25±4.52	47.25±3.19	59.66±2.73	71.33±1.21	14.74±4.38
<i>S. inflata</i>	13.75±1.28	24.66±2.50	39.00±1.78	45.66±3.72	10.63±6.77
<i>S. pallida</i>	28.62±3.50	51.00±4.14	62.33±2.58	71.50±1.51	14.29±6.46
<i>S. variecibatus</i>	29.33±5.20	48.22±5.08	65.16±1.16	74.50±0.54	15.05±5.16
<i>S. lignivora</i>	29.66±1.62	58.50±6.41	76.83±7.47	77.50±0.54	18.03±10.3
<i>S. globosa</i>	17.01±4.69	24.50±1.97	33.83±1.47	46.33±1.50	9.77±2.86
<i>S. dimorphospora</i>	15.50±2.07	30.66±1.86	42.66±3.01	55.00±1.09	13.20±2.05
<i>S. schenckii</i>	17.20±3.35	27.35±2.25	34.16±1.83	43.00±1.78	8.68±1.56
<i>S. schenckii luriei</i>	14.25±3.84	24.83±5.07	35.33±1.03	55.66±1.63	13.78±5.73

S. mexicana type strains grew the fastest with a rate of (19.93±2.15) mm/week. *S. schenckii* and *S. globosa* type strains had growth rates of (8.68±1.56) mm/week and (9.77±2.86) mm/week, respectively. The colony diameters for different isolates at the same temperature are summarized in Table 2.

3.2 Physiological studies

The type strain of *S. lignivora* (CBS 119148) was unable to assimilate sucrose, unlike all of the other strains. All isolates were able to assimilate dextrose and ribitol with the exception of *S. dimorphospora*, *S. inflata*, *S. lignivora*, and *S. mexicana*. With respect to carbon source utilization, the clinical isolates from China were similar to the type strain of *S. globosa* (Table 3).

Table 3 Carbon assimilation associated with *Sporothrix* isolates used in this study

Strain	Sucrose	Raffinose	Ribitol
<i>S. globosa</i> (n=15)	+	-	+
<i>S. mexicana</i>	+	+	-
<i>S. brunneoviolacea</i>	+	+	+
<i>S. humicola</i>	+	-	+
<i>S. inflata</i>	+	-	-
<i>S. pallida</i>	+	-	+
<i>S. variecibatus</i>	+	-	+
<i>S. lignivora</i>	-	-	-
<i>S. globosa</i>	+	-	+
<i>S. dimorphospora</i>	+	-	-
<i>S. schenckii</i>	+	+	+
<i>S. schenckii luriei</i>	+	+	+

Number of tests showing positive (+)/negative (-) as carbon source (+: able to grow sufficiently; -: unable to grow sufficiently)

3.3 Antifungal susceptibility test

Table 4 summarizes the MIC ranges for the *Sporothrix* isolates. Fifteen clinical isolates of *S. globosa* were subjected to antifungal susceptibility testing. These isolates showed a low MIC to KI (MIC range, 31.25–250.00 µg/ml), FLC (MIC range, 0.50–4.00 µg/ml), ITC (MIC range, 0.25–1.00 µg/ml), TRB (MIC range, 0.06–1.00 µg/ml), and AMB (MIC range, 0.13–0.50 µg/ml) (Table 4). ITC, TRB, and AMB had the lowest MICs against *S. schenckii* with values of 2.00, 1.00, and 1.00 µg/ml, respectively, while FLC showed no activity (MIC>64.00 µg/ml). We detected moderate activity for ITC and TRB against the *S. globosa* type strain, with MICs of 1.00 µg/ml, while KI and FLC showed a low activity indicating that they are not effective against this strain. FLC was not effective against *S. mexicana*, *S. humicola*, *S. inflata*, or *S. schenckii luriei* (MIC>64.00 µg/ml).

KI was the most effective antifungal agent against *S. pallida* (MIC=7.80 µg/ml). The MICs of FLU showed moderate activity against clinical isolates of *S. globosa* from China compared with *S. schenckii*, *S. globosa*, and *S. schenckii luriei*. TRB was the most active drug for all the strains tested. AMB was slightly less effective against *S. schenckii luriei* and *S. globosa* compared with *S. globosa* clinical isolates from China.

For ITC, a strain with an MIC of >4.00 µg/ml was considered tolerant, while a strain with an MIC in the range of 1.00–2.00 µg/ml was considered susceptible. Our data showed that ITC was effective against *S. globosa* clinical isolates from China, but was ineffective for *S. schenckii*, *S. globosa*, and *S. schenckii luriei*.

4 Discussion

Different strains comprising the *S. schenckii* complex exhibited variation in colony characteristics. The type strain of *S. globosa* showed terminal clavate obovoid conidia. However, we were unable to observe any dark colony characters, and we presume that the isolate had degenerated, thereby losing its ability to produce these dark mycelia (Marimon *et al.*, 2007). Moreover, pathogenic *Sporothrix* species differed in growth rates and physiological characteristics when compared with environmental isolates, suggesting that these strains have adapted specific, albeit yet to be defined, traits that enable their pathogenicity.

ITC, when administered at a dose of 200 mg orally daily for 3–6 months, is the first antifungal treatment for cutaneous and lymphocutaneous sporotrichosis recommended by the Clinical Practice

Table 4 Minimum inhibitory concentration (MIC) of *Sporothrix* isolates

Strain	Name	MIC (µg/ml)				
		KI	FLC	ITC	TRB	AMB
LC2454, LC2460, LC2466, LC2467, LC2469	<i>S. globosa</i>	31.25–62.50	0.50–2.00	0.50–1.00	0.06–1.00	0.13–0.50
LC2404, LC2405, LC2411, LC2413, LC2419	<i>S. globosa</i>	62.50–125.00	2.00–4.00	0.50–1.00	0.25–1.00	0.13–0.50
LC2432, LC2433, LC2445, LC2447, LC2480	<i>S. globosa</i>	62.50–250.00	1.00–4.00	0.25–1.00	0.13–1.00	0.25–0.50
CBS 120341	<i>S. mexicana</i>	62.50	>64.00	4.00	0.25	8.00
CBS 124561	<i>S. brunneoviolacea</i>	62.50	4.00	1.00	2.00	1.00
CBS 118129	<i>S. humicola</i>	31.25	>64.00	0.06	2.00	8.00
CBS 239.68	<i>S. inflata</i>	31.25	>64.00	1.00	0.06	0.25
CBS 131.56	<i>S. pallida</i>	7.80	4.00	0.13	0.25	>16.00
CBS 121961	<i>S. variecibatus</i>	31.25	4.00	1.00	0.06	1.00
CBS 119148	<i>S. lignivora</i>	62.50	1.00	>16.00	1.00	8.00
CBS 120340	<i>S. globosa</i>	>500.00	>64.00	1.00	1.00	4.00
CBS 553.74	<i>S. dimorphospora</i>	25.65	1.00	4.00	1.00	4.00
CBS 359.36	<i>S. schenckii</i>	250.00	>64.00	2.00	1.00	1.00
CBS 937.72	<i>S. schenckii luriei</i>	31.25	>64.00	1.00	1.00	4.00

KI: potassium iodide; FLC: fluconazole; ITC: itraconazole; TRB: terbinafine; AMB: amphotericin B

Guidelines for the Management of Sporotrichosis (de Lima Barros *et al.*, 2004; Silva-Vergara *et al.*, 2012; Rodrigues *et al.*, 2014). In this study, ITC showed good activity against all species, except for *S. lignivora*, consistent with the results of Kohler *et al.* (2004). Moreover, TRB exhibited high activity against all the strains tested, as demonstrated by other authors (Marimon *et al.*, 2008).

In this study, we found that *S. globosa* isolates from China were more susceptible to antifungals than the type strain of *S. globosa*. The study included strains isolated from the three different regions of China. Even in this limited sample we found variation in morphological characteristics and susceptibilities to antifungal agents. Therefore, our findings reinforce the importance of identifying *S. schenckii sensu stricto* and of evaluating its antifungal susceptibilities to determine the optimum therapeutic option for each case of sporotrichosis. Thus, it is imperative that species causing infection be identified during diagnosis and that treatment be prescribed accordingly. The results of this study provide fundamental data to assist in the selection of antifungal agents with enhanced activities against selected strains.

Acknowledgements

Dr. Lei CAI from the State Key Laboratory of Mycology, Chinese Academy of Sciences (Beijing, China) is acknowledged for providing lab space and facilities to carry out this work, and for providing valuable comments on this manuscript. The authors thank the Dermatology Department of the First Hospital of Jilin University, the Beijing University First Hospital, and the Southwest Hospital of the Third Military Medical University for supplying the clinical strains used in this study. The authors thank the CBS-KNAW biodiversity center for supplying the ex-type strains used in this study.

Compliance with ethics guidelines

Ming-dan ZHAO, Xun ZHOU, Ting-ting LIU, and Zhibang YANG declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

References

- de Lima Barros, M.B., de Oliveira Schubach, A., do Valle, A.C.F., *et al.*, 2004. Cat-transmitted sporotrichosis epidemic in Rio de Janeiro, Brazil: description of a series of cases. *Clin. Infect. Dis.*, **38**(4):529-535. [doi:10.1086/381200]
- de Meyer, E.M., de Beer, Z.W., Summerbell, R.C., *et al.*, 2008. Taxonomy and phylogeny of new wood- and soil-inhabiting *Sporothrix* species in the *Ophiostoma stenoceras-Sporothrix schenckii* complex. *Mycologia*, **100**(4):647-661. [doi:10.3852/07-157R]
- Espinel-Ingroff, A., Chaturvedi, V., Fothergill, A., *et al.*, 2002. Optimal testing conditions for determining MICs and minimum fungicidal concentrations of new and established antifungal agents for uncommon molds: NCCLS collaborative study. *J. Clin. Microbiol.*, **40**(10):3776-3781. [doi:10.1128/JCM.40.10.3776-3781.2002]
- Kohler, L.M., Monteiro, P.C., Hahn, R.C., *et al.*, 2004. *In vitro* susceptibilities of isolates of *Sporothrix schenckii* to itraconazole and terbinafine. *J. Clin. Microbiol.*, **42**(9):4319-4320. [doi:10.1128/JCM.42.9.4319-4320.2004]
- Liu, T.T., Zhang, K., Zhou, X., 2014. Molecular identification of *Sporothrix* clinical isolates in China. *J. Zhejiang Univ.-Sci. B (Biomed. & Biotechnol.)*, **15**(1):100-108. [doi:10.1631/jzus.B1300136]
- Marimon, R., Cano, J., Gené, J., *et al.*, 2007. *Sporothrix brasiliensis*, *S. globosa*, and *S. mexicana*, three new *Sporothrix* species of clinical interest. *J. Clin. Microbiol.*, **45**(10):3198-3206. [doi:10.1128/JCM.00808-07]
- Marimon, R., Serena, C., Gené, J., *et al.*, 2008. *In vitro* antifungal susceptibilities of five species of *Sporothrix*. *Antimicrob. Agents Chemother.*, **52**(2):732-734. [doi:10.1128/AAC.01012-07]
- Oliveira, D.C., Lopes, P.G., Spader, T.B., *et al.*, 2011. Antifungal susceptibilities of *Sporothrix albicans*, *S. brasiliensis*, and *S. luriei* of the *S. schenckii* complex identified in Brazil. *J. Clin. Microbiol.*, **49**(8):3047-3049. [doi:10.1128/JCM.00255-11]
- O'Reilly, L.C., Altman, S.A., 2006. Macrorestriction analysis of clinical and environmental isolates of *Sporothrix schenckii*. *J. Clin. Microbiol.*, **44**(7):2547-2552. [doi:10.1128/JCM.00078-06]
- Rodrigues, A.M., de Hoog, G.S., de Cássia Pires, D., *et al.*, 2014. Genetic diversity and antifungal susceptibility profiles in causative agents of sporotrichosis. *BMC Infect. Dis.*, **14**(1):219. [doi:10.1186/1471-2334-14-219]
- Silva-Vergara, M.L., de Camargo, Z.P., Silva, P.F., *et al.*, 2012. Disseminated *Sporothrix brasiliensis* infection with endocardial and ocular involvement in an HIV-infected patient. *Am. J. Trop. Med. Hyg.*, **86**(3):477-480. [doi:10.4269/ajtmh.2012.11-0441]
- Tan, J.W., Liu, W., Wan, Z., *et al.*, 2013. Reclassification of 33 clinical strains of *Sporothrix* from northern China based on phenotypic and molecular characters. *Mycosystema*, **32**(2):161-167 (in Chinese).
- Trilles, L., Fernández-Torres, B., dos Santos Lazéra, M., *et al.*, 2005. *In vitro* antifungal susceptibilities of *Sporothrix schenckii* in two growth phases. *Antimicrob. Agents Chemother.*, **49**(9):3952-3954. [doi:10.1128/AAC.49.9.3952-3954.2005]
- Yu, X., Wan, Z., Zhang, Z., *et al.*, 2013. Phenotypic and molecular identification of *Sporothrix* isolates of clinical origin in Northeast China. *Mycopathologia*, **176**(1-2):67-74. [doi:10.1007/s11046-013-9668-6]

Zhang, J.D., Lin, J.P., 2008. Clinical analysis of 316 cases of cutaneous sporotrichosis. *Chin. J. Mycol.*, 3(4):207-210 (in Chinese).

Zhou, X., Rodrigues, A.M., Feng, P., et al., 2014. Global ITS diversity in the *Sporothrix schenckii* complex. *Fungal Divers.*, 66(1):153-165. [doi:10.1007/s13225-013-0220-2]

中文概要

题目: 申克孢子丝菌复合体在形态学和生理生化的研究

目的: 研究申克孢子丝菌复合体在形态学、糖同化和抗真菌药物敏感性的差异, 探讨不同种类孢子丝菌的形态、生理生化及抗真菌药物敏感的特性。

创新点: 首次对我国球形孢子丝菌与其它孢子丝菌模式菌株在形态学、生理生化和抗真菌药敏方面进行比较, 从而找出我国孢子丝菌与模式菌株的差异和相同点。

方法: 实验选用 15 株从我国三个不同地区分离的球形孢子丝菌和 11 株购买的孢子丝菌模式菌株 (表 1), 将这 26 株孢子丝菌菌株分别接种到 2% 的马铃薯葡萄糖琼脂平板培养基上, 通过在不同时间段观察菌落生长特征, 测量菌落直径, 镜下观察菌丝、孢子形态, 测定最小抑制浓度 (MIC) 并进行评价。

结论: 实验显示: 根据顶端分生孢子的形态 (图 2)、不同时期菌种的菌落直径 (图 1)、糖同化实验 (表 2 和表 3) 可进行简单鉴定我国球形孢子丝菌与孢子丝菌复合体; 特比萘芬有较好的体外抑菌活性, 而碘化钾、氟康唑、伊曲康唑、两性霉素 B 对不同的菌株的抗菌敏感性不同 (表 4)。综上所述, 我国孢子丝菌临床菌株与孢子丝菌复合体在表型、生理生化及体外抗真菌药物敏感性上均有不同程度的差异。

关键词: 球形孢子丝菌; 表型特点; 抗真菌药物敏感性