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Research Article

THE CONSERVATION OF FUNGI - AN IMPORTANT ASPECT FOR INDUSTRIAL MICROBIOLOGY

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ABSTRACT
Fungi are known to colonize, multiply and survive in diversified habitats, viz. water, soil, air, litter, dung, foam etc. Fungi are ubiquitous and cosmopolitan in distribution. The movement for fungal conservation must have some basic infrastructure, including components independent from learned societies. There must also be an awareness of how to work in the political arena. The fungal cultures are prepared and preserved for the sake of conservation and hence its further utilization is carried out in the field of microbial biotechnology. It has been found that most of the fungi isolated from soil, or from substrates in the soil, i.e., plant debris, grow well on Corn Meal Agar (CMA). Maintaining and preserving fungal cultures are essential elements of systematic and biodiversity studies. Because fungi are such a diverse group, several methods of cultivation and preservation are required to ensure the viability and morphological, physiological, and genetic integrity of the cultures over time. This paper aims to monitor and safeguard fungal resources worldwide, using the word 'resources' in the broadest sense to encompass natural, human, and collection resources.

INTRODUCTION
The kingdom of fungi contains 1.5 million fungal species, of which 74,000 species are named. Many of the described species are known only as dead herbarium material and around 5% of species are isolated as pure cultures. Geographic location, climatic conditions, micro-habitat, substrate type, distribution of fauna and flora are all important factors contributing to fungal distribution around the world.

The present review article deals with fungal conservation, which is very necessary for industrial microbiology branch and has tremendous scope. The well-being of fungi is necessary for sustainable life on this planet.

Fungi (for example host-specific species known only on rare endemic plants) are often treated as part of the problem (a threat to the plant) rather than recognized as themselves being in need of protection. In many countries there is no explicit legal protection for fungi.

However, the start of the modern fungal conservation movement is in its infancy. The European Council for Conservation of Fungi (now the fungal conservation group of the European Mycological Association) was established in Oslo in 1985, and that event marked the start of the modern fungal conservation movement. Thereafter, specialist groups for "lichens" and "fungi" were set up in the Species Survival Commission of the International Union for Nature Conservation (IUCN), the Australasian Mycological Society formed a continental-level fungal conservation group, and the ground-breaking volume Fungal Conservation: issues and solutions (Moore et al., eds, Cambridge University Press, 2001) drew worldwide attention to the topic.

The movement for fungal conservation must have some basic infrastructure, including components independent from learned societies. There must also be an awareness of how to work in the political arena. In this respect, important and exciting developments have occurred in fungal conservation over the past few years. As in 2005, a pioneering workshop was organized by the European Council for Conservation of Fungi in Córdoba, Spain. This was one of the earliest uses (perhaps the first) for fungi of the IUCN conservation status evaluation system.

In 2007, three prototype specialist committees were established for conservation of fungi inadequately covered by the IUCN's specialist groups of that time:

Mildews, Moulds & Myxomycetes

Non-lichen-forming Ascomycetes

Rusts & Smuts
In 2008, The Mycological Society of America established a continental-level fungal conservation group for North America.
In 2009, The Species Survival Commission of the IUCN formally recognized fungi as needing fully separate representation within the Commission's structure. The Commission also decided to increase the number of Specialist Groups representing fungi to five by adopting the prototype specialist committees described above, and re-defining the old "Fungal Specialist Group" so that it henceforth covered the larger basidiomycetes.
In 2009, The Mycological Committee for Asia, at its Asian Mycological Congress established a continental-level fungal conservation group for Asia.

MATERIALS AND METHODS

The fungal cultures are prepared and preserved for the sake of conservation and hence its further utilization is carried out in the field of microbial biotechnology. A wide range of media are used for growing fungi.

Preparation of CMA (Cornmeal Agar)

Suspend 17 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121ºC for 15 minutes. Cool to 50ºC, mix well and dispense into Petri dishes. The prepared medium should be stored at 8-15°C. The color is opaque and white. The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

Preparation of OA (Oatmeal Agar)

Heat 30 g oat flakes in 1 l distilled water to boiling for 2 h. Filter through cloth and fill up to 1 l. Add 15 g agar and sterilize at 121°C for 15 min.

PCA - Potato Carrot Agar

Potato extract	250ml
Carrot extract	250ml
Agar	15 g
Distil water	500 ml

Preparation

Add potato extract (obtained boiling for 5 min 40 g of potatoes in 1 l of water and filtering off) and carrot extract (obtained boiling for 5 min 40 g of carrots in 1 l of water and filtering off), water and agar and sterilize for 15 min at 120°C.

Fungal Culture Method

Different cultures of fungi can be isolated from different compost and soil samples of any region by serial dilution method. The soil samples are collected in sterile polythene bags. One gram of sample is suspended in 10 ml of sterilized distilled water. The soil suspension can be further diluted up to 102-106 times. One ml of this dilute suspension is then transferred to individual Petri plates containing culture medium. The fungal cultures are further purified from bacterial contaminants by using 10 mg/L combination of penicillin and streptomycin (1:1 ratio) in the medium. All the isolates of fungi are identified by microscopic examination Independent colonies of each identified isolate is picked up and transferred to media containing agar slants for culture maintenance. The cultures are stored in a refrigerator at 4°C for further studies.

Long term preservation methods for fungal conservation

Sclerotization

Some fungi develop sclerotia or other long-term survival propagules in culture as well as in nature; preserving such structures, usually at 3°-5°C, is a good way to preserve fungal strains.

Oil Overlay

A low-cost and low-maintenance method for preserving cultures growing on agar slants is oil overlay. Cultures can be kept for several years or, in exceptional cases, up to 32 years at room temperature or 15°-20°C.

Immersion in Distilled Water

Another inexpensive and low-maintenance method for storing fungal cultures is to immerse them in distilled water. Apparently, the water suppresses morphological changes in most fungi.

Organic Substrata

Over the years, researchers have developed practical, effective, and ingenious methods of preserving fungi on various organic substrata such as wood chips, cereal grains and straw, filter paper, and insect and plant tissues.

Soil or Sand

Some fungi can be preserved easily and successfully for many years in dry, sterile soil or sand. This low maintenance and cost-effective method is appropriate for fungi.

Silica Gel

The silica gel method can be used to preserve sporulating fungi if facilities for freeze-drying or for storage in liquid nitrogen are not available.

Freezing

Most fungal cultures frozen at -20° to -80°C in mechanical freezers remain viable.

Liquid Nitrogen

Storage in liquid nitrogen is an effective way to preserve many, if not most, organisms, including those that cannot be lyophilized.

Lyophilization

Lyophilization, or freeze-drying, a low-cost form of permanent preservation, is not appropriate for all fungi. In fact, the technique is used primarily with species that form numerous, relatively small propagules.

DISCUSSION

Most fungi thrive on Potato Dextrose Agar (PDA), but this can be too rich for many fungi, so that excessive mycelial growth is obtained at the expense of sporulation. It has been found that most of the fungi isolated from soil, or from substrates in the soil, i.e., plant debris, grow well on Corn Meal Agar (CMA), a relatively weak medium compared to PDA. Similarly, wood-inhabiting fungi and dematiaceous (dark pigmented) fungi often sporulate better on CMA or Oat Agar, both of which have less easily digestible carbohydrate than PDA. Cellulose-destroying fungi and spoilage fungi retain their ability to produce cellulase when grown on a weak medium such as Water Agar (WA) or Potato Carrot Agar (PCA) with a piece of sterile filter paper, wheat straw or lupin stem placed on the agar surface. The introduction of pieces of tissue, such as filter paper, wheat straw, rice, grains, leaves or dung, often produces good sporulation dependent on the organism grown.

Collectively, these events constituted huge progress for fungal conservation. Conservation groups have also started to appear in national mycological learned societies, and even in one or two places at a local level. In terms of taxonomic coverage the reform and enlargement of the IUCN Species Survival Commission fungal specialist groups mean that there are now identifiable teams responsible for promoting conservation of all fungal groups.

Threats to fungi throughout the globe are of concern since they are not only beautiful but also play a significant role in human welfare. Moore et al. 148 have suggested the following steps for fungal conservation: (i) Conservation of habitats,

(ii) In-situ conservation of non-mycological reserves/ecological niches, and (iii) Ex situ conservation especially for saprotrophic species growing in culture.

One of the tools that would help in conservation is, inventorization. In most countries checklists of fungi are not available however such projects are now operative under the umbrella of IUCN. To help culture

collections centers maintain appropriate standards, the World Federation for Culture Collections (WFCC) has formulated guidelines which outline the necessary requirement 150.

The selection of preservation technique for fungi not only depends upon the success of the method but also upon the use of the organism, time, facilities and resources available. Long-term stability is considered together with the required availability of the culture without delay. A collector may select a continuous growth method which is to be backed up with one that reduces the possibility of change during storage. For example, growth techniques allow strain drift.

Use of synthetic medium places selective pressure on the organism, allowing variants to dominate. Mineral oil storage is a simple method of storage that retains viability of fungi for many years but places strains under selective pressure because of the special conditions of storage. Water storage technique may allow growth depending upon the method adopted. The procedure is to cut agar plugs from the edges of actively growing cultures and placing them in sterile distilled water in screw cap bottles. The nutrients available in the agar will allow growth until oxygen is depleted in the storage container. Soil storage involves inoculation of spores or mycelium suspended in sterile distilled water into sterile soil of approximately 20% moisture content. This method of storage can retain viability for 10 to 20 years. Silica gel storage methods are suitable methods for fungal spores that remain viable for periods up to and over 20 years. Freeze drying entails freezing of the organism and its desiccation by the sublimation of ice under reduced pressure. Cryopreservation is the method of storage at ultra low temperatures, which is the most successful method for retention of both the viability and characteristics of fungi.

The challenge of monitoring and safeguarding the Earth´s fungal resources is a daunting one that must be confronted.

CONCLUSIONS

Here we wish to explore these issues further and extend them, by proposing the establishment of an international collaborative MycoAction Plan. The Plan presented here is intended as a draft to stimulate debate and encourage action at a variety of levels: the worldwide, regional, national and personal. It aims to monitor and safeguard fungal resources worldwide, using the word ‘resources’ in the broadest sense to encompass natural, human, and collection resources.

We can be satisfied with the increased profile of fungi now achieved in biodiversity science (e.g. Schulze and Mooney, 1994; Heywood, 1995; Raven and Williams, 2000) and international systematic initiatives (e.g. Blackmore and Cutler, 1996; Rodriguez, 2000). However, we must now build on this recognition, if another opportunity is not to be exploited to its full potential, and recognize that this is something that each of us has to be proactive.

REFERENCES

Moore DM, Nauta MM, Evans SE and Rotheroe M (2001). Fungal Conservation: issues and solutions. Cambridge: Cambridge University Press.

Schulze ED and Mooney HA (1994). Biodiversity and Ecosystem Function. Berlin: Springer Verlag.

Heywood VH (1995). Global Biodiversity Assessment. Cambridge: Cambridge University Press.

Raven PH and Williams T (2000). Nature and Human Society: the quest for a sustainable world. Washington DC: National Academy Press.

Blackmore S and Cutler D (1996). Systematics Agenda 2000: the challenge for Europe. Linnean Society Occasional Publication No. 1.) Cardigan.

Rodriguez LO (2000). Implementing the GTI: recommendations from the Diversitas core programme element 3 including an assessment of present knowledge of key species groups. *International Union of Biological Sciences.*