

TITLE: GENETIC CHARACTERIZATION OF MEDICALLY RELEVANT SPOROTHRIX SPECIES USING AFLP MARKERS

AUTHORS: CARVALHO, J.A.¹; TERRA, P.P.D.¹; PINHEIRO, B.G.²; CAMARGO, Z.P.^{1,2}; RODRIGUES, A.M.²

INSTITUTION: ¹DEPARTAMENTO DE MEDICINA, PROGRAMA DE PÓS-GRADUAÇÃO EM INFECTOLOGIA, UNIVERSIDADE FEDERAL DE SÃO PAULO, SÃO PAULO, SP (RUA BOTUCATU, 862, EDIFÍCIO DE CIÊNCIAS BIOMÉDICAS, 8º ANDAR, CEP 04023-901, SÃO PAULO – SP, BRASIL); ²DEPARTAMENTO DE MICROBIOLOGIA, IMUNOLOGIA E PARASITOLOGIA, DISCIPLINA DE BIOLOGIA CELULAR, PROGRAMA DE PÓS-GRADUAÇÃO EM MICROBIOLOGIA E IMUNOLOGIA, UNIVERSIDADE FEDERAL DE SÃO PAULO, SÃO PAULO, SP (RUA BOTUCATU, 862, EDIFÍCIO DE CIÊNCIAS BIOMÉDICAS, 8º ANDAR, CEP 04023-901, SÃO PAULO – SP, BRASIL)

ABSTRACT:

Sporotrichosis is a mycosis mostly caused by a group of thermomorphogenic fungi embedded in a clinical clade (i.e., *Sporothrix brasiliensis*, *S. schenckii*, *S. globosa*, and *S. luriei*). The infection is caused after a traumatic inoculation of *Sporothrix* propagules into the (sub)cutaneous tissue of mammals. *Sporothrix* species have been reported around the world, mostly in regions with humid (sub)tropical and temperate climates. During the last decades, vast zoonoses have been ongoing in Brazil with Rio de Janeiro as the epicenter of this cat-transmitted epidemic. In this scenario, the development of new molecular markers is mandatory to explore the genetic diversity and track *Sporothrix* expansion during outbreaks. The purpose of this study was to analyze the genetic diversity of *Sporothrix* originating from different geographic regions of Brazil, using AFLP (Amplified fragment length polymorphism) markers. *Sporothrix* spp. genome sequences were retrieved from Genbank and were *in silico*-digested with *EcoRI* and *MseI* using the software AFLP*inSilico*. Only fragments of 50-500 bp were considered. A total of 256 combinations of selective pairs of primers (based on 2 selective nucleotides) were employed to generate 2,304 fingerprints profiles with different fragment numbers and sizes (0 to 56 fragments/per combination). *In silico* analysis highlighted 6 combinations to be tested in a subset of 15 medically relevant *Sporothrix* isolates. *In vitro*, AFLP presented strict concordance with *in silico* results considering the number and size of the amplicons resolved during capillary electrophoresis. A total of 58 AFLP markers were unambiguously scored in the electropherograms using the software BioNumerics v.7.6. Dendrograms were constructed (UPGMA, Jaccard Distance) based on a banding match table and isolates were correctly clustered according to the taxon name. Intraspecific variation was observed for all agents, including *S. brasiliensis*, a species previously known to be clonal. Based on the data above we recommend the use of 3 different AFLP combinations to explore genetic diversity and taxonomy in *Sporothrix*. Independent experiments with similar results showed that the AFLP is highly reproducible, making this technique interesting to evaluate the genetic diversity and the spreading of *Sporothrix*. The present content in this study will enable the development of strategies to control the disease and will contribute to further studies about diversity in *Sporothrix*.

Keywords: *Sporothrix*, sporotrichosis, genetic diversity, AFLP, genetic characterization

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