Fast and accurate identification of *Sporothrix brasiliensis* using the MALDI-ToF MS methodology

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Introduction and Purpose

Sporotrichosis caused by *Sporothrix brasiliensis* is an emerging disease in Brazil, due to zoonotic transmission from cats. Towards better understanding of eco-epidemiology and targeted treatment, the identification at species level is crucial. The currently available commercial databases for mass spectrometry are unable to identify S. brasiliensis at the species level. In this work we aimed to construct and validate a database for *S. brasiliensis* identification from colonies grown on solid media.

Methods

Eighteen *S. brasiliensis* strains isolated from patients from six different Brazilian states, from July/2013 to September/2021, were analyzed. First, seven strains, previously identified as *S. brasiliensis* by whole genome sequencing (WGS) at the U.S. Centers for Disease Control and Prevention - Mycotic Disease Branch were cultivated in Sabouraud Broth for six days at 37ºC to prepare protein extracts for spectra acquisition in the Microflex™ LT/SH MALDIToF system, according to the manufacturer's instructions.

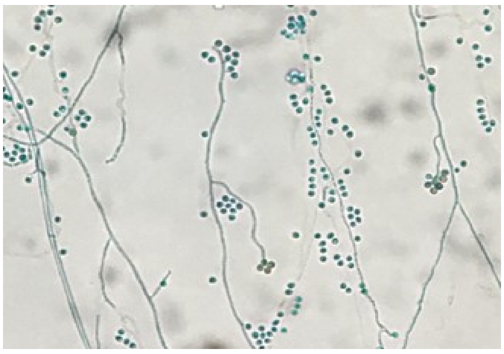

Figure 1 – Microscopy of *Sporothrix brasiliensis*

The obtained spectra were curated and added to a local MALDI-ToF MS library. In order to check the accuracy for species identification, the remaining 11 *S. brasiliensis* strains were used. Eigth of these were formerly identified using WGS and three by ITS region nucleotide sequencing. Isolates were cultivated on Sabouraud Dextrose agar for five days at 37ºC (figure 1). For protein extraction on target plate, after smear preparation and drying the formic acid solution, another drop of 70% formic acid was added. Smears were allowed to dry and matrix solution was added before analysis with the Microflex system.

Results

High quality spectra were successfully obtained and curated (Figure 2). Before adding the spectra to the database, *S. brasiliensis* isolates couldn't be identified to the species level by the Microflex system, all results indicated *S. schenkii* (score ‹ 1.8). When the eleven isolates were retested using the new database, nine were identified as *S. brasiliensis* with scores ranging from 2.01 to 2.33. One of the strains was identified as *S. brasiliensis* with a lower score (1.78) and one couldn't be identified at species level.

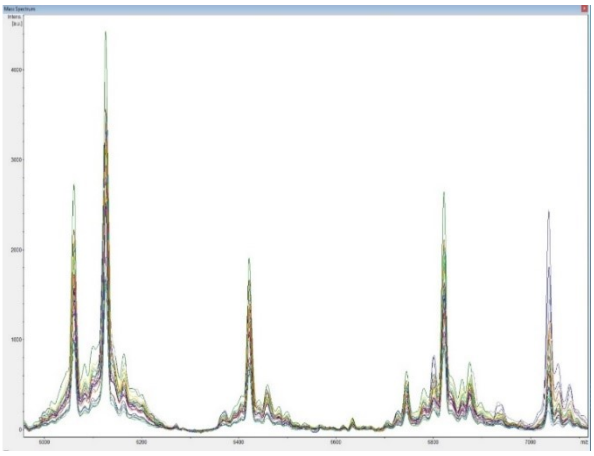

Figure 2 – Spectra of seven strains of *Sporothrix brasiliensis* by mass spectrometry

Conclusions

Insertion of the spectra in the database allowed the correct identification of 81.8% (9/11) of *S. brasiliensis* isolates to the species level using a simple plate extraction method.