

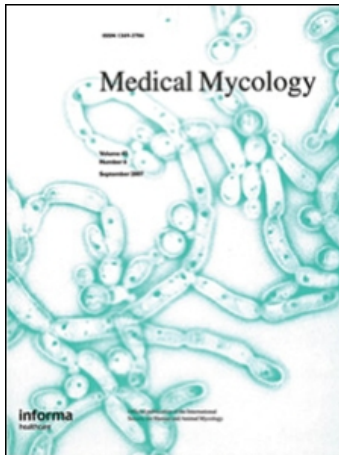
This article was downloaded by: [International Society for Human and Animal Mycology]

On: 3 February 2009

Access details: Access Details: [subscription number 769171546]

Publisher Informa Healthcare

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



## Medical Mycology

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713694156>

### Heterogeneity of proteins expressed by Brazilian *Sporothrix schenckii* isolates

Geisa Ferreira Fernandes <sup>a</sup>; Cristiane Candida Do Amaral <sup>a</sup>; Alexandre Sasaki <sup>a</sup>; Patrício Martinez Godoy <sup>bc</sup>; Zoilo Pires De Camargo <sup>a</sup>

<sup>a</sup> Cellular Biology Sector, Department of Microbiology, Immunology and Parasitology, Federal University of São Paulo (UNIFESP), Brazil <sup>b</sup> Infectious and Parasitic Diseases Sector, Federal University of São Paulo (UNIFESP), Brazil <sup>c</sup> IMC, Universidad Austral de Chile, Valdivia, Chile

First Published on: 31 January 2009

**To cite this Article** Fernandes, Geisa Ferreira, Do Amaral, Cristiane Candida, Sasaki, Alexandre, Godoy, Patrício Martinez and De Camargo, Zoilo Pires(2009)'Heterogeneity of proteins expressed by Brazilian *Sporothrix schenckii* isolates',*Medical Mycology*,

**To link to this Article:** DOI: 10.1080/13693780802713216

**URL:** <http://dx.doi.org/10.1080/13693780802713216>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.informaworld.com/terms-and-conditions-of-access.pdf>

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

# Heterogeneity of proteins expressed by Brazilian *Sporothrix schenckii* isolates

GEISA FERREIRA FERNANDES\*, CRISTIANE CANDIDA DO AMARAL\*, ALEXANDRE SASAKI\*, PATRÍCIO MARTINEZ GODOY†‡ & ZOILO PIRES DE CAMARGO\*

\*Cellular Biology Sector, Department of Microbiology, Immunology and Parasitology, Federal University of São Paulo (UNIFESP), Brazil, †Infectious and Parasitic Diseases Sector, Federal University of São Paulo (UNIFESP), Brazil, and ‡IMC, Universidad Austral de Chile, Valdivia, Chile

The profiles of proteins present in the exoantigens of Brazilian *Sporothrix schenckii* isolates were studied and compared by electrophoresis (SDS-PAGE). Thirteen isolates from five different regions of Brazil (1,000 to 2,000 km apart) and ten from a more limited region (200 to 400 km apart within the state of São Paulo) were cultured in Sabouraud, M199 and minimum (MM) media. Qualitative and quantitative differences in the expression of proteins, which varied according to the medium and the isolate, were observed. Fractions with the same MW but varying in intensity were detected, as well as fractions present in 1 isolate but absent in others. Dendrograms were constructed and isolates grouped based on the fractions obtained, irrespective of the intensity. The results showed that Brazilian *S. schenckii* isolates express different protein profiles, a feature also present in isolates from a more restricted region. The exoantigens were found to have a maximum of 15 protein fractions, ranging in MW from 19–220 KDaltons depending on the medium used for the cultures. These data show the great heterogeneity of Brazilian *S. schenckii* protein expression.

**Keywords** *Sporothrix schenckii*, exoantigens, proteins, SDS-PAGE

## Introduction

*Sporothrix schenckii*, a dimorphic fungus, is found worldwide and is the agent of sporotrichosis, a chronic subcutaneous infection characterized by the development of lymphatic nodules in humans and animals [1–5]. In general, this fungus is identified *in vitro* by the morphological features of its colonies, conidia and other structures. It is a hyaline fungus with a saprophytic filamentous phase characterized by sympodial conidia in radial flower-like clusters and/or thick-walled, dark brown conidia borne laterally along the hyphae in a sleeve-like manner. The conidia are hyaline

or slightly pigmented, usually obovoid, and measure 2–8 µm long by 1.5–2.5 µm wide. In addition, practically all the isolates produce another type of conidia, which are thick-walled, dark brown, and usually borne individually on short denticles along the sides of the vegetative hyphae. These conidia, which are regarded as sessile, are 2–6 µm long by 2–3.5 µm wide [4,6].

Recently, it has been demonstrated that the traditional *S. schenckii* species shows a high genetic variability, and new species have been described within the '*Sporothrix schenckii* aggregate' [7–9]. *S. brasiliensis* is one of these new species and to our knowledge has been only found in Brazil [8,9]. These species were considered new in the light of the results of sequence analysis of three protein-coding loci (chitin synthase, β-tubulin and calmodulin). Although a Spanish group led by Dr Josep Guarro proposed classifying the Brazilian *S. schenckii* strains as *S. brasiliensis* [8,9], in this paper we will refer to this fungus by its traditional name (*S. schenckii*) because we did not carry out

Received 5 August 2008; Final revision received 25 November 2008; Accepted 25 December 2008

Correspondence: Z. P. Camargo, Universidade Federal de São Paulo, Departamento de Microbiologia, Imunologia e Parasitologia, Disciplina de Biologia Celular, 04023-062 Rua Botucatu, 862/8º andar, São Paulo, SP, Brazil. Tel: +55 11 5576 45 23. Fax: +55 11 5571 58 77. E-mail: zpcamargo@unifesp.br

molecular phylogenetic analysis of our isolates and identification was inferred from DNA sequence data from the three different loci mentioned above.

The ideal method for correct identification of microorganisms, particularly in clinical laboratories, is one that requires minimum preparation of the samples and allows the samples to be analyzed directly, quickly and accurately at relatively low cost [10]. Recent advances in analytical instrumentation have been achieved through the use of spectroscopic methods based on physico-chemical principles. The most commonly used methods are pyrolysis-mass spectrometry (PyMS) [11], Fourier transform infrared spectroscopy (FTIR) [12,13] and UV resonance Raman spectroscopy [14].

As sporotrichosis is not a reportable disease in Brazil, there is little information concerning its incidence in different states. Brazil is a continental country with an area of 8,514,204.8 km<sup>2</sup> and is divided into five regions with distinct climates, namely, the North, Northeast, Midwest, Southeast and South. Parts of the southeastern and southern regions are influenced by polar air masses, whereas the North and Northeast are influenced by hot air masses and have high annual average temperatures. The strains analyzed in the present study were isolated in 14 of the 26 Brazilian states, indicating that the prevalence of sporotrichosis in the country is higher than we had been predicted.

In recent years we have collected 151 *S. schenckii* isolates from the five Brazilian regions and stored them in our fungal culture collection. When studied by RAPD, these showed a very high genetic diversity, which could not, however, be correlated with either clinical forms of sporotrichosis or geographic region (personal communication). Despite the clinical importance of *S. schenckii*, little is known about the basic biology, population structure and antigenic composition of isolates of this fungus from different Brazilian geographic areas.

Fungal antigens are of great interest because of their use as reagents for the diagnosis of fungal diseases. In general they contain a very small proportion of antigenic material, and the bulk of the preparations are non-antigenic. As researchers very often use different isolates in their studies, results from different laboratories cannot be compared. Therefore, it is of interest to show that *S. schenckii* isolates from different regions and even from the same area are not homogeneous in terms of their protein/glycoprotein expression, making it important to use the same isolate in comparative studies. In this study we used SDS-PAGE to compare the protein patterns of 13 isolates of

*S. schenckii* from the five regions of Brazil. The isolates came from patients with different clinical forms of sporotrichosis, as well as from the environment and from one animal (cat) which were grown in three different culture media. Ten other *S. schenckii* isolates from a more limited geographic area (the state of São Paulo; area = 248,209.4 km<sup>2</sup>) were also studied to determine whether they were more closely related to each other in terms of protein expression than the isolates from distant regions of Brazil. A further reason for using electrophoresis was to achieve better characterization of each isolate of *S. schenckii* in terms of its protein/glycoprotein pattern and to identify the most common molecules among the isolates and/or the specific molecules for each of them.

## Material and methods

### Isolates

Thirteen *S. schenckii* isolates (11 clinical, 1 environmental and 1 cat) from the five different geographic regions of Brazil were used in this study. Another 10 *S. schenckii* isolates from the state of São Paulo were also included. The main characteristics of these isolates, available in the culture collection of the Mycological Division of the Cellular Biology Department, UNIFESP, São Paulo, SP, Brazil are shown in Table 1. The isolates were maintained by periodic transfer, every three months, on Sabouraud-dextrose agar medium (SAB) at room temperature. All of them were identified as *S. schenckii* by their colony morphology on potato dextrose agar (PDA) and brain-heart infusion agar (BHI), as well as by their microscopic appearance, i.e., septate hyaline hyphae, conidiophores, and typical conidia when cultured at 25°C. The dimorphic nature of *S. schenckii* was demonstrated by converting the fungus to the yeast form at 37°C and observing the typical oval to cigar-shaped yeast cells.

### Culture media

*S. schenckii* cultures were grown in minimal medium (MM), Sabouraud medium (SAB) and Gibco medium 199 (M199), as proposed by Mendonza *et al.* [15].

### Inoculum and culture exoantigens

Each isolate was grown at room temperature (20–25°C) for 7 days on SAB and the total growth from three slants (approximately  $2 \times 10^6$  cells; viable cells >85%) was used to inoculate three 500ml flasks containing one of each of the three media. The initial inoculation of all isolates was carried out on the same day so that all

**Table 1** Characteristics of the *Sporothrix schenckii* isolates according to source, clinical forms of sporotrichosis and geographic origin.

Isolate no.	Source and clinical form of Sporotrichosis	Geographic area
Ss 14	Human-disseminated	Southeast (Minas Gerais)
Ss 16	Human-lymphocutaneous	Northeast (Piauí)
Ss 27	Human-lymphocutaneous	South (Paraná)
Ss 42	Human-fixed	Northeast (Ceará)
Ss 48	Human-lymphocutaneous	Midwest (Goiás)
Ss 51	Human-fixed	North (Pará)
Ss 52	Human-fixed	Southeast (São Paulo)
Ss 53	Cat	South (Rio Grande do Sul)
Ss 61	Soil	Southeast (São Paulo)
Ss 68	Human-fixed	Southeast (Rio de Janeiro)
Ss 99	Human-lymphocutaneous	Southeast (Rio de Janeiro)
Ss 104	Human-lymphocutaneous	Midwest (Mato Grosso)
Ss 106	Human-lymphocutaneous	Southeast (Minas Gerais)
Ss 01	Cat	São Paulo-São Paulo
Ss 123	Human-fixed	Campinas-São Paulo
Ss 118	Human-fixed	Campinas-São Paulo
Ss 101	Human-lymphocutaneous	Bauru-São Paulo
Ss 103	Human-lymphocutaneous	Bauru-São Paulo
Ss 111	Human-fixed	São Paulo-São Paulo
Ss 112	Human-fixed	Ribeirão Preto-São Paulo
Ss 148	Human-lymphocutaneous	São Paulo-São Paulo
Ss 52	Human-fixed	São Paulo-São Paulo
Ss 58	Human-fixed	Botucatu-São Paulo

cultures were in the same growth phase. The flasks were incubated at room temperature (20–25°C) with constant agitation (50 rpm) for 10 days in a rotary shaker (ETICA Equipamentos, São Paulo, Brazil). The cultures were killed with Merthiolate (ethylmercurithiosalicylic acid, sodium salt, 0.2 g/l), and the supernatants from each sample culture in each of the three media were filtered through 0.45 µm Millipore membranes (cutoff 10 kDa), dialyzed at 4°C against distilled water (3 changes over 48 h at 4°C) and concentrated 15–20 times in a vacuum system. Protein concentrations present in the filtrates were determined by the method described by Bradford [16].

#### *Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)*

Protein patterns of the *S. schenckii* exoantigen samples (2 µg of protein per slot) from all cultures were analyzed by SDS-PAGE using 10% gels, as described by Laemmli *et al.* [17]. The gels were stained with silver stain, and the relative molecular weights of the fractions were estimated using standard broad range molecular weight markers (Gibco). Analysis of similarity was carried out using the Gel Compar II program. The bands for the different isolates were compared and screened for the

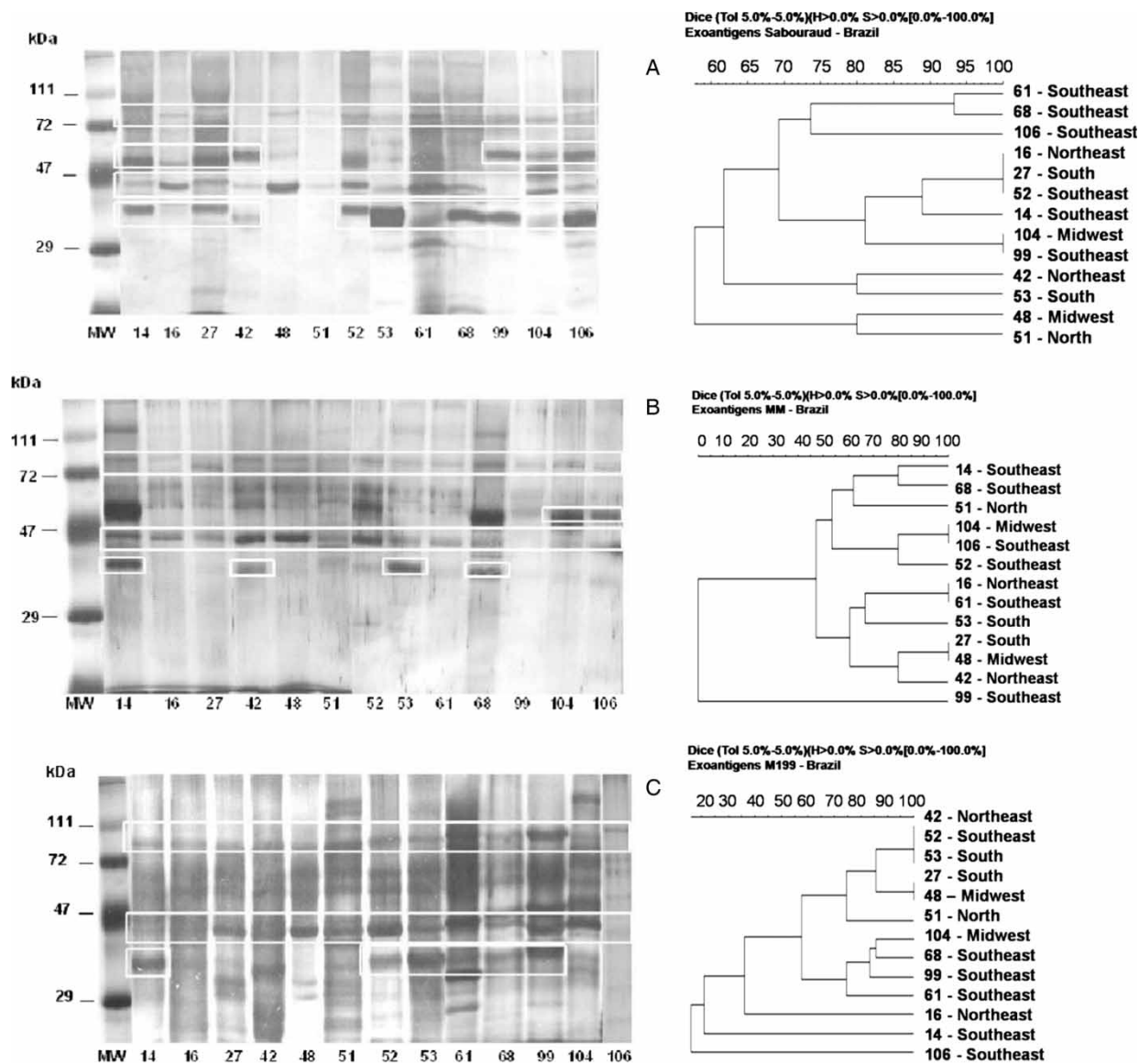
presence or absence of specific bands. These data were then analyzed to obtain an estimate of similarity for each pair of isolates, which was calculated using the Dice coefficient. The similarity matrix was used to construct a dendrogram with the unweighted pair-group method with arithmetic averages (UPGMA).

## Results

### *Protein/glycoprotein profiles of the exoantigens in different media*

Protein concentrations of exoantigens from the 23 different *S. schenckii* isolates ranged from 110–1800 µg/ml<sup>-1</sup> and no correlation between protein concentration and the presence or amount of a specific protein was observed. When exoantigens were analyzed individually by SDS-PAGE, the protein concentration was adjusted to give a total of 2 µg in each sample in order to compare the results.

When the 13 Brazilian *S. schenckii* isolates were analyzed by SDS-PAGE, it was found that the protein profiles of all exoantigens from the three media were complex and exhibited protein fractions varying in density and number. Practically every *S. schenckii* isolate exhibited a distinct protein/glycoprotein profile, showing highly variable protein expression. These characteristics could be observed more easily in the exoantigens from SAB and M199 media. The most diverse protein expressions were observed in exoantigens from the SAB medium. A fraction in the range of 46 kDa was observed in 12 of the 13 exoantigens, but the intensity of the fraction varied according to the isolate. In addition, a fraction of 74 kDa was observed in 8 of the 13 exoantigens, which also varied in intensity with the isolate. Furthermore, a high-intensity fraction of approximately 38 kDa was observed in 7 of the 13 exoantigens and with lesser intensity in 4 of the 13. However, this protein was not present in two of the exoantigens. A band of 54 kDa was also present in 8 of the 13 exoantigens. In the exoantigens from the MM medium, a band in the 80 kDa range was present in all 13 exoantigens, and another in the 46 kDa range was present in 12. An intense band of 58 kDa was present in four of the 13 exoantigens, and another band of 39 kDa was present in four. In the exoantigens from the M199 medium, proteins of approximately 76 kDa and 46 kDa were present in almost all samples, but their intensity varied in each isolate. In addition, an intense band of 33 kDa was observed in six of the 13 exoantigens. Their respective dendrograms are shown on the right side of each figure (Fig. 1A, B, C).



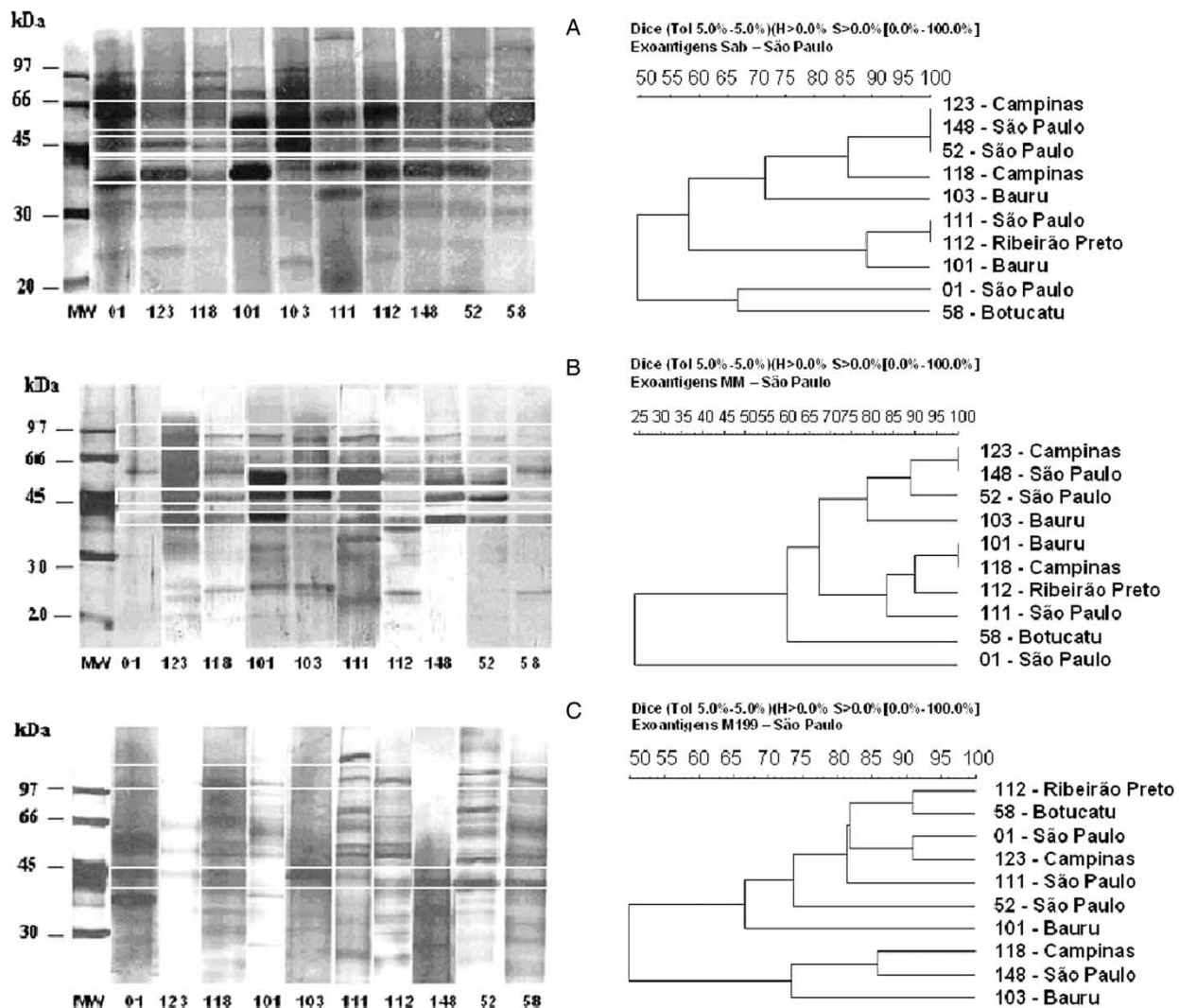
**Fig. 1** Electrophoretic separation in 10% SDS-polyacrylamide gel of 13 Brazilian *Sporothrix schenckii* isolates from different geographic areas. (A) Exoantigen produced in Sabouraud medium; (B) exoantigen produced in Minimal medium; and (C) exoantigen produced in Medium 199. Their respective dendrograms are shown on the right hand side. The molecular weight ladder (MW) is shown on the left.

Analysis of the exoantigens from 10 *S. schenckii* isolates from São Paulo State revealed that the protein profiles varied according to the medium used for the culture. In the SAB medium, two intense fractions of 53 kDa and 38 kDa were observed in eight of the 10 *S. schenckii* exoantigens, and another less intense fraction of 45 kDa was observed in nine. In the MM medium an intense 40 kDa band was observed in seven of the 10 exoantigens, with intense fractions in the 45 kDa and 54 kDa ranges were observed in 7 and 4, respectively. A sharp band of 80 kDa was observed in 9 exoantigens. In the M199 medium, we could observe that all

*S. schenckii* isolates exhibited a higher number of protein fractions, although the most prominent bands in almost all exoantigens (9 of the 10) were a fraction in the 45 kDa range and a sharp 98 kDa band. Their respective dendrograms are shown on the right side of each figure (Fig. 2A, B, C).

## Discussion

Because exoantigens are valuable for the immunoidentification of fungal pathogens and for solving taxonomic problems, knowledge of protein profiles of



**Fig. 2** Electrophoretic separation in 10% SDS-polyacrylamide gel of 10 Brazilian *Sporothrix schenckii* isolates from São Paulo State. (A) Exoantigen produced in Sabouraud medium; (B) exoantigen produced in Minimal medium; and (C) exoantigen produced in Medium 199. Their respective dendrograms are shown on the right hand side. The molecular weight ladder is shown on the left.

pathogenic fungi is crucial both to allow these proteins to be used in serological tests and to gain an understanding of the antigenic variability of these exoantigens. There are a number of studies of the protein and antigen composition of *S. schenckii* in the literature. De Bievre and Prevot [18], studying protein extracts of the filamentous and yeast forms, found differences in their protein composition and described four bands that were specific to the yeast form. Albornoz *et al.* [19], analyzing diverse antigenic preparations of *S. schenckii*, showed the presence of two fractions that were detected in serological tests. Scott and Muchomore [20] demonstrated the importance of certain fractions for the diagnosis of cutaneous and extracutaneous forms of sporotrichosis.

Our results clearly show that almost all the *S. schenckii* isolates in the present study exhibit their own protein/glycoprotein profiles. This must be borne in mind by researchers when results from different laboratories are compared, as is the case in pathogenicity studies or the standardization of antigenic preparations for diagnosis purposes, to mention only a few examples. The culture medium plays a very important role in inducing the production of different molecules in the same isolate. The availability of a suitable, defined medium for the culture and production of *S. schenckii* antigens would facilitate future studies into the metabolic, antigenic and physiological characterization of this fungus. Furthermore, should the researcher be interested in a specific molecule, the

particular medium that favored its secretion could be chosen.

Several studies have demonstrated the usefulness of electrophoretic separation of proteins in clarifying variations within or between fungal species. Many groups of fungi have been studied taxonomically by electrophoresis, including dermatophytes [21], *Candida* [22], *Aspergillus* [23] and *Fusarium* [24]. Species such as *Blastomyces dermatitidis* [25], *Histoplasma capsulatum* [26] and *Fonsecae pedrosoi* [27] and a group of dermatophytes [28] have also been characterized by isoelectric focusing. Numerical taxonomy methods have been used to interpret the results of electrophoresis [29–31].

The potential value of electrophoresis when studying and comparing *S. schenckii* isolates from different geographic areas of Brazil has been shown in the present study. The pattern observed, comprising 15 protein fractions with MWs ranging from 19–220 kDaltons, could be used as a basis for future comparison of isolates. We were able to observe by visual analysis that the protein profile of the 13 Brazilian isolates varied in the number and intensity of bands. Some fractions were produced in great quantities in certain isolates and in lesser quantities in others, a finding that did not vary with the medium in which the exoantigen was produced. The similarity between the isolates was determined using numerical analysis, and the protein patterns were compared two by two, taking into account the presence or absence of the fractions rather than the differences in band densities. The latter varied between the isolates but, remarkably, does not appear to be related to their geographic origin. When isolates from a more limited region – the state of São Paulo – were analyzed, practically the same results were obtained. Our findings suggest that it is not possible to group different *S. schenckii* isolates according to the qualitative or quantitative expression of proteins. In the case of *S. schenckii* from the five different regions of Brazil, various isolates could be grouped in the same cluster. For example, isolates numbers #16, #27, #52, #14, #104 and #99, from the northeastern, southern, southeastern and midwestern regions (exoantigens produced in the SAB medium), which are separated by 1,000–2,000 km, were grouped together. In the case of *S. schenckii* isolates from the restricted area of São Paulo State, isolates numbers #112, #58, #01, #123, #111, #52 and #101, which were cultured in the M199 medium and came from cities 100 to 400 km apart, could be grouped in the same cluster.

This analysis of the proteins expressed by different *S. schenckii* isolates shows that Brazilian isolates are indeed genetically diverse and that the majority of

them express a distinct protein profile. These findings agree with our previous study using RAPD, in which no correlation with geographic area or clinical forms was found.

## Acknowledgements

The authors wish to thank the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior (CAPES) for financial support.

**Declaration of interest:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

## References

- Almeida-Paes R, Pimenta MA, Pizzini CV, et al. Use of mycelial-phase *Sporothrix schenckii* exoantigens in an enzyme-linked immunosorbent assay for diagnosis of sporotrichosis by antibody detection. *Clin Vac Immunol* 2007; **14**: 244–249.
- Barros MBL, Schubach AO, Valle CF, et al. Cat-transmitted sporotrichosis epidemic in Rio de Janeiro, Brazil: description of a series of cases. *Clin Infec Dis* 2004; **38**: 529–535.
- Galhardo MCG, Oliveira RMZ, Valle ACF, 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**: 141–151.
- Mesa-Arango AC, Reyes-Montes MR, Pérez-Mejía A, et al. Phenotyping and genotyping of *Sporothrix schenckii* isolates according to geographic origin and clinical form of sporotrichosis. *J Clin Microbiol* 2002; **40**: 3004–3011.
- Nascimento RC, Almeida SR. Humoral immune response against soluble and fractionate antigens in experimental sporotrichosis. *FEMS Immunol Med Microbiol* 2005; **43**: 241–247.
- Lopes-Bezerra LM, Schubach A, Costa RO. *Sporothrix schenckii* and sporotrichosis. *An Ac Bras Cien* 2006; **78**: 293–308.
- de Meyer EM, de Beer ZW, Summerbell RC, et al. Taxonomy and phylogeny of new wood- and soil-inhabiting *Sporothrix* species in the *Ophiostoma stenoceras-Sporothrix schenckii* complex. *Mycologia* 2008; **100**: 647–661.
- Marinon R, Cano J, Gené J, et al. *Sporothrix schenckii*, *S. globosa*, and *S. Mexicana*, three new *Sporothrix* species of clinical interest. *J Clin Microbiol* 2007; **45**: 3198–3206.
- Marinon R, Gené J, Cano J, et al. Molecular phylogeny of *Sporothrix schenckii*. *J Clin Microbiol* 2006; **44**: 3251–3256.
- Goodacre R, Kell DB. Pyrolysis mass spectrometry and its applications in biotechnology. *Curr Opin Biotechnol* 1996; **7**: 20–28.
- Goodacre R. Characterization and quantification of microbial systems using pyrolysis mass spectrometry: introducing neural networks to analytical pyrolysis. *Microbiol Eur* 1994; **2**: 16–22.
- Goodacre R, Timmins EM, Rooney PJ, Rowland JJ, Kell DB. Rapid identification of *Streptococcus* and *Enterococcus* species using diffuse reflectance-absorbance Fourier transform infrared spectroscopy and artificial neural networks. *FEMS Microbiol Lett* 1996; **140**: 233–239.

- 13 Helm D, Labischinski H, Schallehn G, Naumann D. Classification and identification of bacteria by Fourier transform infrared spectroscopy. *J Gen Microbiol* 1991; **137**: 69–79.
- 14 Nelson WH, Manoharan R, Sperry JF. UV resonance Raman studies of bacteria. *Appl Spectrosc Rev* 1992; **27**: 67–124.
- 15 Mendoza M, Díaz AM, Hung MB, Zambrano EA, Díaz E, De Albornoz MC. Production of culture filtrates of *Sporothrix schenckii* in diverse culture media. *Med Mycol* 2002; **40**: 447–454.
- 16 Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976; **72**: 248–259.
- 17 Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* (London) 1970; **227**: 680–685.
- 18 de Bievre C, Prevot RA. Sur la composition protéique des phases levuriformes et filamenteuses de *Sporothricum schenckii* étudiée para electrophorese en gel d'acrylamide. *C R Acad Sci Paris* 1967; **265**: 537–539.
- 19 Albornoz MB, Cabral N, Villanueva E. Antigenic structure of *Sporothrix schenckii*. *Bol Oficina Sanit Panamericana* 1980; 296–299.
- 20 Scott EN, Muchomore HG. Immunoblot analysis of antibody responses to *Sporothrix schenckii*. *J Clin Microbiol* 1989; **27**: 300–304.
- 21 Schechter Y, Landau JW, Dabrowa N, Newcomer VD. Comparative disc electrophoretic studies of proteins from dermatophytes. *Sabouraudia* 1966; **5**: 144–149.
- 22 Schechter Y. Comparative electrophoresis and numerical taxonomy of some *Candida* species. *Mycologia* 1972; **64**: 841–853.
- 23 Sorenson WG, Larsh HW, Hamp S. Acrylamide gel electrophoresis of proteins from *Aspergillus* species. *Am J Bot* 1971; **58**: 588–593.
- 24 Glynn AN, Reid J. Electrophoretic patterns of soluble fungal proteins and their possible use as taxonomic criteria in the genus *Fusarium*. *Can J Bot* 1969; **47**: 1823–1831.
- 25 Hall NK, Deighton F, Larch HW. Use of an alkali soluble, water-soluble extract of *Blastomyces dermatitidis* yeast-phase cell walls and isoelectrically focused components in peripheral lymphocyte transformations. *Infect Immun* 1978; **19**: 411–415.
- 26 Lancaster MV, Sprouse RF. Analytical and preparative isotachopheresis of histoplasmin purified derivative components *Anal Biochem* 1977; **77**: 158–167.
- 27 Ibrahim-Granet O, De Bievre C, Roman F, Letoffe S. Comparative electrophoresis, isoelectric focusing and numerical taxonomy of some isolates of *Fonsecaea pedrosoi* and allied fungi. *J Med Vet Mycol* 1985; **23**: 253–263.
- 28 Jeffries CD, Reiss E, Ajello L. Analytical isoelectric focusing of secreted dermatophyte proteins applied to taxonomic differentiation of *Microsporum* and *Trichophyton* species (preliminary studies). *Sabouraudia* 1984; **22**: 369–379.
- 29 Berry JA, Franke RG. Taxonomic significance of intraspecific isoenzyme patterns of the slime mold *Fuligo septica* produced by disc electrophoresis. *Am J Botany* 1973; **60**: 976–986.
- 30 Latgé JP, Boucias DG. Intraspecific variability in *Conidiobolus obscurus*. *J Gen App Microbiol* 1984; **30**: 135–150.
- 31 Snider RD, Kramer CL. An electrophoresis protein analysis and numerical taxonomic study of the genus *Taphrina*. *Mycologia* 1974; **66**: 754–772.