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Storage of Stock Cultures of Filamentous Fungi, Yeasts, and Some Aerobic Actinomycetes in Sterile Distilled Water

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Castellani's procedure for maintaining cultures of filamentous fungi and yeasts in sterile distilled water was evaluated. Four hundred and seventeen isolates of 147 species belonging to 66 genera of filamentous fungi, yeasts, and aerobic actinomycetes were maintained in sterile distilled water at room temperature over periods ranging from 12 to 60 months in four independent experiments. Of the 417 cultures, 389 (93%) survived storage in sterile distilled water. The selection of good sporulating cultures and sufficient inoculum consisting of spores and hyphae suspended in sterile distilled water were the most important factors influencing survival in water over a longer period of time. The technique was found to be simple, inexpensive, and reliable.

Several methods have been proposed for maintaining culture collections of fungi. Among these, dispersal of spores in sterile soil (1), sterile mineral oil overlays (3), deep freezing (4), ultra-low temperature freezing (9), and lyophilization (7) are the most favored. With the exception of the sterile soil and sterile mineral oil overlay techniques, the other methods involve time and expensive equipment.

Castellani (5, 6) reported maintenance of several cultures of human pathogenic fungi and yeasts in sterile distilled water for 12 months without any apparent changes in their morphology or physiology. A slightly modified version of Castellani's method, wherein physiological salt solution was substituted for distilled water and screw-capped bottles were used in place of cotton-plugged test tubes, was described by Benedek (2). Hejtmankova-Uhrova (8) reported successful maintenance of 73 strains of fungi belonging to 13 genera in sterile distilled water for 12 months.

The present study comprises four independent experiments. They were initiated at different times to evaluate Castellani's technique of maintaining fungal cultures in sterile distilled water. The results of these experiments are presented.

MATERIALS AND METHODS

Four hundred and seventeen isolates of 147 species belonging to 66 genera were included. Of these genera, 48 were of filamentous fungi, 15 were yeast genera, and 3 were aerobic actinomycetes.

The viability of fungal cultures stored in sterile distilled water was tested by four independent workers. Even though many of the species and genera investigated were common to all four experiments, the individual isolates selected by each worker differed in each experiment. Some of the genera were represented by several species, and each species in turn included several isolates. In some cases, on the other hand, the genus and species were represented by a single isolate.

The first experiment was initiated in 1969, and covered only 16 isolates of 16 species belonging to 15 genera. The viability of these isolates maintained in sterile distilled water was tested only once, that is, after 60 months of storage. No attempt was made to revive these cultures between 1969 and 1974.

Similarly, the second and third experiments were started in 1970 and 1972, and their results were read only once. The second experiment included 18 isolates of 17 species belonging to 12 genera. The third experiment covered 48 species belonging to 27 genera. Fifty-three isolates were included in the third experiment. The cultures stored in sterile distilled water from these two experiments were revived at the same time that those from the first experiment were cultured for viability.

The fourth experiment, which was started in 1973, included a greater number of cultures than were used in the three previous experiments. One hundred and twenty-six species of 58 genera were represented by 330 isolates. These 330 water cultures were revived after 12 months along with the other cultures from the three previous experiments.

Cultures were inoculated onto slants of potato dextrose agar in screw-capped tubes (20 by 150 mm) and were incubated at 25 C for 2 weeks. Six to seven milliliters of sterile distilled water was pipetted aseptically onto each 2-week-old culture. The spores and fragments of hyphae were dislodged by lightly scraping the aerial growth with the same pipette, and the resultant suspension was withdrawn and trans-

ferred to a sterile glass 1-g vial. The cap of the vial was tightened to prevent evaporation of the water. The labeled vials then were stored at 25 C on laboratory shelves.

For revival of cultures, 0.2 to 0.3 ml of the suspension was withdrawn with a sterile pipette and transferred to a fresh slant of PDA. The cultures were

TABLE 1. *Viability of water-stored cultures after 60 months (experiment 1)*[a]

Species	Viable isolates/ no. tested
Yeasts	
Candida albicans	1/1
Cryptococcus neoformans	1/1
Geotrichum candidum	1/1
Pathogenic molds and actinomycetes	
Cladosporium werneckii	1/1
Epidermophyton floccosum	0/1
Leptosphaeria senegalensis	1/1
Nocardia asteroides	1/1
Petriellidium boydii	1/1
Phialophora jeanselmei	1/1
P. verrucosa	1/1
Trichophyton schoenleinii	1/1
Saprophytes	
Alternaria sp.	1/1
Aspergillus versicolor	1/1
Aureobasidium pullulans	1/1
Cladosporium sp.	1/1
Syncephalastrum sp.	1/1

[a] Total number of genera = 15; total number of species = 16; number of viable isolates/number tested = 15/16; viability = 94%.

TABLE 2. *Viability of water-stored cultures after 48 months (experiment 2)*[a]

Species	Viable isolates/ no. tested
Yeasts	
Candida albicans	1/1
C. tropicalis	1/1
Rhodotorula sp.	1/1
Saccharomyces cerevisiae	1/1
Torulopsis glabrata	1/1
T. capitatum	1/1
Pathogenic molds and actinomycetes	
Actinomadura madurae	1/1
Aspergillus fumigatus	1/1
Epidermophyton floccosum	1/1
Microsporum audouinii	1/1
Sporothrix schenckii	2/2
Trichophyton mentagrophytes	1/1
T. rubrum	1/1
T. verrucosum	0/1
T. violaceum	1/1
Saprophytes	
Acremonium sp.	1/1
Streptomyces sp.	1/1

[a] Total number of genera = 12; total number of species = 17; number of viable isolates/number tested = 17/18; viability = 94%.

TABLE 3. *Viability of water-stored cultures after 24 months (experiment 3)*[a]

Species	Viable isolates/ no. tested
Yeasts	
Candida albicans	2/2
C. krusei	1/1
C. parapsilosis	1/1
C. stellatoidea	1/1
C. tropicalis	1/1
Cryptococcus laurentii	2/2
C. neoformans	1/1
Geotrichum candidum	1/1
Saccharomyces cerevisiae	1/1
Torulopsis glabrata	1/1
Trichosporon cutaneum	1/1
Pathogenic molds and actinomycetes	
Blastomyces dermatitidis	2/2
Cladosporium bantianum	1/1
Coccidioides immitis	1/1
Curvularia lunata	1/1
Dactylaria gallopava	2/2
Epidermophyton floccosum	1/1
Fonsecaea pedrosoi	1/1
Madurella grisea	1/1
Microsporum audouinii	1/1
M. canis	1/1
M. cookei	1/1
M. gypseum	1/1
M. vanbreuseghemii	1/1
Nocardia asteroides	1/1
Paracoccidioides brasiliensis	0/1
Phialophora jeanselmei	1/1
P. verrucosa	1/1
Rhizopus arrhizus	1/1
R. oryzae	0/1
Sporothrix schenckii	1/1
Trichophyton concentricum	0/1
T. gallinae	1/1
T. mentagrophytes	2/2
T. rubrum	1/1
T. schoenleinii	1/1
T. simii	1/1
T. terrestre	1/1
T. violaceum	1/1
Saprophytes	
Alternaria sp.	1/1
Aspergillus tamarii	1/1
A. ustus	1/1
Curvularia sp.	1/1
Fusarium sp.	1/1
Helicostylum piriforme	0/1
Helminthosporium sp.	1/1
Paecilomyces sp.	1/1
Trichoderma viride	1/1

[a] Total number of genera = 27; total number of species = 48; number of viable isolates/number tested = 49/53; viability = 92%.

TABLE 4. *Viability of water-stored cultures after 12 months (experiment 4)*[a]

Species	Viable isolates/ no. tested
Yeasts	
Candida albicans	5/5
C. curvata	1/1
C. guilliermondii	3/3
C. krusei	2/2
C. lipolytica	1/1
C. parapsilosis	3/3
C. stellatoidea	2/2
C. tropicalis	2/2
Cryptococcus albidus	10/10
C. diffluens	1/1
C. laurentii	3/3
C. luteolus	1/1
C. neoformans	12/12
C. terreus	2/2
C. uniguttulatus	1/1
Debaromyces hansenii	3/3
Endomycopsis fibuligera	1/1
Geotrichum candidum	3/3
Hansenula californica	2/2
H. petersonii	1/1
H. polymorpha	2/2
Kluyveromyces fragilis	2/2
Pichia sp.	4/4
P. acaciae	1/1
P. media	1/1
Pityrosporum pachydermatis	1/1
Prototheca sp.	1/1
P. wickerhamii	10/11
P. zopfii	5/5
Rhodotorula sp.	1/1
R. glutinis	2/2
R. rubra	5/5
Saccharomyces cerevisiae	1/1
Sporobolomyces sp.	1/1
Torulopsis glabrata	4/4
Trichosporon cutaneum	3/3
T. capitatum	3/3
T. fermentans	1/1
T. penicillatum	1/1
Total	108/109
Pathogenic molds and actinomycetes	
Actinomadura pelletieri	1/2
Ajellomyces dermatitidis	2/2
Arthroderma ciferrii	1/1
A. uncinatum	1/1
Aspergillus fumigatus	1/1
A. niger	1/1
Cladosporium bantianum	1/1
C. carrionii	5/5
C. werneckii	17/17
Conidiobolus coronatus	0/1
Epidermophyton floccosum	1/1
Exophiala pisciphilus	1/1
E. salmonis	1/1
Fonsecaea pedrosoi	4/4
Histoplasma capsulatum	1/1
Madurella mycetomi	1/2
Microsporum audouinii	1/1
M. canis	4/5
M. cookei	2/2
M. distortum	1/1
M. ferrugineum	1/1
M. gypseum	2/2
M. persicolor	5/6
M. racemosum	1/1
M. vanbreuseghemii	1/1
Mucor pusillus	1/1
Nannizzia incurvata	2/2
Nocardia asteroides	5/5
N. caviae	1/1
Petriellidium boydii	2/2
Phialophora dermatitidis	1/1
P. gougerotii	4/4
P. jeanselmei	9/9
P. parasitica	1/1
P. verrucosa	2/2
Piedraia hortai	0/1
Rhinocladiella mansonii	2/2
Scolecobasidium humicola	3/3
Scopulariopsis brevicaulis	1/1
Sporothrix schenckii	2/2
Streptomyces griseus	1/2
Trichophyton ajelloi	1/1
T. equinum	1/1
T. gallinae	13/23
T. georgiae	1/1
T. megninii	2/2
T. mentagrophytes	12/13
T. rubrum	5/5
T. schoenleinii	1/1
T. soudanense	2/2
T. terrestre	3/3
T. tonsurans	1/1
T. verrucosum	2/2
T. violaceum	0/1
T. yaoundei	0/1
Total	138/158
Saprophytes	
Absidia sp.	2/2
Acremonium sp.	1/1
Alternaria sp.	2/2
Aspergillus clavatus	1/1
A. glaucus	1/1
A. nidulans	4/4
A. terreus	1/1
A. versicolor	1/1
Aureobasidium pullulans	21/22
Beauveria sp.	1/1
Ceratocystis fimbriata	1/1
C. megalobrunnea	1/1
C. stenoceras	1/1
Chaetomium sp.	1/1

TABLE 4. *Continued*

Species	Viable isolates/ no. tested	Species	Viable isolates/ no. tested
Cladorrhinum foecundissimum	1/1	*Penicillium* sp.	1/1
Cladosporium sp.	1/1	*P. claviforme*	1/1
Cunninghamella sp.	2/2	*Phoma* sp.	1/1
Drechslera sp.	1/1	*Rhinocladiella anceps*	1/1
Exophiala brunnea	2/2	*R. atrovirens*	3/3
Hyalodendron sp.	1/1	*Syncephalastrum racemosum*	1/1
Nigrospora sp.	1/1	*Torulomyces* sp.	1/1
Oidiodendron sp.	1/1	*Trichoderma viride*	2/2
Paecilomyces sp.	2/2	Total	62/63

[a] Total number of genera = 58; total number of species = 126; number of viable isolates/number tested = 308/330; viability = 93%.

incubated at 25 C for 3 weeks and were observed periodically for growth. Those cultures that did not grow by the end of 3 weeks were retested with the same procedure. When no growth was observed after the second subculture, the isolates were recorded as not viable.

RESULTS AND DISCUSSION

It became clear from the results of the four experiments (Tables 1 to 4) that the viability of the isolates ranged from 92 to 94%. None of the cultures were found to be contaminated by bacteria or other fungi. All the yeast cultures survived. In addition, several fungi that are notoriously difficult to maintain over an extended period, such as *Aureobasidium pullulans*, showed a better survival rate when stored in water than on conventional media.

About 6 to 8% of the 417 cultures did not survive storage in water. These belonged to the following genera and species: *Madurella mycetomi*, *Paracoccidioides brasiliensis*, *Trichophyton concentricum*, *T. gallinae*, *T. megninii*, *T. verrucosum*, and *T. yaoundii*. All of these species are poor sporulators. On routine media like Sabouraud dextrose agar or potato dextrose agar, the above-mentioned species do not sporulate regularly. A reexamination of the storage vials of these species revealed that the inoculum had been too scanty in most cases. When inocula were adequate in regard to size, and consisted of several wefts of hyphae, even some of the poorly sporulating or nonsporulating species like *T. violaceum*, *T. schoenleinii*, and *M. ferrugineum* survived storage very well.

To insure success, care must be taken to select actively sporulating isolates and to suspend adequate amounts of spores and pieces of hyphae in sterile distilled water for storage in vials. Care must also be taken not to allow the water to evaporate from the vials.

Some of the revived cultures of species with perfect forms were tested for their mating ability after storage in water. The species like *Arthroderma ciferrii* and *Petriellidium boydii* readily formed ascocarps in abundance on oatmeal salts agar. In case of heterothallic species like *Arthroderma uncinatum* and *Nannizzia incurvata*, numerous ascocarps were formed on oatmeal salts agar when the revived cultures were crossed with the opposite mating type strains. In the case of other imperfect species, the revived cultures were comparable to the originals with respect to morphology and sporulation. The storage in water showed supression of pleomorphic changes in many cases, and, in cases of perfect species, showed no loss of mating competence.

Results of the present study clearly show that the method of maintaining fungal cultures in sterile distilled water for extended periods of time is simple, inexpensive, and reliable. The method offers many advantages for laboratories that maintain small culture collections for reference or teaching purposes. The water culture technique is simple, as is the technique for reviving these cultures. The storage space required for the vials is minimal, and, since the vials are stored at room temperature, expensive refrigeration is not needed. The revival technique is less messy than that for cultures stored under mineral oil.

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