Fungi preservation in distilled water[*]
Preservação de fungos em água destilada[*]

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Abstract: BACKGROUND - Mycology collections are used to preserve fungi for future studies or to obtain extracts, drugs and others. Fungal preservation in culture media by successive sampling requires care and is not always easy. Novel techniques are necessary to maintain viable fungi for longer periods.
OBJECTIVE – To evaluate the efficacy of preservation of fungi in distilled water for 12 months.
METHODS – 43 species of fungi were maintained in glass flasks with sterile distilled water. Two hundred to 250 _l of the liquid were monthly collected and inoculated in potato dextrose agar to evaluate growth and viability of the fungi.
RESULTS – All fungi grew every month during one year.
CONCLUSIONS – Preservation of fungal strains in distilled water allowed, in a short time interval, to prove viability and sporulation capacity of the fungi. Preservation of fungi in distilled water is a cheap and practical method to maintain a mycology collection.
Keywords: Culture; Distilled water; Fungi

Resumo: *FUNDAMENTOS – As micotecas são coleções de fungos conservadas para estudo futuro ou para a obtenção de extratos, drogas e outros fins. A conservação em meios de cultura por repiques sucessivos exige cuidados e nem sempre é fácil. Há necessidade de novas técnicas para a manutenção de fungos viáveis por longos períodos.*
OBJETIVO – avaliar a eficácia da preservação de fungos em água destilada num período de 12 meses.
MÉTODOS – 43 espécies de fungos foram mantidas em frascos de vidro com água destilada estéril. Mensalmente eram colhidos de 200 a 250μl do líquido e inoculado em ágar batata dextrose para avaliar o crescimento e viabilidade dos fungos.
RESULTADOS – houve crescimento dos fungos em todos os meses pelo período de um ano.
CONCLUSÕES – A preservação de cepas pelo método da água destilada possibilitou, nesse curto espaço de tempo, provar a viabilidade e a capacidade de esporulação das cepas submetidas ao estudo. A conservação de fungos em água destilada é método barato e prático para manutenção de micoteca.
Palavra-chave: Água destilada; Cultura; Fungos

INTRODUCTION

Fungi have a great importance in the field of microbiology, for they affect all living beings. There is a myriad of infections caused by these agents, ranging from superficial mycoses to diseases with high morbidity and mortality rates.[1] On the other hand, various medications, conservants and other drugs are obtained from fungi.[1] Given the increase of diseases with immunological compromising, such as the Acquired Immunodeficiency Syndrome, many opportunistic fungal infections have become prevalent.[2]

Maintenance of these fungi in certain culture media requires a lot of care, because they are quickly consumed and require frequent samplings, demanding time and allowing contamination and decrease in virulence.[3,4] Due to these difficulties, alternative methods for fungal conservation have been developed, e.g., mineral oil,[5] sand,[6] silica gel,[5] dried host tissues[5] and distilled water.[7,8,9] Liophilization[10] and conservation in liquid nitrogen[11,12] are other alternatives, even though results are varied.

At the Dermatology Department at Padre Bento Hospital Complex in Guarulhos, fungi are maintained by means of the periodical sampling technique.[2] Given the difficulties of this technique, the present study was carried out to evaluate the viability of various colonies maintained for a one-year period in distilled water.

MATERIAL AND METHODS

The studied mycology collection is composed of 43 fungal strains obtained from patients with diverse mycoses, preserved with periodical samplings in potato dextrose water. Samples of these strains were taken and put in several sterile glass flasks containing 4 mL of also sterile distilled water, which were identified and hermetically closed with special rubber tops with aluminum belts (Figure 1). Collected fungi were: *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus sp*, *Aureobasidium sp*, *Aureobasidium hediomar*,

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Basidiobolus ranarum, Circella sp., Cladosporium bantianum, Cladosporium carrionii, Conidiobolus coronatus, Cunninghamella sp, Epidermophyton floccosum, Exophiala dermatitides, Exophiala jeanselmei, Fonsecaea compacta, Fonsecaea pedrosoi, Fusarium sp, Geotrichum candidum, Histoplasma capsulatum, Madurella grisea, Microsporum canis, Microsporum gypseum, Mucor sp, Paracoccidioides brasiliensis, Penicillium nonatum, Penicillium sp, Phialophora verrucosa, Phaeoannellomyces werneckii, Scopulariopsis sp, Rhinocladiella aquaspersa, Rhizopus sp, Scedosporium apiospermum, Scytalidium hialinum, Scytalidium lignicola, Sporothrix schenckii, Trichosporon beigelii, Trichophyton mentagrophytes var. interdigitale, Trichophyton mentagrophytes var. mentagrophytes, Trichophyton raubitschekii, Trichophyton rubrum, Trichophyton schoenleinii, Trichophyton tonsurans, Trichophyton verrucosum, Trichophyton violaceum.

In 30-day intervals, after assepsy with 70% alcohol and in the presence of a flame, samples of the fungi-containing fluids were obtained with insulin syringes (200 to 250 μL), seeded in potato dextrose and put in a conservatory under a temperature ranging from 25 to 30 ºC. Colony growth was observed weekly and material collection was performed monthly for microscopic analysis (staining with lactophenol cotton blue). Microcultivation was performed monthly in a slide, following a standard technique.[13] Identification of fungi followed the international rules for each genus and species.[13] Cultures and microcultivations were photographed for follow-up. This procedure was followed for 12 months.

RESULTS

All 43 fungi conserved in distilled water were recovered in collections for culture. Table 1 shows fungi identified in the cultures obtained from fluid contained in flasks with distilled water in twelve collections.

Figure 2 depicts examples of cultures in potato dextrose Agar of some of the fungi recovered from distilled water. Fungal macroscopic and microscopic morphological features were maintained.

DISCUSSION

Unlike most bacteria, fungi are beings with a slow growth velocity in culture media. On the other hand, many times there is contamination by bacteria or other fungi, which harms conservation of the colonies. Existing techniques for maintenance of mycology collections are work-demanding, expensive and, many times, inefficient. Thus, development of novel forms of fungal preservation for prolonged periods is necessary.[3]

Mycology laboratories are responsible for the diagnosis of fungal infections, as well as conservation of standard strains. Biochemical assays for the identification of more primitive fungi, such as leavens, are known.[2] Nevertheless, up to this moment there is no specific biochemical method for the identification of filamentous fungi. It is done through both macroscopic and microscopic examination, in which the following features are observed: colony growth; color; texture; pulverulent or not, cottonous, filamentous; frutification body; micro or macroconidium and others.[13] Because fungi differ among themselves, a mycology collection is necessary in a mycology laboratory. These collections are constituted by fungal specimens and have led many researchers to improve their techniques of preservation of each of the species of interest for specific areas, hence creating world collections such as the IMI (International Mycological Institute) Kew England; ATCC (American Type Culture Collection) Maryland, USA; CBS (Centralbureau Voor Schimmelcultures) Baar – Delft, Holland; Institute For Fermentation (IFO), Osaka, Japan; Japan Collection of Microorganismos (JMC) Wako, Saitama, Japan;

FIGURE 1: Complete set of the mycology collection in distilled water

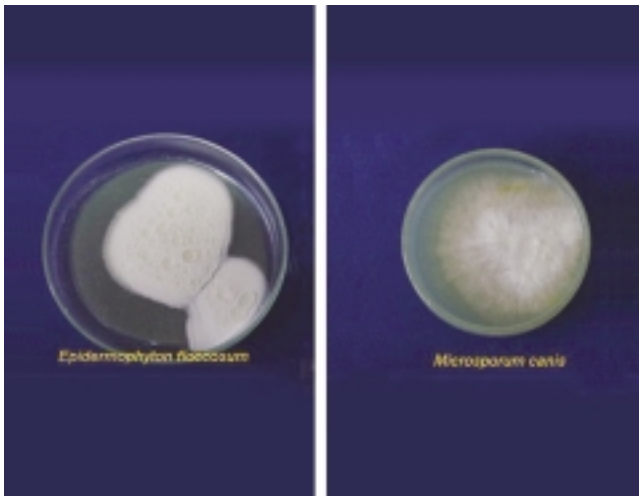

FIGURE 2: Fungal cultures obtained from distilled water

TABLE 1: Fungi recovered from spores conserved in distilled water in a 12-month period

Preservation - distilled water	period from August 2002 to July 2003 - by the distilled water method.											
Period of study	Aug-02	Sep-02	Oct-02	Nov-02	Dec-02	Jan-03	Feb-03	Mar-023	Apr-03	Mar-03	Jun-03	Jul-02
microorganism under study												
Aspegillus fumigatus	1	1	1	1	1	1	1	1	1	1	1	1
Aspergillus niger	1	1	1	1	1	1	1	1	1	1	1	1
Aspergillus sp	1	1	1	1	1	1	1	1	1	1	1	1
Aureobasidium sp	1	1	1	1	1	1	1	1	1	1	1	1
Aureobasidium hediomar	1	1	1	1	1	1	1	1	1	1	1	1
Basidiobolus ranarum	1	1	1	1	1	1	1	1	1	1	1	1
Circinella	1	1	1	1	1	1	1	1	1	1	1	1
Cladosporium bantianum	1	1	1	1	1	1	1	1	1	1	1	1
Cladosporium carrionii	1	1	1	1	1	1	1	1	1	1	1	1
Conidiobolus coronatus	1	1	1	1	1	1	1	1	1	1	1	1
Cunninghamella sp	1	1	1	1	1	1	1	1	1	1	1	1
Epidermophyton floccosum	1	1	1	1	1	1	1	1	1	1	1	1
Exophiala jeanselmei	1	1	1	1	1	1	1	1	1	1	1	1
Fonsecaea compacta	1	1	1	1	1	1	1	1	1	1	1	1
Fonsecaea predosoi	1	1	1	1	1	1	1	1	1	1	1	1
Fusarium sp	1	1	1	1	1	1	1	1	1	1	1	1
Geotrichum candidum	1	1	1	1	1	1	1	1	1	1	1	1
Histoplasma capsulatum	1	1	1	1	1	1	1	1	1	1	1	1
Madurella grisea	1	1	1	1	1	1	1	1	1	1	1	1
Microsporum canis	1	1	1	1	1	1	1	1	1	1	1	1
Microsporum gypseum	1	1	1	1	1	1	1	1	1	1	1	1
Mucor sp	1	1	1	1	1	1	1	1	1	1	1	1
Paracoccidioides brasiliensis	1	1	1	1	1	1	1	1	1	1	1	1
Penicillium nonatum	1	1	1	1	1	1	1	1	1	1	1	1
Penicillium sp	1	1	1	1	1	1	1	1	1	1	1	1
Phialophora verrucosa	1	1	1	1	1	1	1	1	1	1	1	1
Phaeoannellomyces werneckii	1	1	1	1	1	1	1	1	1	1	1	1
Scopulariopsis sp	1	1	1	1	1	1	1	1	1	1	1	1
Rhinocladiella aquaspersa	1	1	1	1	1	1	1	1	1	1	1	1
Rhizopus sp	1	1	1	1	1	1	1	1	1	1	1	1
Scedosporium apiospermum	1	1	1	1	1	1	1	1	1	1	1	1
Scytalidium hialinum	1	1	1	1	1	1	1	1	1	1	1	1
Scytalidium lighinicola	1	1	1	1	1	1	1	1	1	1	1	1
Sporothrix schenckii	1	1	1	1	1	1	1	1	1	1	1	1
Trichosporon beigelii	1	1	1	1	1	1	1	1	1	1	1	1
Trichophyton mentagrophytes interdigitale	1	1	1	1	1	1	1	1	1	1	1	1
Trichophyton mentagrophytes var. mentgrophytes	1	1	1	1	1	1	1	1	1	1	1	1
Trichophyton raubitschekii	1	1	1	1	1	1	1	1	1	1	1	1
Trichophyton rubrum	1	1	1	1	1	1	1	1	1	1	1	1
Trichophyton schoenleinii	1	1	1	1	1	1	1	1	1	1	1	1
Trichophyton tonsurans	1	1	1	1	1	1	1	1	1	1	1	1
Trichophyton verrucosum	1	1	1	1	1	1	1	1	1	1	1	1
Trichophyton violaceum	1	1	1	1	1	1	1	1	1	1	1	1

Mycotheque de L'Université Catholique de Luvain (MUCL) Luvain-le-Nueve, Belgium, and others.[5,13]

Subcultivation of fungal samples, from the flasks with prime-isolated strains, met desired goals, being active when, with the aid of an insulin syringe, aliquots containing a quantity equivalent to the variable quantity of 200-250 µl of distilled water containing a suspension of spores were taken. The mentioned quantity was inoculated in a chosen substract, proper for cultivation (potato dextrose agar). By using the proposed methodology, strains kept in a penicillin flask were noticed to occupy a minimal space (Figure 1) in comparison to conditioning in test tube, as in the periodical sampling method, besides the fact that test tubes are fragile, damageable by any slight impact, which disseminates microorganisms and contaminates the environment or even the professional.

With the distilled water technique, the procedure used to obtain preserved microorganisms can be performed several times, using always the same flask, until total use of the liquid. Moreover, space occupied by the whole set of flasks was optimized in relation to the space needed in traditional collections for successive samplings in test tubes. It has also been observed that there is no need to wear out the fluid and remove the preserved strain for its subcultivation, acoording to the original method described by Castellani.[7]

Our mycology collection became a simple kit containing microorganisms necessary for research or clinical interest, kept in penicillin flasks to the side of slides prepared for microcultivation (Figure 1) with their respective strains, showing micelia and frutification bodies.

Preservation of strains by the distilled water method has made it possible, in 12 months, to maintain viability and sporulation capacity.

Macroscopic observation of the strains under study was possible through the utilization of the above-mentioned method. Strains kept their characteristics as described in the literature,[2,5] even those that had already lost their properties because of successive samplings.

Regarding microscopic analyses of the strains, as to viability of micro and macroconidia, mycelia and frutification bodies, it was noticed that even several samplings did not alter features inherent to wild strains. In order to confirm such features, we used the microcultivation technique, which showed that microstructures of the studied organisms were typical as assessed by methods used for identification.

CONCLUSIONS

Based on the experiment, it can be concluded that:

1. Strain preservation by the distilled water method has made it possible, in a short period of time, to prove the viability and sporulation capacity of the strains submitted to this study.
2. Maintenance technique in sealed flasks containing distilled water has proven to be efficable for preserving the characteristics of sporulation of fillamentous fungi of medical interest.
3. Conservation of fungi in flasks with distilled water has easy handling, storage and transportation, besides being more economic the traditional method of successive samplings. ❑

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