# PRESERVATION OF DERMATOPHYTES UNDER MINERAL OIL.

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**Key words:** Dermatophytes, mineral oil, preservation.

*RESUMEN*

***[Preservación de dermatofitos bajo aceite mineral]***

*Se analizaron 113 cepas de dermatofitos pertenecientes a la colección de la Micoteca URM del Departamiento de Micología - Universidade Federal de Pernambuco. Los cultivos fueron mantenidos bajo aceite mineral esterilizado, durante un periodo entre 4 y 36 años. Un 88% de estas cepas, estaban viables y con sus caracteristicas morfológicas conservadas. Los resultados obtenidos revelan que este metodo de preservación es adecuado para este grupo de hongos.*

*SUMMARY*:

*One hundred and thirteen samples of dermatophytes belonging to the culture collection of the URM Universidade Federal de Pernambuco were examined. They were maintained under sterilized mineral oil during a 4 to 36 year period. A total 88% of these samples were viable and preserved their normal morphological characteristics. This indicates that the method of preservation in mineral oil is effective and can be used for this group of fungi.*

## INTRODUCTION

Several techniques such as lyophilization (1), liquid nitrogen (2, 3), silica gel (4), mineral oil (5), sterile soil (6), and sterile water (7, 8) have been used for long-term preservation of fungus species. Although effective, each one of them has some limitations. Equipment for liquid nitrogen and lyophilization is expensive and cultures cannot be stored again after being opened.

Soil storage may induce mutation with changes in morphological characteristics and pathogenicity (9). Using silica gel, viability decreases with time of maintenance; consequently, periodic checking and preparation of new cultures onto silica gel are necessary (4). Preservation in sterile water is useful for yeasts and many filamentous fungi (10).

The use of mineral oil for preservation of fungus cultures was introduced by Sherf (5) in 1945. Since then it has been widely employed (11, 12, 13, 14, 15, 16, 17, 18). The advantage of this method is the possibility of longevity of the cultures, low maintenance costs, and its availability of having insect free cultures (4).

At the "Departamento de Micologia, Universidade Federal de Pernambuco, Brasil" this method has been used since its culture collection began in 1954. Today 3360 cultures of many different species are maintained at the Department. The success of this method of preservation of several groups such as dematiaceous ***Hyphomycetes, Basidiomycetes,*** and ***Fusarium*** has been proved (19, 20, 21).

The objective of this paper is to demonstrate the general condition of dermatophyte cultures preserved under mineral oil for several years.

## MATERIALS AND METHODS

One hundred and thirteen samples of dermatophyte fungi maintained at the culture collection of the Departamento de Micologia/Universidade Federal de Pernambuco/Brazil ("Micoteca URM") were studied. The cultures, varying from 4 to 36 years in age, were kept in a Sabouraud plus yeast extract medium (S + Y) and immersed in mineral oil. Most of these fungi were isolated from soil, skin lesions, or scalp and were provided by researchers or from research Institutions.

Inoculum from each oil culture was transferred to a liquid medium (20 g glucose, 10 g peptone, 3 g meat extract, 1000 ml distilled water)for 10 days, and after growth was inoculated into Agar Sabouraud plus yeast extract medium in tubes for 10 to 14 days. Samples from developed colonies were stained with Amann blue for light microscope observation of morphological characters of vegetative and reproductive structures. The identity of each species was confirmed by comparing the material under study with published descriptions (22, 23).

In order to induce sporulation of those cultures which produced only vegetative phases, suspensions of mycelial mass from each culture were transferred to Petri dishes containing garden soil and one of the following baits: horse hair, human hair and nail pieces, all previously sterilized, as modified from Vanbreuseghem technique (24). The plates were kept at room temperature (30°C ± 2) for 20-30 days. Sporulating colonies were transferred to Agar Sabouraud yeast extract medium. All cultures well developed and sporulated were chosen for preservation under three different methods: mineral oil, lyophylization (1), and sterilized water (7).

## RESULTS AND DISCUSSION

From the 113 cultures under study (Table 1), 88% (100) were preserved in good conditions and 12% were not alive after 4-36 years of storage under mineral oil. Most of the viable cultures (52) sporulated in a Sabouraud plus yeast extract medium, and the general aspect of colonies and the morphology of conidia were normal, appearing stable regardless of the duration of storage. However, some cultures sporulated only after being submitted to the modified Vanbreuseghem technique. This was the case of the following cultures: ***Microsporum gypseum*** (1614; 1750; 1751; 1752; 1753; 1754; 1756; 1760; 1761); ***M. canis*** (2660); ***Trichophyton mentagrophytes*** (260; 469; 724; 2240), ***T. rubrum*** (179; 596; 607), ***T. tonsurans*** (178; 450; 610; 679; 680; 691; 700; 731; 735; 771; 1666), ***T. violaceum*** (1842 ); ***T. proliferans*** (2709) and ***Epidermophyton floccosum*** (2668). It was observed that 17% of the viable cultures were not still able to sporulate (Table 1). The reasons why the method works for some but not all cultures are not clear.

Little & Gordon (14) reported the survival of three ***T. tonsurans*** cultures after 6 to 12 years of storage under mineral oil. In this work ***T. tonsurans*** was the species with the highest number of non viable cultures. The difficult preservation of this species could be explained by its affinity for scalp and hairs, which makes it less able to live on synthetic media. Mineral oil storage has been considered a success for preservation of cultures of fungi, especially due to positive results regarding longevity (17, 21) but it was observed that 12% of the studied samples did not show signs of viability. Al Doory (25) mentioned that 87% of some dermatophytes he studied were well preserved after three years under mineral oil. However, cultures of ***T. concentricum, T. gallinae*** and ***T. violaceum*** did not survive more than 1 - 2 years.

In the present case, a 25 years old culture of ***T. violaceum*** ( 1842), was able to sporulate when induced by the modified Vanbreuseghem (24) technique, while cultures of ***T. concentricum*** (699) and ***T. gallinae*** (598), although developing mycelium did not sporulate. It is possible that another technique or changes in cultural conditions would induce sporulation of these cultures.

In general it was observed that viability of the cultures was high (88%) for a storage period from 4 to 36 years. As expected, viability decreased with time but the difference observed between short and long term storage was only a few percentage points (table 2).

Although considered a messy technique by some (17), preservation in mineral oil is an easy and inexpensive process. Contamination is greatly reduced, which allows the repeated use from a single storage tube. However, care must be taken to preserve cultures with a high number of spores, in order to assure enough material for further use after long term storage.

From the positive results obtained for most of the cultures, it can be concluded that mineral oil is still an efficient technique to preserve the viability and sporulation capability of many dermatophyte fungi.

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**Table 1**
**Number of dermatophyte cultures stored under mineral oil at 28 C + 2 for 4 to 34 years.**

| Species | Number of cultures | | | | | |
|---|---|---|---|---|---|---|
| | Tested | Not viable | Viable | Sporulating | Not sporulating | Viability % |
| *Epidermophyton floccosum* (Marz)Lang & Miloch (a) | 5 | 1 | 4 | 4 | 0 | 80 |
| *Microsporum audouinii* Gruby (b) | 2 | 0 | 2 | 0 | 2 | 100 |
| *M. amazonicum* Morais, Borelli & Feo (c) | 1 | 0 | 1 | 1 | 0 | 100 |
| *M. canis* Bodin (d) | 4 | 1 | 3 | 3 | 0 | 75 |
| *M. fulvum* Uriburu (e) | 2 | 0 | 2 | 2 | 0 | 100 |
| *M. gypseum* (Bodin) Guiat & Grigorakis (f) | 33 | 2 | 31 | 30 | 1 | 93 |
| *M. nanum* Fuentes (g) | 2 | 0 | 2 | 0 | 2 | 100 |
| *M. racemosum* Borelli (h) | 0 | 0 | 9 | 9 | 0 | 100 |
| *M. praecox* Rivalier (i) | 1 | 0 | 1 | 0 | 1 | 100 |
| *Tricophyton album* Sab (j) | 2 | 0 | 2 | 0 | 2 | 100 |
| *T. concentrichum* Blanchard (k) | 1 | 0 | 1 | 0 | 1 | 100 |
| *T. gallinae* (Megnin) Silva & Benham (l) *(=M.gallinae)* | 1 | 0 | 1 | 0 | 1 | 100 |
| *T. glabrum* Sab. (m) | 1 | 0 | 1 | 0 | 1 | 100 |
| *T. gloriae* Ajello (n) | 1 | 0 | 1 | 1 | 0 | 100 |
| *T. mentagrophytes* (Robin) Blanch (o) | 13 | 0 | 13 | 13 | 0 | 100 |
| *T. phaeseoliforme* Borelli (p) | 1 | 0 | 1 | 1 | 0 | 100 |
| *T. proliferans* English & Stockdale (q) | 1 | 0 | 1 | 1 | 0 | 100 |
| *T. rubrum* (Cast.) Sab. (r) | 9 | 1 | 8 | 8 | 0 | 100 |
| *T. schoenleinii* (Lebert) Lang & Miloch (s) | 1 | 0 | 1 | 0 | 1 | 100 |
| *T. soudanense* (Joyeux ) Vanbreu-seghem (t) | 1 | 0 | 1 | 0 | 1 | 100 |
| *T. tonsurans* Malmsten (u) | 18 | 8 | 10 | 9 | 1 | 55 |
| *T. verrucosum* Bodin (v) | 2 | 0 | 2 | 0 | 2 | 100 |
| *T. vinosum* Sab. (x) | 1 | 0 | 1 | 0 | 1 | 100 |
| *T. violaceum* Sab. (z) | 1 | 0 | 1 | 1 | 0 | 100 |
| **TOTAL** | **113** | **13** | **100** | **83** | **17** | |

* Letters = number of cultures at Micoteca URM - a) 569, 2668, 2873,3172, 3195; b) 229, 701; c) 2732; d) 465, 2660, 2766, 2877; e) 2710, 2740; f) 342, 1614, 1623, 1750, 1751, 1752, 1753, 1754, 1756, 1757, 1759, 1760, 1761, 1762, 1775, 1786, 1787, 1788, 1789, 1790, 1791, 1793, 1794, 1992, 2482, 2708, 2730, 2738, 2739, 2741, 2771, 2779, 2876; g) 1368, 2090; h) 2711, 2712, 2728, 2729, 2731, 2733, 2735, 2736, 2742; i) 2734; j) 298, 594; k) 699; l) 598; m) 614; n) 2798; o) 260, 469, 676, 724, 2240, 2772, 2774, 2777, 2781, 2782, 2784, 2785, 2875; p) 2082; q) 2709; r) 179, 596, 607, 719, 1665, 2236, 2669, 2874; s) 245; t) 636; u) 242, 450, 521, 610, 679, 680, 691, 700, 730, 731, 734, 735, 738, 769, 771, 885, 1277, 2822; v) 723, 727; x) 612; z) 1842.

**Table 2**
**Viability of cultures maintained under mineral oil considering three storage periods**

| Storage period (years) | # of studied cultures | # of viable cultures | viability (%) |
|---|---|---|---|
| 04-08 | 37 | 37 | 92 |
| 09-25 | 06 | 05 | 83 |
| 26-36 | 70 | 61 | 87 |
| TOTAL | 113 | 100 | - |
| AVERAGE | | | 87 |

## REFERENCES


1. **Raper, K. B. & Alexander, D. R.** (1945). Preservation of mold by lyophil process. Mycologia 37: 499-525
2. **Hwang, S.W.** (1966). Long-term preservation of fungus cultures in liquid nitrogen refrigeration. Appl. Microbiol. 14: 784-788
3. **Hwang, S. W.** (1968). Investigation of ultra-low temperature for fungal cultures I. An evaluation of liquid-nitrogen storage for preservation of selected fungal cultures. Mycologia 60: 613-621
4. **Smith, D. & Onions, H. S.** (1983). A comparison of some preservation techniques for fungi. Trans. Br. Mycol. Soc. 81: 535-540
5. **Sherf, D.** (1943). A method for maintaining *Phytomonas sepedonica* for long periods without transfer. Phytopath. 23: 330-332
6. **Bakerspiegel, A.** (1953). Soil as a storage medium for fungi. Mycologia 45: 596-604
7. **Castellani, A.** (1939). Viability of some pathogenic fungi in distelled water. J. Trop. Med. and Hyg. 24: 270-276
8. **Baldasserini, G. & Munguelluzzi, C.** (1965). L'impiego dell'acqua destillata per il mantenimento in coltura dei dermatofiti e dei lieviti Mycophathol. et Mycol. Appl. 27: 110-112
9. **Booth, C.** (1971). I. Introduction to general methods. II. Fungal culture media. In : Methods of Microbiology. vol 4 (ed. C. Booth) London, Academic Press.pp.1-194
10. **McGinnis, M. R.; Padhye, A. A., & Ajello, L.** (1974). Storage of stock cultures of filamentous fungi, yeasts, and some aerobic Actinomycetes in sterile distilled water. Appl. Microbiol. 28: 218-222
11. **Buell, C. B. and Weston, W. H.** (1947). Application of the mineral oil conservation method to maintaining collections of fungus cultures. Amer. J. Bot. 34: 555-561
12. **Norris, D.** (1944). Preservation of tube cultures of fungi and bacteria with liquid parafin. J. Austral. Agr. Sci. 10: 77
13. **Stebbing, M. E. & Robbins, W. J.** (1949). Mineral oil fungus cultures. Mycologia 41: 632-636
14. **Little, G. N. & Gordon, M. A.** (1967). Survival of fungal cultures maintained under mineral oil for twelve years. Mycologia 59: 733-736
15. **Perrin, W. P.** (1979). Long-term storage of cultures of wood-inhabiting fungi under mineral oil. Mycologia 84: 867-869
16. **Fennel, O. T.** (1960). Conservation of fungi cultures. Bot. Rev. 26: 79-141
17. **Smith, D., & Onions, A. G. S.** (1983). The preservation and maintenance of living fungi. Kew, CMI 51.
18. **Kobayashi, T.** (1984). Maintaining cultures of *Basidiomycetes* by mineral oil method. I. Bull. for and For. Rod. Res. Inst. 325: 141-147
19. **Fernandes, M. J. S.** (1982). Preservaçao dos fungos Dematiaceae em óleo mineral. I. Rev. Microbiol. 13: 211-214
20. **Cavalcanti, M. A. Q.** (1990). Viabilidade de culturas de *Basidiomycotina* preservados em oleo mineral. Rev. Lat. Amer. Microb. 32: 265-268
21. **Lima, D. M. M.** (1991). Preservaçao de especies de Fusarium sob óleo mineral. Pesq. agropec. bras. 26: 853-855
22. **Ajello, L.** (1968). A taxonomic review of the dermatophytes and related species. Sabouraudia 6: 147-159
23. **Verna, L. C., & Herrero, F. J.** (1952). Micologia, Morfologia, Biologia Experimentation. Buenos Aires, El Ateneo.
24. **Vanbreuseghem, R.** (1966). Guide pratique de mycologie Midicale et Vitirinaire. Paris, Masson.
25. **Al-Doory, Y.** (1968). Survival of dermatophyte culture maintained on hair. Mycologia 69: 721-723