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Preservation of fungal cultures under liquid paraffin

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Preservation of 363 cultures belonging to Zygomycetes, Besidiomycetes and Hyphomycetes under liquid paraffin was evaluated. The survival percentage of oil preserved cultures ranged between 91 and 50 depending upon the group of fungi preserved and the storage period. The viable cultures grow more vigorously with better sporulation in comparison to those maintained by periodic transfer.

Key words : Preservation, fungal cultures, liquid paraffin

INTRODUCTION

The preservation of fungi in pure and viable forms, so that all their properties and potential are retained, is essential because of their increase utility in biotechnology. Maintenance and preservation of pure microbial cultures are important for any collection be it small–personal or big service collection. Various methods have been empolyed to meet the demand (Dade, 1960 ; Fennell, 1960 ; Onions, 1971, 1977 ; Smith and Onions, 1983).

The selection of the preservation method adapted depends on the availability of funds and man power. Although freeze drying (for sporulating) and preservation under liquid nitrogen (sporulating as well as mycelial cultures) are most reliable their adaptation may not be possible because of high cost. Preservation under sterile water (Lal and Mathur, 1989) though less cumbersome, less expensive and less messy suffers inherently for lesser durations. Preservation under liquid paraffin is a method which may be reliable at the same time less costly, and may be adapted where funds are limited. In order to study the utility of this method, purified strains belonging to Basidiomycetes (208), Hyphomycetes (89) and Zygomycetes (66) were preserved under liquid paraffin in the year 1993, 1995 and 1987 respectively and were tested for their retrieval after 9, 7 and 15 years respectively in the Indian Type Culture Collection, IARI, New Delhi.

MATERIALS AND METHODS

The cultures were grown on slopes of appropriate media in screw capped universal bottles of 30 ml capacity at suitable temperatures. The media used were potato dextrose agar (PDA), yeastral PDA, potato-carrot-agar (200, 200-.20) and Czapek Dox agar. After attaining growth (about 14 days incubation period), they were covered by 1 cm of sterile liquid paraffin (heavy, medical grade). Approximate quantity of liquid paraffin required to cover the cultures were dispensed in test tubes and autoclaved twice at 121°C for 20 min and then dried in an oven at 60°C till the turbidity was lost. It was then poured over the cultures under sterile conditions and stored at 13-15°C in a cold room.

Viability of the cultures was tested by transferring a small portion of the preserved cultures to fresh media slopes and incubating at suitable temperature. Growth was observed after fifteen days. Cultures showing no growth after repeated subculturing were considered to be non-viable.

RESULTS AND DISCUSSIONS

A majority of fungal cultures responded well to this method of preservation (Table 1). However, strainal differences were quite evident within a species as was also reported by Little and Gordon (1967) and Lal *et al.*, (1989). The strains of some species gave 100% retrieval while in others it was not so.

Species observed with 100 per cent viability under the class Zygomycetes are *Mucor genevensis, M. hiemalis, M. variabilis, M. circinelloides, M. recemosus, Rhizopus oryzae, R. stolonifer, R. microsporus, Absidia glauca, A. coerulea, A. spinosa, A. blakesleeanus, A. corymbifera, A. cylindrospora, Gongronella butleri, Cunninghamella elegans, C. echinulata, C. verticillata, C. blakesleeanus, C. bainieri, Actionmucor elegans, A. repens, Dichotomocladium hesseltine, Circinella muscae, Zygorhynchus moelleri.*

Under the class Hyphomycetes the species are *Trichoderma viride, Trichothecium roseum, Bipolaris papendorfii, Drechslera holmii, D. spicifera, D. sorokiniana, D. rostrata, D. tetramera, D. turcica, D. hawaiienesis, Curvularia lunata, C. senegalensis, Cladosporium cladosporioides, C. sphaerospermum, Epicoccum purpurascens, Myrothecium roridum, Trichophyton simii, Gliomastix murorum, Alternaria alternata, A. solani, A. brassicae, Sclerotium rolfsii, Beauveria bassiana, Metarhizium anisopliae, Nomuraea rileyi, Gonatobotryis simplex, Geotrichum candidum, Tritirachium oryzae, Botrytis cinerea, Stachybotrys atra, Phaeoisa-riopsis griseola, Stemphylium vesicaria, Periconia byssoides, Aspergillus flavus, A. niger, A. stellatus, A. oryzae, A. tamarii, A. parasiticus, A. clavatus, A. ochraceous, A. deflectus and A. fischeri.*

Table 1 : Viability of cultures preserved under liquid paraffin.

Organisms	Storage Period (Yr.)	Percentage of Retrieval
Zygomycotina	15	85
Basidiomycotina	9	
Pleurotus		78.3
Agaricus bisporus		61
Agaricus bitorquis		50
Lentinus		50
Coprinus		71.5
Othes		88.8
Deuteromycotina		
Hyphomycetes	7	87
Aspergilli	15	91

Under the class Basidiomycetes the species are *Pleurotus sajorcaju, P. sapidus, P. ostreatus, P. eryngii, P. flabellatus, P. pulmonaris, P. fossulatus, P. milleri, P. citrinopileatus, Pleurotus Florida, Polystictus xanthopus, Polyporus graminocephalus, Ganoderma lucidum, Schizophyllum commune, Auricularia polytricha, Calocybe indica, Lentinus edodus, Collybia velutipes, Coprinus kimurae, C. comatus, C. cinereus.*

It was observed thermotolerant fungi *Thermomucor pusillus, Volvariella volvaceae* and *V. diplasia* do not respond well to oil preservation.

Little and Gordon (1967) reported 86.5 per cent survival after six years and 75.6 per cent after twelve years of storage at room temperature (18-20°C in winters and 32-34°C in summers). Thirty two years survival of 81 per cent cultures preserved in mineral oil was observed by Smith and Onions (1983).

The subcultures obtained from the preserved cultures of Zygomycetes, Hyphomycetes, Aspergilli grew more vigorously and gave better and profuse sporulation as compared to those maintained by periodic tranasfer, whereas cultures of *Pleurotus, Agaricus, Lentinus* and *Coprinus* thus preserved, showed slow growth as were also observed by Buell and Weston (1947). The subcultures generally gave appressed mycelial growth and normal growth was restored only after 2-3 transfers. Morphological transformations as reported by Little and Gordon (1967) were not observed. This method of preservation certainly reduces the frequency of subculturing, chances of contamination and mite infection, with a majority of cultures surviving for long periods. This method seems to be most suitable for small culture collections and also individuals who have to maintain sizeable number of cultures for their research.

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