**Objectives:**We aimed to characterize Uruguayan clinical and environmental isolates of *Sporothrix* spp. using mass spectrometry and to compare the results with classical phenotypic identification.

**Materials & Methods:
Isolates**Our laboratory receives clinical samples of fungal infections, as well as environmental isolates, for identification. We have maintained *Sporothrix* spp. isolates since 1960, and new strains continue to be added. We currently have a total of 38 preserved *Sporothrix* spp. strains, all initially identified by phenotypic characteristics. The strains were plated on Petri dishes with Sabouraud dextrose agar and incubated at 28°C for 14 days. Colonies were observed macroscopically at 7 and 14 days. Each strain was identified based on the morphological characteristics (macroscopic and microscopic) of the mycelial phase, for which slide cultures were performed.

**Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry (MALDI-TOF MS)**The ethanol/formic acid extraction procedure was followed according to the manufacturer's instructions (Bruker Daltonik GmbH, Bremen, Germany). *Sporothrix* spp. isolates were loaded into a MALDI-TOF mass spectrometer for automated measurement and data interpretation. Spectra were analyzed using MALDI Biotyper™ 3.0 software (Bruker Daltonik, Bremen, Germany) and the CDC MSI and MicrobeNet databases. All isolates were analyzed in triplicate.

The Bruker *Escherichia coli* bacterial test standard (No. 255343) was used for calibration and optimization of instrument parameters, according to the manufacturer's instructions. The MALDI Biotyper was calibrated accordingly.

Results were interpreted based on the following criteria: a score ≥1.7 was considered acceptable for identification at both the species and genus levels. Scores between 1.5 and 1.6 were considered acceptable for genus-level identification only, and scores below 1.5 were deemed unreliable. To establish a different genus or species, a minimum difference of 10% between the highest score and the next closest score was required.

**Results:
Phenotypic Identification**Macroscopic examination revealed a variety of rough, cerebriform colonies with cream, ochre, brown, and black colorations. All isolates developed conidia with a typical acyclic distribution, daisy-shaped and pleuronic. Thirty isolates presented pyriform or oval microconidia, while the remaining eight exhibited round conidia.

**MALDI-TOF MS**We report the first *Sporothrix* spp. spectra for all preserved strains circulating in Uruguay. Spectral analysis using the Bruker MALDI-TOF database identified 35 strains as *S. schenckii*, while 3 strains remained unidentified. Of the 35 identified strains, 24 achieved a high score. Spectral analysis using the MSI database identified non-*schenckii* species, including *S. brasiliensis*, *S. globosa*, *S. variecibatus*, *S. humicola*, among others (Table 1).

**Conclusions:**The results obtained are consistent with the Bruker MALDI-TOF database, as it does not include spectra for non-*schenckii* species. We believe that low scores obtained with Bruker could be key indicators suggesting the presence of non-*schenckii* species. This represents the first experience using MALDI-TOF MS for *Sporothrix* spp. that successfully identifies the circulation of non-*schenckii* species in Uruguay. Additional studies using genomic sequencing are required to confirm these findings.