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Long-Term Preservation of Fungal Isolates in Commercially Prepared Cryogenic Microbank Vials

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Since 1994, 6,198 yeasts and 391 molds belonging to 25 and 37 species, respectively, were stored in Microbank cryogenic vials at ≥−130°C in liquid nitrogen and at −70°C in a freezer. All of the isolates, with the exception of 45 yeasts and 15 dermatophytes, were recovered from both storage temperatures. Good reproducibility was demonstrated for amphotericin B, fluconazole, and voriconazole MICs determined for random isolates.

Long-term preservation of fungal strains is essential for their in-depth study; however, both the viability and the stability of living cells should be ensured during the preservation period. Fungal isolates are usually preserved in water at room temperature (10), an easy and economical procedure introduced for fungi by Castellani in 1939 (5). Because the stability of fungal cells was not ensured by this simple procedure, other methods have been suggested, such as preservation in soil or on oil- or water-covered slants, cryopreservation either in liquid nitrogen or at low temperature (−20 and −70°C) (2–5, 7, 9–11, 14, 16), and lyophilization (the freeze-drying procedure) (1, 15). Cryopreservation in liquid nitrogen and lyophilization are the methods recommended and used by the American Type Culture Collection (1). Although lyophilization of living cells provides a mechanism for stabilizing these cells for long periods of time, this procedure is cumbersome and lengthy and requires expensive equipment. On the other hand, storage in liquid nitrogen vapor (above the liquid at ≥−130°C) is a more convenient and less expensive alternative for long-term storage of living cells. Storage above the liquid nitrogen prevents leakage of the liquid nitrogen into the vials.

The Microbank system (Prolab Diagnostics, Austin, Tex.) consists of sterile vials that contain 25 porous, colored beads and a cryopreservative fluid; this system was originally developed for storage of bacterial cells (8). The beads are acid washed, and their porous nature allows the cells to adhere to the bead surface; the beads serve as carriers for the cells being stored (Microbank package insert). When an isolate is stored in this way, 25 or more identical cultures can be preserved. The purpose of this study was to evaluate the preservation in Microbank sterile vials of yeast and mold clinical isolates that were received from 1994 to the end of 2002 at the VCU Medical Center (Richmond, Va.) and the Valme University Hospital (Seville, Spain). Two temperatures (≥−130°C [liquid nitrogen vapor] and −70°C [freezer]) were evaluated.

Fresh, pure cultures of 6,198 yeast and yeast-like organisms and 391 molds (Table 1) were grown on either Sabouraud dextrose agar (for yeasts) or potato dextrose agar (for molds) at 35°C; some isolates of dermatophytes, *Histoplasma capsulatum, Blastomyces dermatitidis,* and *Alternaria* spp. were incubated at 30°C. Yeast and yeast-like isolates were incubated for 48 to 72 h, and molds were incubated for 7 to 15 days. Each isolate was stored in accordance with the directions of the manufacturer. For each isolate, the cryogenic fluid of two Microbank vials was inoculated with the fungal growth to a density approximately equivalent to a McFarland standard of 3 or 4. The inoculated fluid was mixed four or five times to emulsify the suspension and to bind the cells to the beads. The extraneous cryogenic fluid was then removed, leaving the inoculated beads as free of liquid as possible to prevent the beads from sticking together during freezing but allowing a thin layer of suspension to stay at the bottom of the vial. The vials were then held overnight at −70°C. After overnight freezing, one of the vials was stored in liquid nitrogen vapor (≥−130°C) and the other was left at −70°C.

The viability and purity of the strains were monitored immediately after storage, at 1 and 6 months after storage, and once a year subsequently as follows. One of the inoculated beads was removed under aseptic conditions with a sterile needle, and each vial was returned immediately to the corresponding low temperature; the bead was then inoculated onto either Sabouraud dextrose agar or potato dextrose agar for at least 20 days. Both the viability and the morphological characteristics of each culture were observed.

Each mold isolate was considered viable if the rate of growth present was the same as that of the original culture and if the morphology and color of the colony matched the fungal identification documented for each strain. All of the mold strains, with the exception of 15 (of 61) dermatophyte isolates, were recovered each time from both storage temperatures and showed the initial colony characteristics, growth rates, and morphologies (Table 1). Only isolates of *B. dermatitidis, H. capsulatum,* and *Alternaria* spp. required more than one bead for harvesting; they required two to four beads. These results are in agreement with those described by Chandler (6), who preserved 50 uncommon molds for 18 months in Microbank vials and found that only one bead was necessary for the recovery of most isolates. Each yeast strain was considered viable if growth was present; the identification and purity of

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TABLE 1. Fungal isolates preserved between 1994 and 2002

Species	No. of isolates stored	No. (%) of isolates not recovered
Yeasts and yeast-like organisms		
Candida albicans	4,453	5
Candida ciferrii	5	0
Candida dubliniensis	42	28
Candida famata	28	0
Candida glabrata	359	0
Candida guilliermondii	33	0
Candida humicola	6	0
Candida kefyr	10	0
Candida krusei	279	5
Candida lambica	20	0
Candida lipolytica	5	0
Candida lusitaniae	43	0
Candida parapsilosis	352	3
Candida rugosa	3	0
Candida tropicalis	401	2
Candida zeylanoides	14	0
Cryptococcus neoformans	112	1
Cryptococcus albidus	3	0
Cryptococcus laurentii	9	0
Hansenula anomala	4	0
Sporobolomyces salmonicolor	1	0
Trichosporon beigelii	2	0
Rhodotorula rubra	6	1
Rhodotorula glutinis	1	0
Saccharomyces cerevisiae	7	0
Total yeast and yeast-like organisms	6,198	45 (0.7)
Moniliaceous molds		
Aspergillus fischeri	1	0
Aspergillus flavus	24	0
Aspergillus fumigatus	117	0
Aspergillus niger	10	0
Aspergillus nidulans	9	0
Aspergillus terreus	27	0
Aspergillus sydowii	1	0
Aspergillus versicolor	1	0
Fusarium incarnatum	1	0
Fusarium moniliforme	5	0
Fusarium solani	10	0
Fusarium oxysporum	5	0
Paecilomyces lilacinus	9	0
Rhizopus arrhizus	7	0
Rhizomucor pusillus	1	0
Trichoderma longibrachiatum	4	
Dematiaceous molds		
Alternaria spp.	4	0
Bipolaris hawaiiensis	3	0
Bipolaris spicifera	3	0
Cladophialophora bantiana	8	0
Cladosporium cladosporioides	1	0
Curvularia spp.	4	0
Dactylaria gallopava	3	0
Exophiala jeanselmei	6	0
Exophiala spinifera	2	0
Phoma sp.	1	0
Scedosporium apiospermum	17	0
Scedosporium prolificans	10	0
Wangiella dermatitidis	9	0
Dimorphic molds		
Blastomyces dermatitidis	5	0
Histoplasma capsulatum	5	0
Penicillium marneffei	17	0
Dermatophytes		
Epidermophyton floccosum	4	2
Microsporum canis	12	3
Microsporum gyseum	6	1
Trichophyton mentagrophytes	26	2
Trichophyton rubrum	13	7
Total molds	391	15 (3.8)

yeasts were also randomly validated on CHROMagar medium. A very small percentage of yeasts (0.7%) were not recovered; *Candida dubliniensis* had the lowest recovery rate (33%; 28 of the 42 isolates were not recovered). The stability was validated by determining the antifungal susceptibilities of random samples of yeasts (200 isolates) and molds (50 isolates) stored at both temperatures. Amphotericin B, fluconazole, and voriconazole MICs were determined by following NCCLS guidelines (documents M27-A2 [for yeasts] and M38-A [for molds]) (12, 13) before storage and 6 months and 4 years after preservation. MICs for the isolates after storage were either the same as, or within three dilutions of, the MICs before storage, which is the criterion used in NCCLS studies to obtain percentages of intra- and interlaboratory reproducibilities as well as for establishing quality control MIC ranges (12, 13). In general, the effects of both storage temperatures on the stability and viability of stored isolates were similar, which is fortunate because most laboratories have a −70°C freezer.

The advantage of using the Microbank system over other cryogenic systems (4, 7, 9, 11, 14, 16) is its commercial availability. The time-consuming procedure of preparing other preservative devices such as drinking straws (16) or cryogenic fluid is avoided; Microbank vials are stored at room temperature prior to use. The harvesting of individual isolates is easier than that described by Pasarell and McGinnis (14), in which a portion of the frozen culture is chipped and subcultured. Because vials should not be outside the low-temperature device for more than 3 min to avoid thawing, it is recommended that the frozen vials be placed in an insulated cryoblock during harvesting.

In conclusion, the Microbank system appears to be an easy, convenient, economical, and effective tool for the preservation of fungal isolates other than dermatophyte and *C. dubliniensis* strains. Longer monitoring of isolates and storage of other species would further validate the reliability of this system for the cryogenic preservation of yeast and mold strains. Also, the stability of fungal cells should be further assessed by molecular parameters.

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