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Preservation of cultures under silica gel and paraffin oil - a comparision

MADHU KULSHRESHTHTA AND A. K. SARBHOY
Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi 110012

Culture collections are important resources for fungi. The present investigation was envisaged to compare two techniques i.e. silica gel and mineral oil after 20 and 30 years respectively. After 20 and 30 years, on subculturing the fungi from submerged oil culture was found to survive for a longer time than silica gel technique. Out of 76 fungi only 10 fungi could survive on silica gel while 28 fungi survived under mineral oil.

Key words : Preservation techniques, culture, mineral oil, silica gel

INTRODUCTION

Culture collections are important resources (Hawksworth, 1985; Kirsop, 1983) for fungi there are several techniques employed so far which retain both the viability and stability of these organisms. Fungi are ubiquitous in nature and play an important role, in the biodeterioration of economically important materials of storage products. The present investigation was envisaged to compare two techniques i. e. silica gel and mineral oil after 20 and 30 years respectively.

Growing cultures are particularly subject to airborne contamination. In addition microbial spores, carried by mites may contaminate the cultures and in some instances overgrow the stored organism so that it lost. At the very least, contaminant will necessitate re-isolation of the organism. This is often difficult and if it fails, it may be necessary to find a replacement. The techniques that will maintain long term genetic stability and enable the storage of a wider range of fungi are : Silica gel storage and Mineral oil storage.

MATERIALS AND METHODS

Forty two cultures were maintained on potato dextrose agar medium.

Mineral oil storage

Cultures are prepared on McCartney bottles and covered to a depth of about 1 cm of paraffin of specific gravity. The depth of 1 cm is quite critical (Fennell, 1960) because if the oil was deeper, the fungus may not receive sufficient oxygen and die. If depth is less, exposed mycelium or agar on the sides of container may allow moisture to evaporate and the culture will dry out (Dade, 1960). Mites which may cause contaminants in cultures, cannot penetrate the layer of oil. The screw caps on the tubes are left loosened and the tubes kept in incubator. Cultures preserved in this way should be checked for viability at an interval of 2 to 3 months.

Silica gel storage

This technique was introduced at Commonwealth Agricultural Bureau International Mycological

Institute for preservation of fungi by Onions (1971).

Fungi can successfully dried by this technique while adding spore suspesions to the desiccant silica gel without indicator.

For this technique glass homeopathic bottles or similar with tight caps (rubber seal) were used. Fill bottles with silica gel and sterilize for 90 min. at 180°C. Store in a dessicator until required. Prepare a 5% solution of skimmed milk powder in distilled water, dispense in 2 ml aliquots and autoclaved for 10 min. at 121°C and then store at 4°C. A dense suspension of a fresh culture (mycelium or spores) in skimmed milk was prepared and cool silica gel containers on ice for at least 30 min. Add suspension to the silica gel until culture three quarters is wetted then return bottle to ice for at least 15 min. Leave at room temperature until crystals are readliy separated. Screw cap down firmly and store at room temperature at 4°C. A small sample of crystals can be taken as when reqiured for testing.

RESULTS

After 20 and 30 years, on subculturing the fungi from submerged oil culture of fungi, it was found that out of 76 fungi only 10 fungi could survive on silica gel while 28 fungi survived under mineral oil.

In silica gel only six hyphomycetes and 3 mucorales were able to survive after 20 years while in mineral oil storage only twenty three hyphomycetes and no mucorales were able to survive. None of the Ascomycetes and Coelomycetes could survive in the present studies (Table 1).

Table 1 : Fungi that survived after 20 and 30 years respectively when stored in Silica gel and Mineral oil.

Fungi	Silica gel	Mineral oil
Hyphomycetes		
Aspergillus niger	×	-
Aspergillus carbonum	×	(+)
Aspergillus umbrosus	×	-
Aspergillus terreus	×	-
Aspergillus aurentus	×	-
Aspergillus carbonarius	×	(+)
Aspergillus acanthosporium	×	(+)
Aspergillus luchuensis	×	(+)
Aspergillus sydowi	×	(+)
Aspergillus terreus	×	(+)
Aspergillus flavus	×	-
Aspergillus sp.	(+)	×
Beltrania sp.	×	-
Curvularia lunata	×	(+)
Curvularia sp.	(+)	×
Fusarium moniliforme	×	(+)
Fusarium moniliforme var. *subglutinans*	(+)	×
Epicoccum nigrum	×	-
Graphium sp.	(+)	-
Penicillium frequentans	×	(+)
Penicillium sp.	-	(+)
Penicillium sp.	(+)	×
Pithomyces chartarum	×	-
Alternaria sp.	×	-
Trichoderma harzianum	×	(+)
Trichoderma sp.	×	-
Sporotrichium sp.	×	(+)
Stachybotrys sp.	×	-
Mucorales		
Absidia spinosa	(+)	×
Gilbertella persicaria	×	-
Mucor sp.	(+)	×
Rhizopus arrhizus	(+)	×
Thamnidium elegans	×	-
Ascomycetes		
Chaetomium bostrychodes	×	-
Chaetomium sp.	×	-
Khuskia oryzae	×	-
Coelomycetes		
Colletotrichum sp.	×	-
Pestalotia sp.	×	-

- No growth : (+) Presence of fungal growth ; x Not cultured

DISCUSSION

For preservation of fungi the criteria upon which the decision is normally made are usually the collections, the importance of the individual isolates (Smith and Onions, 1983a). Each isolate is valuable and in some instances may be irreplaceble. Continous growth techniques are unsuitable because of the high risk or variations therefore, storage methods which offer genetic and metabolic stability are essential.

Some of the isolates showed deterioration when stored by many techniques but by far the most variation in the strains was seen in those stored

under oil. Silica gel storage proved almost as valuable as centrifugal freeze drying for the retention of characteristics but fewer strains survived the technique (Smith and Onion, 1983b). *Neurospora* species have been successfully stored for several years by this means (Perkins, 1962). A wide range of fungi have kept their viability in silica gel at CABIMI though it is not a suitable technique for the preservation of mycelial cultures, Oomycetes, *Pythium* or *Phytophthora* (Onions, 1977; Smith and Onions, 1983 a, b), *Helminthosporium maydis* retained its pathogenicity for 12 months when kept in a dehydrated state on silica gel crystals (Sleesman *et al.*, 1974).

Mineral oil technique reduced the metabolic rate of fungi due to limited supply of oxygen. So it has been suceessful with many group of fungi and significantly extended storage periods between transfer to fresh media. Reported storage periods without transfer of up to 32 years for some genera of fungi (Smith and Onions, 1983b), 27 years for wood inhabiting fungi (Perrion, 1979), 21 years of 88 percent of a range of fungi stored at CABIMI (Onions, 1977). Some fungi have been found to be sensitive to storage under oil, the Saprolegniaceae and some water moulds only survive 12-30 months (Reischer, 1949). Onions (1977) recommended 6 monthly subculturing of the CABIMI collection of water moulds and a 2 yearly cycle for some sensitive strains (Smith and Onions, 1983b).

Therefore, from the present study it was revealed there is no technique full proof and one technique is good for sporulating and other is for mycelial fungi. In view of this it is recommended that in preservation of fungi several techniques should be selected for different groups of fungi which suits for their storage.

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