# 1

# *Resource centres*

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## 1.1 Nature of the resource

Although culture collections of filamentous fungi date back to the late nineteenth century and one of the largest of them, the Centraalbureau voor Schimmelcultures (CBS) in The Netherlands was established in 1904, rather little interest had been shown in their funding, and proposals to set up such collections often received only token support. With the advent of biotechnology the search for microorganisms which have properties suitable for commercial exploitation has led to a renewed interest in culture collections because of the appreciation of the valuable resource they represent.

The term 'filamentous fungi' is used for species producing filament-like hyphae. It includes almost the entire fungal kingdom and is used in contradistinction to 'yeasts', which are essentially unicellular fungi with vegetative cells capable of repeated budding. The yeast fungi are not considered further here as they are treated in a companion volume in this series. While some fungus culture collections maintain both filamentous fungi and yeasts, most specialise in one growth form or the other. This is a consequence of the different uses made of them (and so of their relevance to particular industries), the diverse growth conditions, and the dissimilar ways in which they are currently characterised (physiologically and biochemically in the yeasts, but largely morphologically in all other fungi).

Precisely how many strains of filamentous fungi are maintained in the living state in culture collections throughout the world is unknown. However, the number certainly exceeds 170 000 scattered through over 200 collections, and it has been estimated that around 7000 different species are represented (Hawksworth, 1985*a*). The physiological and

biochemical attributes of the majority of these strains have not yet been determined. They constitute a vast and as yet largely unexploited resource, and it is for this reason that workers in various areas of biotechnology and related industries have begun to take such interest in culture collections in the 1980s.

Culture collections are, however, much more than places for the deposit and supply of cultures. They are almost always managed by specialist mycologists and thus form part of larger centres of mycological expertise. The resource centres consequently provide expert advice on filamentous fungi and their properties, their identification and preservation. In addition, training and specialist services to industry on a contract or consultancy basis are available from the larger collections, a number of which also act as depositories for patent strains. The aim of this book is to describe the nature of the resources and services available from culture collections of filamentous fungi for the benefit of biotechnologists and other potential users.

## 1.2 Biotechnological applications

The oldest established uses of filamentous fungi are those concerned with food for man. Larger fleshy fungi (particularly mushrooms) collected from pastures and forests have been eaten by man from the earliest times. The number of edible species probably exceeds 500, but many are restricted to particular geographical regions. Remarkably, not more than 20 species are currently exploited commercially (e.g. *Agaricus brunnescens*, 'mushrooms'; *Lentinula edodes*, 'shii-take'; *Tricholoma matsutake*, 'matsu-take'; *Volvariella volvacea*, 'padi straw mushroom'). Opportunities clearly exist for developing techniques to extend the numbers of species cultivated commercially. These could be of considerable value as a supplementary food source in less-developed countries. An important review of the cultivation of edible mushrooms is given by Chang & Hayes (1978), and of their sources by Wu (1987).

Many fermented foodstuffs and drinks, especially in Asia, are based on filamentous fungi (Hesseltine, 1965; Batra & Millner, 1976; Steinkraus, 1983). The most important of these are species of *Aspergillus* (e.g. *A. oryzae*, Koji), *Monascus* (e.g. *M. purpureus*, 'red rice', ang-kak), *Mucor* (e.g. *M. rouxii*, 'Chinese rice') and *Rhizopus* (e.g. *R. oligosporus*, 'tempeh'). The role of certain *Penicillium* species, such as *P. camembertii* and *P. roquefortii*, in cheese production also has an extremely long history (Pitt, 1980).

These long-established uses in food production are being extended by

biotechnologists to the production of single-cell protein (SCP) for use in both human foods and animal feeds (Birch, Parker & Worgan, 1976; Smith, 1981). The filamentous fungi are grown in large fermenters with appropriate nutrients, which may be waste materials such as wood chips. *Fusarium*-based products (Anderson & Solomons, 1984) are now on sale for human consumption in the UK, and other genera with potentially useful species include *Chaetomium*, *Paecilomyces* and *Trichoderma*. In these applications, strain selection is of special importance, as some species of all these genera produce harmful mycotoxins. Where biodegradation of wastes rather than production of feedstuffs is the desired end-product of the process, toxin production may not be a significant consideration. Various cellulases and lignase enzymes are produced by conidial and mycelial phases of wood-rotting Hymenomycetes, such as the *Sporotrichum* species.

Fungi also have applications in the detoxification of wastes, and in the extraction of metals from dilute solutions (Eggins & Allsopp, 1975).

Filamentous fungi are a rich source of metabolites. Over 3000 have been characterised, and novel compounds continue to be discovered (Turner, 1971; Turner & Aldridge, 1983). Some 1600 of these have already been found to have antibiotic or antitumour properties. The biological activities of many fungi are still unknown, however, and representatives of only a small proportion of known genera have yet been screened. The cephalosporins and penicillins are of vital importance as antibiotics. Other important products are the anti-fungal griseofulvin (from *Penicillium griseofulvum*), ergot alkaloids (from *Claviceps purpurea*), plant growth hormones (gibberellins, from *Fusarium moniliforme*), and the cattle growth stimulator zearalenone (from *F. graminearum*). Exciting attributes are still coming to light. For example, the immunosuppressant cyclosporin from *Tolypocladium inflatum* was only discovered in 1970, but is now transforming the safety of transplant surgery, and the cytocalasins from *Hypoxylon* and related species found in 1967 are proving of value in isolating nuclei for genetic engineering.

Fungi are also sources of industrial chemicals. Examples of these are citric and gluconic acids (*Aspergillus niger*), vitamins (riboflavin from *Eremothecium ashbyii*), polysaccharides (pullulan from *Aureobasidium pullulans*), and enzymes (rennin from *Rhizomucor pusillus*, lipase from *Penicillium roquefortii*, protease from *Aspergillus oryzae*, cellulase from *Trichoderma viride*). A wide variety of biochemical conversions and modifications of molecules such as sterols can also be carried out by fungi. Bennett (1985) and Onions, Allsopp & Eggins (1981) provide

useful introductions to the diverse industrial uses of fungi. As the activity of strains maintained in culture collections becomes better documented, potential new sources of valuable compounds will be identified.

Particularly exciting are the possibilities for genetic engineering using filamentous fungi (Bennett & Lasure, 1985). These are only just starting to be explored, but somatic hybridisation between allied species and the potential for the transfer into bacteria of genes making valuable metabolites present many new possibilities. An enzyme from *Trichoderma* has recently been cloned and expressed in yeast (Ardsell *et al.*, 1987).

With world concern over the possible harmful effects of agricultural pesticides, increasing emphasis is being placed on the use of fungi as both mycoherbicides (strains of *Colletotrichum gloeosporioides*) and mycoinsecticides (species of *Beauveria, Hirsutella, Metarhizium, Verticillium*). Several products based on the mass inoculation of targets by conidia are now commercially available.

Increased precision in the identification at the strain level of mycorrhizal fungi associated with both forest trees and orchids, and the use of biotechnological methods to improve the effectiveness of strains, have considerable promise for plantation forestry productivity and the orchid industry.

## 1.3 Conservation

Culture collections may be considered as germ plasm resources equivalent to seed banks, botanical gardens and zoos, in that they conserve representatives of the world's microorganisms and make them available to others for propagation and use. The maintenance of a fully comprehensive range of strains of the world's filamentous fungi is a daunting task never likely to be achieved by a single culture collection. In general, collections hold strains relevant to the interests of past and present staff scientists and to the applied field of the funding body, or to meet the needs of the region in which they are located. Most strains in individual collections are thus not replicated in other collections. As a result of the different specialisations of collections, they may be seen as components of a collective resource.

By using this resource, especially when assisted by the computerised information systems now being developed (Chapter 2), biotechnologists are able to obtain strains from a wide variety of substrata and regions without having to mount expensive collecting expeditions. Fur-

thermore, some filamentous fungi are extremely rare in nature and their rediscovery by isolation from soils or other materials may be far from easily accomplished. A considerable number of fungi described in culture are only known from the original isolation.

Culture collections have an obligation to conserve this massive resource for posterity and are engaged in developing increasingly successful long-term methods to attain this objective. Confidential short-term conservation of cultures for research workers in industry and universities is also undertaken by culture collections which provide a 'safe-deposit' facility (Chapter 7). This service reduces the risk of loss due to contamination or accident during experimental investigations and also limits the possibility of genetic change that can arise from inappropriate or poor preservation procedures, such as periodic transfer used in many research laboratories.

The value of culture collections is enhanced both by the acquisition of new isolates through the work of collection staff and by depositors submitting isolates. The policy on acquisitions varies considerably between collections (Chapter 3, Section 3.2.1) and some are not in a position to accept all cultures offered to them, because of limited staff and facilities. However, all research workers are urged to ensure that living cultures on which published research results are based are permanently conserved in a service culture collection. If this is not done, future workers may not be able to extend or verify the results, in the latter case rendering the publication of doubtful value. Much elegant biochemical work on filamentous fungi has already been based on strains which are not now available for further study.

## 1.4 Types of culture collections

Existing culture collections were established with a variety of objectives and priorities to fulfil different functions. Collections may generally be regarded as belonging to one of the following four categories, but it must be stressed that these are not mutually exclusive as some of the larger collections operate in more than one mode.

### 1.4.1 *Service collections*

Service culture collections have as their main objective the provision of authenticated cultures to all who request them.

Most service collections maintain a broad spectrum of fungi (and often other groups of organisms as well), actively solicit particular strains cited in the literature, and are concerned with the development of long-

term preservation methods (Chapter 4). Many are also International Depositary Authorities (IDAs) for patent strains (Chapter 6). While the income from the sale of cultures by the largest service collections is not inconsiderable, many strains are retained for conservation purposes and are rarely sold. Collections cannot recover their full costs from such sales. Consequently, service collections are generally supported by substantial grants from public funds in recognition of the fundamental service they provide to pure and applied biology.

The ability of service collections to check the identity of strains both on deposit and during the course of maintenance and preservation procedures is vital to a collection's ability to supply authenticated strains. Many collections have expert taxonomic mycologists on staff, or are associated with institutions with such scientists, in order to be confident of this aspect of quality control.

The major service collections produce catalogues of the strains held, are involved in advisory and training work to varying extents, conduct research on systematics and preservation, and often undertake identification work. Some also have facilities for biochemical, biodeterioration, or other specialised work and these are listed in Section 1.5 below.

### 1.4.2 *In-house collections*

In-house collections are those established primarily to serve the needs of the organisation of which they form a part, whether it be a government department, research laboratory or an industrial company. In most cases, in-house collections established in publicly funded institutes supply cultures to others, but only as a secondary function and subject to them having the manpower to do so. Catalogues are rarely available, charging may or may not be implemented, and other services are related to the activities of the parent body. Several major industrial companies have very large in-house collections, and highly sensitive strains used in commercial operations or the subject of active research are kept under strict security. However, some companies make isolates available from time to time to other researchers when this will not endanger commercial operations. As catalogues are seldom available to the public, the use of such in-house collections by others is necessarily limited.

### 1.4.3 *Research collections*

Individual research workers regularly build up substantial collections of cultures related to their own research work. These collections

are often of particular importance as they contain strains with unusual attributes that may be cited in publications by the researcher. Such collections are often maintained only by short-term maintenance procedures (repeated transfers, domestic deep-freeze) and the parent institution may have no commitment to their long-term preservation. There are consequently risks of loss due to the use of inadequate preservation methods, and the collection can become endangered when the research worker changes employment or research interests, completes a higher degree or retires.

It is very important that research workers deposit subcultures of key isolates with major service collections for long-term preservation. Collections can hold them in 'Reserve' or as 'Safe-Deposits' (Chapter 7, Section 7.2.1) if the researcher does not want them to be generally available until his or her work is completed and published, but in the meantime they will be expertly maintained.

Many research workers make available selected isolates to colleagues and others working in the same field on a free or exchange basis.

#### 1.4.4 *Laboratory suppliers*

Several commercial laboratory suppliers include a few filamentous fungi among the lists of living organisms they have available for sale. These are usually single representatives of species widely used for teaching purposes at schools or at undergraduate level. Prices are usually competitive with those of service collections due to the large-volume sales of a very few strains. In the case of the filamentous fungi, strains maintained by such suppliers are limited to the extent that they are likely to be of little value to research workers in biotechnology and related fields.

## 1.5 Resource centres

### 1.5.1 *Locating resource centres*

The number of culture collections that maintain strains of filamentous fungi is certainly in excess of 200, and many of these are included in the second edition of the *World Directory of Collections of Cultures of Microorganisms* (McGowan & Skerman, 1982) [available from The Secretary, UNEP/UNESCO/ICRO Panel on Microbiology, Swedish University of Agricultural Sciences, S-750 07 Uppsala, Sweden (book form or in microfiche), and also from UNEP]. These collections vary greatly in coverage, size and the level of services they provide. In the following sections, brief details are given on collections holding more

than 500 strains, with extended treatment of those with over 10 000 strains. The information given here has been supplied especially for this publication by the collections; only those with over 500 strains and that wished their work to be listed are included. Information on further collections, and additional data on those listed is provided by McGowan & Skerman (1982), Hawksworth (1985*a*) and, for European collections, by the European Culture Collections' Organisation (1984).

Locating the resource centre, or centres, most likely to be able to provide particular strains or services is not always an easy task. Although the species held in many centres are listed in the *World Directory*, the details of strains are not included. Computerised databases play a key role in alleviating the present problem (Chapter 2), but the service collections listed below may also be contacted for advice on the location of particular strains. Additionally, the international or national organisations concerned with culture collections, microbiology, or biotechnology may be approached, and further information on these is provided in Chapter 8.

The acronyms adopted below are those used in the *Directory* of the World Data Center.

### 1.5.2 *Asia*

**Culture Collection Department CCCCM**
Institute of Microbiology
Academia Sinica (AS), Beijing, China
Tel.: 28-5614
*Founded:* 1951 *Strains of fungi:* 4750
*Status:* Under the leadership of the China Committee for Culture Collections of Microorganisms. Since 1979 this collection has acted as the Centre for General Microbiological Culture Collections (CGMCC) in the Committee.
*Coverage:* All groups. Collection, preservation and distribution of cultures of microorganisms with significance to industry, taxonomy, biochemistry, genetics and education.
*Notes:* Catalogue available.

**Institute of Applied Microbiology IAM**
The University of Tokyo, 1-1-1 Yayoi
Bunkyo-ku, Tokyo 113, Japan
*Founded:* 1953 *Strains of fungi:* 1000

*Coverage:* All groups.

*Catalogue:* Strains are listed in the Japan Federation for Culture Collections (JFCC) *Catalogue of Cultures* (available from the Business Center for Academic Societies Japan, 16-3 Hongo 6-chome, Bunkyo-ku, Tokyo 113). An independent catalogue is published.

*Notes:* The collection was established to promote research in basic and applied microbiology by collecting, preserving and distributing authentic cultures. This is one of the main purposes for which the Institute was founded. The collection is a member of the Japan Federation for Culture Collections. Cultures are distributed to institutions, both in Japan and abroad. Preservation is by freeze-drying, L-drying, cryogenic freezing, and serial transfer. Deposits of cultures of scientific interest are welcomed.

**Institute for Fermentation IFO**
17-85 Juso-honmachi
2-chome, Yodogawa-ku, Osaka 532, Japan
Tel.: 06-302-7281
*Founded:* 1944 *Strains of fungi:* 7000
*Status:* Financial support is obtained from the IFO foundation and annual donations from a private company.
*Coverage:* All groups.
*Catalogue:* The IFO *List of Cultures* (7th edition, 1984) is available from the Business Center for Academic Societies Japan, 16-3 Hongo 6-chome, Bunkyo-ku, Tokyo 113, Japan.

**Japan Collection of Microorganisms JCM**
The Institute of Physical and Chemical Research (RIKEN),
Wako
Saitama 351-01, Japan
Tel.: 0484-62-1111 ext. 6000; Telex: 02962818 (RIKENJ)
*Founded:* 1980 *Strains of fungi:* 1000
*Status:* Government supported.
*Coverage:* All groups.
*Notes:* Catalogue available. The foundation of the collection was stimulated by national policy on the promotion of life sciences. The collection is expected to function as the key centre in Japan. It collects, preserves and distributes moulds of scientific, industrial, medical and ecological importance, in active cooperation with other collections. Also, since it shares the host institute, RIKEN, JCM contributes to the

activities of the World Data Center on Microorganisms (WDC) (see pp. 49–51).

### 1.5.3 *Australasia*

**Australian National Reference Laboratory in Medical Mycology AMMRL**
Royal North Shore Hospital of Sydney
Pacific Highway, St Leonards
New South Wales 2065, Australia
Tel.: (02) 438-7128
*Founded:* 1949 *Strains of fungi:* 750
*Coverage:* All groups of medical interest except Basidiomycotina and Mastigomycotina. Primarily concerned with the maintenance and distribution of fungi of medical importance.
*Catalogue:* A catalogue is available.
*Special services:* The collection provides information on and assistance with diagnostic and technical problems in the field of medical mycology.
*Research:* Engaged in research which is directed towards the study of pathogenic fungi in Australia.
*Training:* Informal training programme for those working in the area of medical microbiology.

**Victoria University of Wellington DBVU**
Microorganism Culture Collection
School of Biological Sciences, Private Bag
Wellington, New Zealand
Tel.: (64) (04) 721-000; Telex: NZ 30882
*Founded:* 1965 *Strains of fungi:* 594
*Coverage:* All groups. The collection contains fungi isolated from diseased plant material, cereal seeds, soil, jet and diesel fuel, bird feathers and air. Mercury resistant strains of *Pyrenophora avenae* and triadimenol and nuarimel resistant strains of *P. teres* and *P. graminea* are held.
*Catalogue:* A catalogue is available.
*Culture sales:* Cultures are exchanged free of charge for cultures from other collections and generally free to teaching institutions. A fee is charged to industry.
*Preservation:* Cultures are stored under sterile mineral oil.