

Low success rate of recovery of *Sporothrix* spp. from a fungal collection using storage through mineral oil technique

Taxa limitada de sucesso na recuperação de Sporothrix spp. oriundos de micoteca utilizando armazenamento em óleo mineral

Anderson Luis Terçola,¹ Melissa Orzechowski Xavier,¹ Beatriz Mendes Roca,¹ Mariana Umpiérrez Vieira,¹ Mariana Rodrigues Trápaga,¹ Livia Silveira Munhoz,¹ Vanice Rodrigues Poester¹

ABSTRACT

Subject: We aimed to evaluate the recovery rate of *Sporothrix* spp. isolates maintained for years through the mineral oil technique. **Methods:** Two hundred and seventeen isolates of *Sporothrix* spp. from the fungal collection of the Mycology Laboratory of Faculdade de Medicina - Universidade Federal do Rio Grande (FAMED-FURG), Southern Brazil, with a mean period of storage of five years, were included in the study. Isolates were subcultured in duplicate on Sabouraud dextrose agar and on brain heart infusion agar. **Results:** Of the 217 isolates, only 16.6% (n = 36) were recovered. The median period of storage was similar between the viable (n = 36) and non-viable (n = 181) isolates, being 5.8 years and 4.7 years, respectively (p > 0.05). **Conclusion:** Considering the low rate of *Sporothrix* spp. recovery using mineral oil, we reject to use this methodology and strongly recommend another storage methods to guarantee the maintenance of viable *Sporothrix* spp. isolates in fungal cultures.

Keywords: fungal storage; dimorphic fungi; sporotrichosis; fungus storage.

RESUMO

Objetivo: avaliar a taxa de recuperação de isolados de *Sporothrix* spp. mantidos por um longo período (anos) em técnica de armazenamento em óleo mineral. **Métodos:** foram incluídos no estudo duzentos e dezessete isolados de *Sporothrix* spp. oriundos da micoteca do Laboratório de Micologia da Faculdade de Medicina - Universidade Federal do Rio Grande (FAMED-FURG), com um período médio de armazenamento de cinco anos. Os isolados foram subcultivados em duplicata em ágar Sabouraud dextrose e em ágar infusão de cérebro e coração. **Resultados:** dos 217 isolados incluídos no estudo, apenas 16,6% (n = 36) foram recuperados. A mediana de tempo de armazenamento foi semelhante entre os isolados viáveis (n = 36) e não viáveis (n = 181), sendo de 5,8 anos e 4,7 anos, respectivamente (p > 0,05). **Conclusão:** considerando a baixa taxa de recuperação de *Sporothrix* spp. utilizando óleo mineral, não se indica o uso dessa técnica, recomendando-se outros métodos de armazenamento para garantir a manutenção de isolados viáveis de *Sporothrix* spp. em culturas fúngicas.

Palavras-chave: armazenamento fúngico; fungos dimórficos; esporotricose; micoteca.

INTRODUCTION

Sporotrichosis, a worldwide mycosis caused by *Sporothrix* species, is nowadays considered as an emergent skin neglected disease in different countries and areas.^{1,2} The three main species associated with more than 90% of clinical cases are *S. schenckii* stricto sensu, *S. globosa*, and *S. brasiliensis*.²⁻⁴ Distinct patterns of virulence, resistance to commercial antifungal drugs, source of infection, among others factors are described between these species, which impact the epidemiological profile of the disease.¹

Prospecting future research on the public health problematic of sporotrichosis, keep isolates viable in fungal collections are a vital step to the scientific area. Many methods are described for fungal collections including storage in sterile distilled water at room temperature,⁵ cryopreservation in nitrogen at a - 80° C⁶ and mineral oil at room temperature.⁷⁻⁹ Due to low cost, the mineral oil method has widely been used to preserve distinct molds and yeasts, showing rates of success in the recovery of the strains around 80%.⁸

¹ Laboratório de Micologia, Faculdade de Medicina, Universidade Federal do Rio Grande, Campus Saúde – Rio Grande (RS), Brasil.

Autora correspondente: Vanice Rodrigues Poester

Rua Visconde de Paranaguá, 102, Centro, CEP.: 96203-900, Rio Grande – Rio Grande do Sul, Brasil

E-mail: vanicerp@gmail.com

Recebido em 05/05/2024 – Aceito para publicação em 04/08/2024.



However, to dimorphic fungi, such as *Sporothrix* spp. cells preservation, mineral oil has been showing discrepancy in results regarding its efficacy,⁹⁻¹¹ therefore, we aimed to evaluate the viability of isolates of *Sporothrix* spp. preserved in mineral oil for a long period of time from a fungal collection in a hyperendemic area of sporotrichosis in southern Brazil.

METHODS

Sporothrix spp. isolates (n = 217) from the fungal collection of the Mycology Laboratory from the Faculty of Medicine of Federal University of Rio Grande (FURG) recovered from clinical cases of human or animal sporotrichosis (n = 215) or isolates recovered from soil (n = 2) during the period of 2010 to 2018 were included in the study.

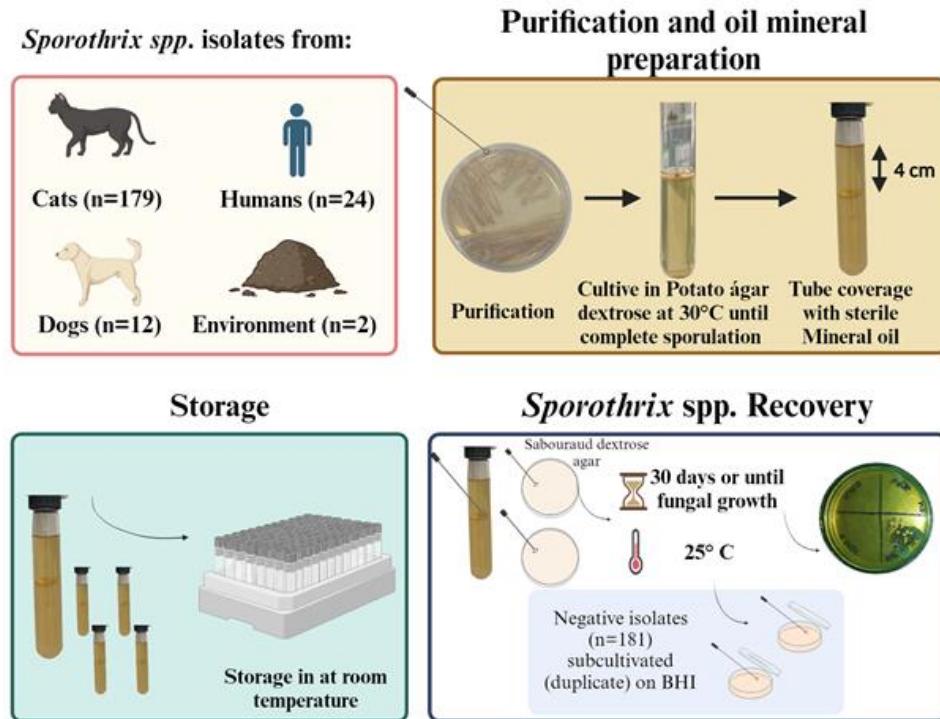
The storage was performed at the diagnosis of the sporotrichosis cases, being the isolates purified and cultured in tubes

with potato dextrose agar, incubated at 30° C for at least 15 days or until complete sporulation. After sporulation colonies were covered by sterile mineral oil, until around 4 cm above the surface, sealed with parafilm and maintained at room temperature (around to 25° C) (Figure 1).

To evaluate the rate of recovery, a fragment of the colonies was cultivated in duplicate in plates with Sabouraud dextrose agar, incubated at 25° C until the fungal growth or for 30 days (Figure 1). The isolates that did not grow were cultured again in duplicate in brain heart infusion agar (25° C until the fungal growth or for 30 days). Data regarding the origin of the isolates (animal, human or environment) and the period of storage were collected.

Results were analyzed by frequency and median. Kruskal Wallis test was performed to evaluate the impact of the origin and period of storage with the recovery rate, using the SPSS 20.0 statistical program (IBM®, New York, USA).

Figure 1. Illustration showing the methods used to analyze the rate of recovery of *Sporothrix* spp. isolates maintained through mineral oil technique. BHI: Brain Heart Infusion ágar.



RESULTS

From the 217 isolates of *Sporothrix* spp., 147 (81%) did not grow, 32 (18%) were contaminated by anemophilous filamentous fungi, 1 (0.5%) was contaminated by bacteria, and 1 (0.5%) by mites' proliferation, being only 16.6% (n = 36) able to be recovered from sterile oil mineral.

The majority of the isolates (n = 179) were from cats (82.5%), 24 from humans (11%), 12 from dogs (5.5%), and 2 (1%) from the environment, and the rate of recovery was

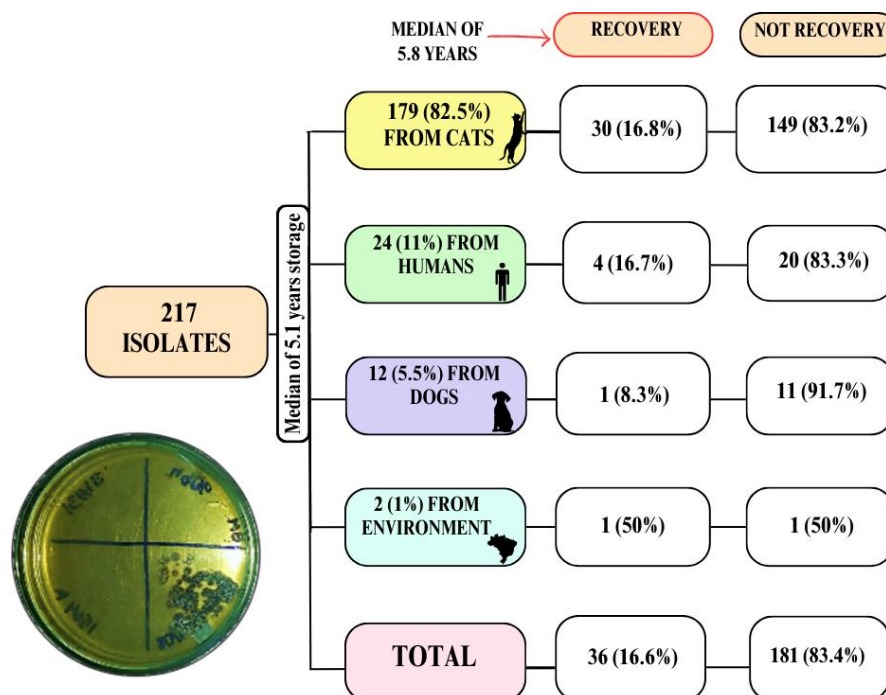
not different between these sources of the isolates ($p > 0.05$) (Figure 2).

The median period of storage of the 217 isolates included in the study was 5.1 years, ranging from 1 to 9 years. The period of storage did not influence the rate of recovery, being viable isolates (n = 36) maintained during a median of 5.8 years, and the non-viable (n = 181) isolates during a median of 4.7 years ($p > 0.05$) (Figure 2).



Todo conteúdo desta revista está licenciado em Creative Commons CC By 4.0.

Figure 2. Rates of *Sporothrix* spp. strains recovery after storage in mineral oil, during a period among 1 to 9 years.



DISCUSSION

Data about the effectiveness of mineral oil applied to *Sporothrix* spp. preservation is poorly available in the scientific literature, and showing disagreement with success rates of recovery ranging from 20%¹⁰ to 85%.¹⁰ Our study contributes with these data and, similar to reported previously,⁹ showed a low effectivity (~ 15%) of mineral oil to preserve *Sporothrix* spp. cells.

Although first studies developed by Borba & Oliveira¹⁰ and Lima & Borba,¹¹ using a fungal collection from Brazil around the '90s, reported high rates of recovery (85% and 69.7%) in isolates stored from a period of 8 to 49 years, another more recent study (in 2022) also developed in Brazil with stored isolates from a period of 34 to 64, showed a recovery rate of 21% more similar to our study (16.6%).⁹

In our study, the majority of non-viable isolates only did not grow, showing the death of *Sporothrix* cells during a long period of storage on mineral oil.

However, we can indicate a possible cause and a limitation of the study, on nine years of storage different people were involved in this process (graduate and post-graduate students, university employees, etc.), which could have caused a variable technique in the initial storage of isolates, mainly regarding the time need for total sporulation of fungi, an essential step for viability in mineral oil. Thus, considering that fungal collections at universities will face changes in Human Resources together with the importance of maintaining isolates viable for long periods to be used in

future research, other preservation methods should be used, such as storage in sterile distilled water and cryopreservation that showed success in preserve *Sporothrix brasiliensis* cells, with a recovery rate of 100%.¹²⁻¹³

CONCLUSION

In conclusion, based on our results mineral oil was a failure method to keep *Sporothrix* spp. isolates viable independently of the period of storage, therefore, we reject to use this methodology and strongly recommend another storage methods to guarantee the maintenance of viable *Sporothrix* spp. isolates in fungal cultures.

Acknowledgments

The authors are grateful to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, numbers 316067/2021-0), Fundação de Amparo à pesquisa do Estado do Rio Grande do Sul (FAPERGS; 24/2551-0000720-2) and to Programa Inova Fiocruz (number PRES-008-FIO-22-2-11). Figure 1 created with BioRender.com.

Conflicts of Interest

All authors declare that they have no conflicts of interest pertaining to this work.



REFERENCES

1. Lopes-Bezerra LM, Mora-Montes HM, Zhang Y, Nino-Vega G, Rodrigues AM, de Camargo ZP, et al. Sporotrichosis between 1898 and 2017: The evolution of knowledge on a changeable disease and on emerging etiological agents. *Med Mycol*. 2018;29:126–43. doi: 10.1093/mmy/myx103.
2. Morgado DS, Castro R, Ribeiro-Alves M, Corrêa-Moreira D, Castro-Alves J, Pereira SA, et al. Global distribution of animal sporotrichosis: A systematic review of *Sporothrix* sp. identified using molecular tools. *Curr Res Microb Sci*. 2022;3:100-40. doi: 10.1016/j.crmicr.2022.100140.
3. Poester VR, Mattei AS, Madrid IM, Pereira JTB, Klafke GB, Sanchotene KO, et al. Sporotrichosis in Southern Brazil, towards an epidemic? *Zoonoses Public Health*. 2018;65:815–21. doi: 10.1111/zph.12504.
4. Rabello VBS, Almeida MA, Bernardes-Engemann AR, Almeida-Paes R, de Macedo PM, Zancopé-Oliveira RM. The historical burden of Sporotrichosis in Brazil: a systematic review of cases reported from 1907 to 2020. *Braz J Microbiol*. 2022;53(1):231-44. doi: 10.1007/s42770-021-00658-1.
5. Castellani A. Viability of some pathogenic fungi in distilled water. *J Trop Med Hyg*. 1939;(42):225-6.
6. Lee JJ. ATCC Preservation Methods: freezing and freeze-drying. *J. Protozool*. 1992;39(5):651. doi: 10.1111/j.1550-7408.1992.tb04871.x.
7. Sherf AF. A method for maintaining *Phytophthora* in culture for long periods without transfer. *Phytopath* 1943;33:330-2.
8. Karabiçak N, Karatuna O, Akyar I. Evaluation of the viabilities and stabilities of pathogenic mold and yeast species using three different preservation methods over a 12-year period along with a review of published reports. *Mycopathologia*. 2016;181:415-24. doi: 10.1007/s11046-016-9985-7.
9. Barreira T, Corrêa-Moreira D, Borba C, Moraes A, Oliveira M. Molecular and phenotypic reidentification of *Sporothrix schenckii* clinical isolates preserved under mineral oil for 34 to 64 years in a culture collection in Brazil. *Curr Res Microb Sci*. 2022;3:100-28. doi: 10.1016/j.crmicr.2022.100128.
10. Borba CM, Silva AMM, Oliveira C. Long-time survival and morphological stability of preserved *Sporothrix schenckii* strains. *Mycoses*. 1992;(35)185-8. doi: 10.1111/j.1439-0507.1992.tb00843.x.
11. Lima F, Borba CM. Viability, morphological characteristics and dimorphic ability of fungi preserved by different methods. *Rev Iberoam Micol*. 2001;18:191-6. doi: 1130-1406/01/10.00.
12. Brilhante RS, Silva NF, Lima RA, Caetano ÉP, Alencar LP, Castelo-Branco DS, et al. Easy storage strategies for *Sporothrix* spp. strains. *Biopreserv Biobank*. 2015;13:131–4. doi: 10.1089/bio.2014.0071.
13. Rabello VBS, Corrêa-Moreira D, Santos C, Pinto TCA, Procopio-Azevedo AC, Boechat J, et al. Preservation methods in isolates of *Sporothrix* characterized by polyphasic approach. *J Fungi (Basel)*. 2022;1:9-34. doi: 10.3390/jof9010034.

Como citar este artigo:

Terçola AL, Xavier MO, Roca BM, Vieira MU, Trápaga MR, Munhoz LS, Poester VR. Low success rate of recovery of *Sporothrix* spp. from a fungal collection using storage through mineral oil technique. *Rev Fac Ciênc Méd Sorocaba*. 2024;26:e66530. doi: 10.23925/1984-4840.2024v26a13.



Todo conteúdo desta revista está licenciado em Creative Commons CC By 4.0.