TITLE: IMPACT OF PRESERVATIVE METHODS IN ISOLATES OF *Sporothrix* spp. CHARACTERIZED BY POLYPHASIC TAXONOMY

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The long-term preservation of microorganisms is essential for the realization of retrospective and prospective studies. The preservative methods should guarantee viability, purity, as well as phenotypic and genotypic stability. However, the selection of the more adequate method needs to take into account the biological characteristics of the species as well as the resources available in the laboratory. Species of *Sporothrix* complex are dimorphic fungi, they are etiologic agents of sporotrichosis, a subcutaneous mycoses widely spread in Rio de Janeiro State, Brazil, a region of hyperendemicity with zoonotic transmission. To elucidate aspects as related to the taxonomy, physiology and virulence of *Sporothrix* spp. it is necessary a long-term maintenance of maintain these fungi in stock for long periods to for future studies. The present study aimed to evaluate the phenotypic and genotypic stability of ten *Sporothrix* spp. isolates after preservation in sterile water, at 4°C and cryopreservation at -70°C for 6, 12, 18, and 24 months. The phenotypic stability was evaluated by tests that distinguish included in the *Sporothrix* species key identification: conidial morphology, colony diameters, thermotolerance and carbohydrate assimilation. Genotypic stability by T3b fingerprinting was performed as well as partial sequencing of calmodulin and *β*-tubulin genes in 0 and 24 months. According to the polyiphasic taxonomy, nine strains are were identified as *S. brasiliensis* and one as *S. schenckii*. All isolates were viable after storage in all methods and times tested, but the preservation in sterile water showed contamination by others fungi when subcultured on potato dextrose agar, which was prevented by fungal re-isolation on Mycosel agar. A change in conidial morphology occurred in one isolate after preservation in sterile water, presenting triangular conidial. Modification in colony diameters occurred in another isolate after preservation in sterile water and at -70°C, but storage at 4°C maintained this parameter stable in all isolates. Analysis of carbohydrate assimilation showed variation after storage in all methods and times evaluated. These variations in colony diameters and carbohydrate assimilation after preservation compromised the identification of species by key identification. Studies about the stability of genotypes of all isolates in different methods of preservation are still in development.

Keywords: Preservation, *Sporothrix* spp., phenotype, genotype

Development Agency: CNPq, FAPERJ, PROEX/CAPES