CRITICAL PROBLEMS OF CULTURE COLLECTIONS

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CRITICAL PROBLEMS OF CULTURE COLLECTIONS

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LEKH R. BATRA and TEIJI IIJIMA, Editors

Invited papers presented at a symposium held September 3, 1983, at Tokyo, Japan and sponsored by the Third International Mycological Congress

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PREFACE

The importance of culture collections is being increasingly recognized in research and development with added emphasis on genetic engineering of biota of all kinds. However, as researchers, teachers, or product developers, we repeatedly find that cultures of new, or otherwise unique taxa published in reputable journals are unavailable for further scrutiny. These cultures apparently never entered a reliable service or reference collection. A clone or strain once lost is lost usually forever. We may isolate the same species again but it will not be the same strain.

Discoveries of new uses of fungus cultures are a recurring phenomena as are the causes of losses of cultures: ignorance or suboptimal appreciation of the importance of saving cultures; inadequate cooperation between individuals or institutions, including nations; unsatisfactory national policy statements on culture collections and absence of means, such as professional society working groups, to foster awareness of usefulness of culture collections, or to carry out national policies; and lastly, inadequate funding. This list of critical needs of culture collections is by no means exhaustive but the combined effect of all is insidious. Each year several valuable collections disappear, either before, or soon after, a related research or development task of a program is completed, because no prior arrangements were made with a major reference or service collection to care for useful scientific treasures.

In my own specialty, agricultural mycology including phytopathology, one of the vexing problems has been to maintain and finance specialized research collections of fungi. Several symposia have addressed this question but consensus on a solution has not emerged. These are the collections with a limited temporal scope, but which are the bases of authoritative commodity-oriented or generic monographs based on genetics, morphology, physiology and phytopathology; for example the Zentmeyer collection

of *Phytophthora* comprising 1700 isolates of 27 species at the University of California (Riverside), a similar collection of *Fusaria* at the Pennsylvania State University, and the privately maintained Emory G. Simmons collection of *Alternaria* and *Stemphylium* at his residence. These collections are most significant when a wide mixture of inocula are a requirement such as, (a) to screen for disease resistance of plant germplasm, and (b) to prepare allergen antigens with a wide spectrum. Most general or reference collections, such as the American Type Culture Collection, do not exclude any past or future significant voucher specimens. However, one cannot expect such a collection to become a "foster parent" to all such specialized collections upon the retirement of the "scientist-parent" or at the termination of a research program. Perhaps professional societies and institutes can play a key role to impress upon agencies concerned (federal, state or non-profit foundations) to finance retention or retrieval of special collections of fungi.

In February 1983 the IMC3 Program Subcommittee requested that I convene and chair a symposium entitled '*Critical Problems of Culture Collections*'. Soon afterwards, informal consultations with colleagues well-versed in the management of culture collections led to the following general theme and scope of the symposium, as published in the IMC3 Abstracts: This symposium addresses the problems associated with the acquisition and maintenance of cultures on a worldwide basis, particularly to suggest ways to improve international awareness, in order to obtain material from underexplored regions (M. A. A. Schipper). Sound decisions can be made only with critically evaluated data received promptly; and computer storage and retrieval of culture collection data can be of assistance for specimen and extraspecimen information such as nomenclature, bibliography, source, history, host range, applications, cataloguing etc. (S. C. Jong). Fungus cultures used as vital tools, standards and measures must remain

pure, viable and unaltered in form or function during storage and distribution. This requires continued research in materials and methods used to preserve cultures (A. H. S. Onions). The industrially or otherwise economically important cultures such as those used to obtain patents, negotiate licensing agreements, or for settling infringements and interferences require special care under national and international law (C. P. Kurtzman and T. Iijima). Lastly, Culture Collections are casualties of financial "austerity". Because it is not possible to predict to what new uses fungi maintained as cultures will be put, it is often difficult to justify expenditure in publicly supported collections beyond the immediate future. In this respect they resemble museums which preserve nonliving evidence of progress in other areas. Robert E. Stevenson will address the question of funding of Culture Collections at the national and international level.

I express my appreciation to the IMC3 Program Subcommittee who asked me to convene and chair this symposium, thus offering me an opportunity to serve the Congress at Tokyo. On behalf of all participants I thank the Board of Trustees, and Dr. Teiji Iijima, Director, Institute for Fermentation, Osaka, for financing the publication and distribution of the symposium proceedings in conjunction with the fiftieth anniversary celebrations of the Institute. The following colleagues kindly reviewed one or more chapters: S. W. T. Batra, C. W. Hesseltine, S. C. Jong, D. F. Farr, A. J. Lyons, K. H. McKnight, T. Myoda, M. J. O'Brien, and M. E. Simpson. I am particularly grateful to M. Pope who assisted in the design and layout and to M. R. Hunt for assistance in critically reviewing the entire proceedings with respect to unformity of style, particularly bibliographies. The bibliography in the appendix was prepared by S. C. Jong and L. R. Batra.

Lekh Raj Batra
February, 1984.
Beltsville, Maryland

CRITICAL PROBLEMS OF CULTURE COLLECTIONS — OPENING REMARKS

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Our convenor and chairman, Dr. Batra, very generously has insisted that I make some introductory remarks for our symposium. I had hoped to be excused today, as I no longer head a major public collection of fungus isolates. However, Dr. Batra is persuasive — and my resistance is low whenever the interests of the international culture collection community are involved.

The real purpose of my few remarks is to give a feeling of historical continuity to the development of the professional strengths that are found today in so many of the large and some of the small centers of fungus culture study and conservation. Having been in the international culture collection picture for 30 years, I, along with a very few others of my generation in this room, have watched with awe as some of these early poor children of the biological world — these orphans — poorly housed, sometimes poorly staffed, always very poorly financed, nevertheless developed into mycological institutions or departments of which we now are so proud — with top professional staffs, strong management, excellent facilities, and at least adequate financial support.

During the 30-year period we have seen the development of dependable methods of long-term conservation; we have moved our data management from the file-card to the computer; we have watched the acknowledged value of

industrial application; and we have helped to establish and train new national and regional culture collection facilities that are beginning to form a network devoted to both local and worldwide biological interests.

All of these topics, with their serious problems of financial, professional, and political support, will be addressed at length today by one or the other of our colleagues.

But just before I stop, may I mention two problem areas that have had special meaning for me through the years.

The first of these is the need for small culture collection facilities organized to serve the particular interests of national and regional development — small but good, with a staff (small but good) specifically trained in the technical and taxonomic work of the culture unit, and so reliable that they are recognized by their institutional colleagues as competent and responsible sources of reference and production cultures and information.

Much work along this line has been done by members of the World Federation for Culture Collections under sponsorship of United Nations agencies. They have been particularly active in organizing regional training courses in culture collection techniques and in establishing several regional and specialized Microbiological Resources Centers (commonly referred to as MIRCENs). I am especially familiar with and proud of the unit based in Thailand, whose culture collection I helped to organize 15 years ago and which now has develped into the Bangkok MIRCEN, with regional service and cooperation responsibilities in several countries of Southeast Asia. I see these national collections and regional MIRCENs as gradually overcoming local support problems and evolving into contributing members of the overall international culture collection community of service and expertise.

The second problem area that always has interested me is the long-term handling, the eventual disposition

of specialized research collections of fungus isolates. The genus specialist who works with living cultures usually can arrange to deposit "Type" strains in major public collections. But where are the depositories for significant numbers of variant isolates that truly define the nature of the expert's species and that may be much more valuable in nontaxonomic applications than many type strains ever will be?

The large public research and resource collections cannot routinely accept and maintain entire collections of retired or deceased experts. Yet the mycological community has been and continues to be faced with the loss not only of expertly characterized reference materials of this sort but also the data files related to them. I know of no systematic plans or policies — national, regional, or international — to prevent such losses, and we can only hope that our talks together can keep the subject moving toward a reasonable solution of the problem.

Enough. Dr. Batra is ready to introduce several most active and influential culture collection experts — all of them accustomed to working together across international borders — and all with similar problems and expert opinions on handling these problems.

COLLECTING MYCOLOGICAL CULTURES AND IMPROVING COOPERATION: PROBLEMS, IMPEDIMENTS, OPPORTUNITIES

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ABSTRACT

Vitality and continuity of collections depend for a major part on knowledge, skill and dedication of the staff, on a well-chosen accession program relevant to the needs of founders/users, and on sound cooperation with others. Financial limitations are not a prerogative of developing countries only. Investments for equipment, etc. must be considered. Cooperation among collections and among investigators and collections is of mutual benefit. Cultures gain value with increase of data on the strains. Cooperation often grows with familiarity, through papers, lectures, meetings, and exchange of catalogues (worldlist, databanks).

The vitality and continuity of a collection depends primarily on the capabilities of the staff, centered on the curator. A stamp collector can place new additions in a shoe-box for future sorting — either by himself or by his grandchildren. However, it is of no use to collect living cultures that cannot be handled immediately. So, first of all, the collector must know his/her own limitations.

Some essential requirements. A curator must have command of:

1. The necessary laboratory equipment and staff.

2. Sufficient knowledge of distinguishing characters and growth conditions of species intended to be included in the collection.
3. Enough dedication to do a lot of often little appreciated work.

Dedication cannot be bought. Knowledge is the result of training and study, to economize here is a mistake. Equipment is a matter of money and should be invested according to the type of collection concerned.

Polar organisms do not require heated rooms.

Freeze-drying apparatus is of no use for a collection of sterile mycelia.

Cryogenic storage should be avoided if a regular supply of liquid nitrogen is uncertain.

Accessioning criteria differ according to the type of collection. Specialized research collections usually concentrate on a small number of species or genera; national and international resource collections contain a diverse range of cultures. Very careful selection of accessioning criteria is vital for survival, especially when funding is limited.

How do collections obtain the desired cultures? Specialists working the collection isolate and identify specimens within their own field. But mostly collecting means "requesting" (1) either a "specific" request in connection with publications or catalogues, or (2) an open request to send cultures, including offers for exchange.

Some attitudes of depositors. Attitudes of *prospective depositors* are rather varied. Young students are sometimes very eager to deposit their cultures in a major collection. They are disappointed when a collection does not accept rather common species or unidentified strains. Unfortunately, some people still appear to be apprehensive about depositing their isolates, possibly because of fear of rivalry, while others fail to recognize the importance of ensured maintenance. Thus type strains of new species,

.....How to obtain the desired cultures.....

subjects of intensive research, are lost because the investigator did not safeguard the maintenance of the cultures. It is very unpleasant, and scientifically not correct, when an author states that strains have been

deposited in a resource collection but fails to actually do so. Sometimes such strains are sent but are not viable on arrival.

Another problem which arises with the accessioning of strains is that depositors and cuators may disagree

..... Sometimes depositors' and curators' taxonomic views do not concur.....

about the correct taxonomic name. However, apart from those isolated cases, most mycologists do cooperate readily. Curators are lucky, when careful investigators after conclusion of their studies present them with a well documented collection. For, as important as the maintenance of well defined cultures, is the collecting of data on the strains.

How to improve and promote cooperation? I have mentioned distrust as a possible impediment. To overcome such problems, one should strive to eliminate any cause for suspicion. Both parties must be clear about their intentions. Cultures sent for identification should not be incorporated in the collection without permission from the sender; similarly cultures sent with a request for expert opinion in cases of suspected new taxa should not be used for other purposes without permisson from the sender. Students at all levels should be shown the value of saving irreplaceable material.

Scientists who intend to deposit valuable cultures for maintenance, will prefer well established collections, with reasonable chances of continuity, over new collections; unless the new collections are specialized or have published a well planned accessioning program that is valued by the depositor. Cooperation is often easier once personal acquaintance has been made. Opportunities to meet are welcome. It is a pity that travel is beoming more and more expensive!

Communication may be simplified by establishing federations or other forms of cooperation between culture collections; fewer addresses, combined information on contents and services; and most probably, more favorable conditions in view of an efficient management. Recently, an investigation has been completed on collections of micro-organisms in The Netherlands. It aims at centralized management, a joint catalogue, and at other cooperative endeavors. A quite young organization is "ECCCO", European Culture Collections Curators' Organization. At the

Conference of the World-Federation of Culture Collections, in 1981, European curators arranged to meet once a year to discuss common problems and common interests. We have met twice and both meetings were much appreciated. Some topics discussed were:

.....Collections should be relevant to the needs of the region.....

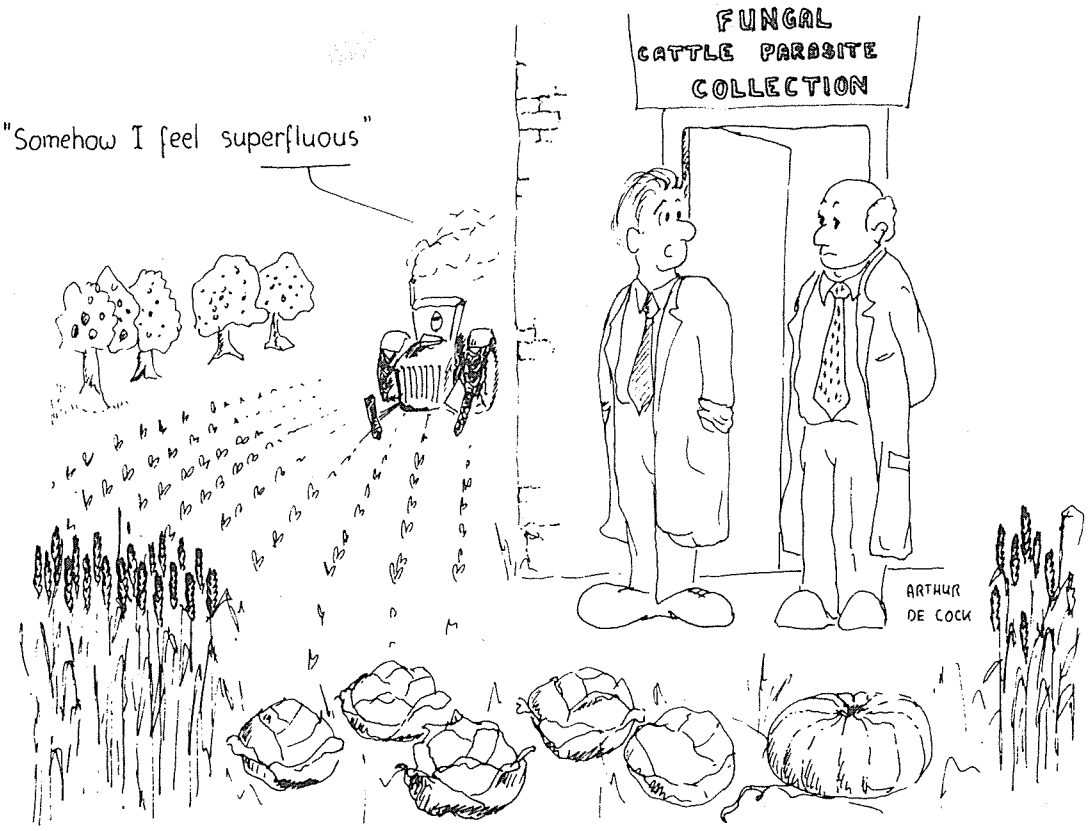
Postal regulations, especially with regard to international exchange of cultures.

Patent deposits, the procedures involved and administrative and technical problems encountered in this field. (See Kurtzman, this symposium.)

Improvements in technical methods of preservation. (See Onions and Smith, this symposium.)

Financial aspects. (See Stevenson, this symposium.)

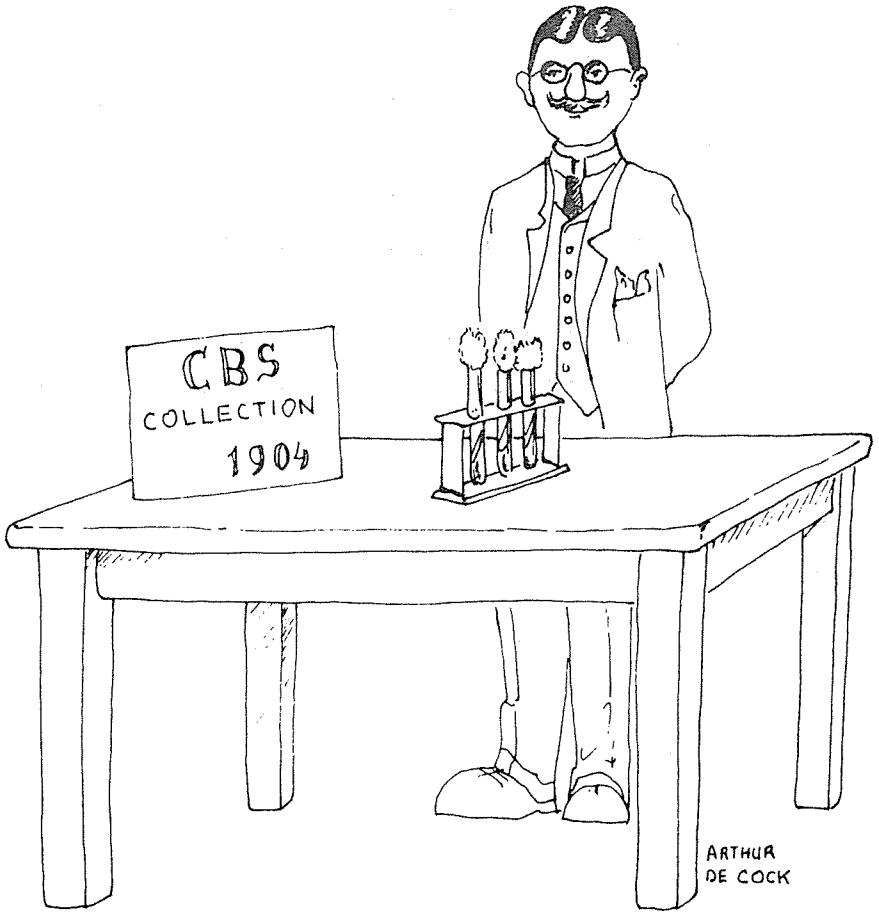
Computerization of data and possibility of coordination. (See Jong, this symposium.)



.....Collections should be relevant to the needs of the region.....

European Culture Collections Curators' Organization members are very positive about this type of cooperation.

Needs of developed and developing countries. Then, I have been asked to give my opinion on the question whether the needs of developed and developing countries are the same? My answer is no! In developing countries, pure research — not aimed at distinct applications — has a very low priority. Collections should never be just stores of cultures, but must be able to provide identification services. They should be able to respond to requests for advice, to assist in research on applied mycology and to



.....All today's "major" collections started small.....

the benefit of the region, and consequently be relevant to the needs of the particular region.

All of today's "major" collections started small, based on the needs of the founders. For example, in Japan applied mycology has grown in response to traditional fermentations. It has developed into a full-grown scientific approach to fermentation processes, of fungal products like antibiotics etc. The Japanese collections reflect this type of industry. Agricultural communities have other needs than cattle-breeders; human, veterinary, or plant diseases may differ widely according to the region.

Collections should cooperate and coordinate, but not imitate! A friend from Indonesia told me recently how she had experimented with native materials for culture media to replace the very expensive potato-agar, recommended by American and European mycologists in her field of research. In the CBS potato-agar was always a much used medium, as it was so very cheap!

Collections that count, are not followers of a uniform, predestined pattern; but each develops an individual character.

DATA MANAGEMENT AT THE AMERICAN TYPE CULTURE COLLECTION

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ABSTRACT

The principal functions of a resource collection of living fungus cultures include not only the accession, preservation, authentication, and distribution of reference and type cultures, but also the creation and manipulation of data files for each culture maintained. The collection also acts as an information clearing house in all the disciplines of mycology, namely to disseminate information pertaining to the availability of strains, nomenclature, classification, characterization, preservation techniques, and applications of fungus cultures to the study and solution of human problems involving food, energy, health and environmental pollution. Data and resource management systems used at the American Type Culture Collections are described. These systems include a Waurax-Centurion minicomputer, one Jacquard 100 multifunction office word processor, one AM Comp/Edit 5810 combined word processor and phototypesetter, two computer-communicating terminal stations (Trendata 4000, 30 CPS, and an IBM 15 CPS) and two portable terminals (CDI and a DEC-writer Correspondent).

INTRODUCTION

In the United States the resource fungus collection, the American Type Culture Collection (ATCC), at present maintains over 17,000 strains of living fungi. During the last five years (1978-1982), a total of 4,993 strains were accessioned (Table 1). Strain data files of 4,388

accessions were compiled, each containing documentation of strain history, isolation source, taxonomic characteristics, genetic informtion, correspondence, reprints and patents, and evaluation of the usefulness of cultures.

Table 1. Taxonomic range of strains accessioned during 1978-1982

	Genera	Species	Strains
Fungi Imperfecti	945	1,441	1,951
Ascomycetes	465	627	1,538
Basidiomycetes	327	452	726
Phycomycetes	236	428	673
Myxomycetes	31	33	105
Total	2,004	2,981	4,993

Name changes for taxonomic reasons were made on 610 previously accessioned strains, and new uses and reference citations were added to 962 catalogued strains. The latest catalogue, published in 1982, lists 14,127 strains, representing 1,218 genera and 5,213 species of fungi (Table 2). A total of 24,790 cultures were distributed for a fee.

Table 2. The range of ATCC fungi listed in 1982 edition catalogue

	Genera	Species	Strain
Imperfecti	552	2,861	6,785
Ascomycetes	326	1,145	3,806
Basidiomycetes	205	670	1,820
Phycomycetes	91	546	1,499
Myxomycetes	16	51	178
Lichens	28	39	39
Total	1,218	5,312	14,127

The system of management of culture data files at the ATCC is presented here as a model requirement involved in culture collection functions.

INFORMATION SYSTEM IN CULTURE REQUISITION

New fungus strains constantly appear in current publications. In order to develop the mycological collection of the ATCC into a more effective resource responsive to the diverse academic and industrial needs of mulit-disciplinary microbiology, active searching and solicitation of new cultures reported in a rapidly growing literature are an essential part of daily curatorial functions.

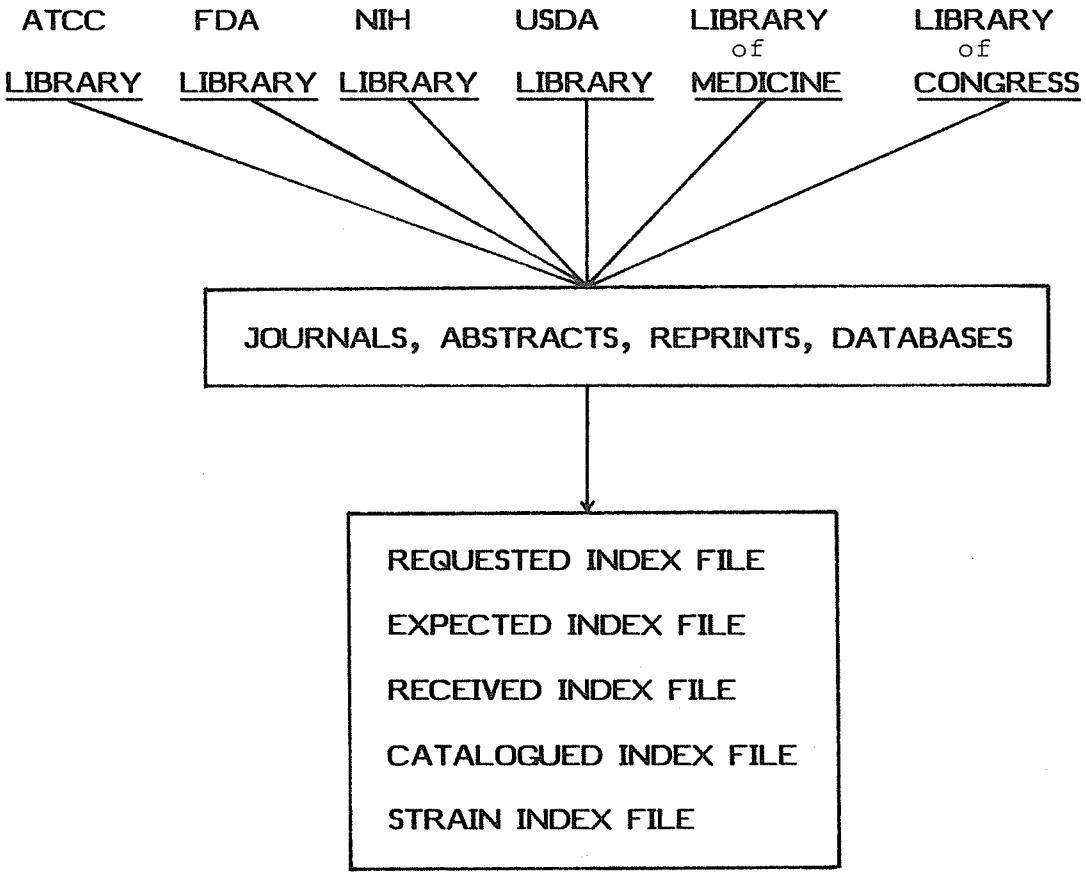
Searching for new cultures. In scanning for the fungus cultures being used in contemporary research, all issues of *Current Contents*, *Microbiology Abstracts*, *Abstracts of Mycology*, and various journals subscribed to by ATCC are reviewed. Requests for reprints of articles that appeared in the abstracts are made. With the help of the Information Scientist, online searches through telecommunication access to various computerized databases such as Lockheed Dialog and the National Library of Medicine MEDLARS systems are used for retrieval of necessary bibliographic information on special groups of fungi. For example, in 1982, various databases were searched for the following subjects:

1) Lytic enzymes active on the cell walls of yeasts and filamentous fungi
2) Hybridomas and monoclonal antibodies involving fungi
3) Edible fungi involved in patents
4) Nitrogen-fixation by fungi
5) Fish diseases caused by fungi
6) Official standards using fungi, especially ATCC cultures
7) Fungi causing allergic reations
8) Post-harvest decay of fruits and vegetables caused by fungi

In addition, the Food and Drug Administration Medical Library, the National Library of Medicine, the National Institute of Health Library, the National Agricultural Library, the Library of Congress, which also provides translation of foreign literature, are nearby (Figure 1).

Solicitation of cultures. Fungi accepted by ATCC must have adequate documentation indicating properties that make them useful to research, teaching or industry. They must be able to be propagated in the laboratory in order for the ATCC scientfic staff to check for authenticity and to preserve them by either freeze-drying or freezing techniques for long-term storage which insure against changes in biological properties. These criteria

FIGURE 1. FLOW OF INFORMATION IN CULTURE REQUISITION



determine which cultures will be requested for deposit by letters to the original investigators in the field. Before the Curator requests cultures, the following files are checked by the Mycology Secretary to avoid duplication of requests:

1) "CULTURE REQUESTED, EXPECTED AND RECEIVED" card index to determine if the culture has been previously requested or received but still in the laboratory for processing.

2) "CULTURE CATALOGUED" card index to determine if the culture has recently been received and processed but is not in the current catalogue and its data file has been sent to the Professional Services Department for inclusion in the next edition of the catalogue.

3) "STRAIN DESIGNATION" card index to determine if the culture is already in the collection. A strain may carry several strain designation numbers. For example, *Aspergillus oryzae* ATCC 1011 =ATCC 4814=ATCC 7561=ATCC 9102=ATCC 12891=CBS 102107=CMI 16266=CMI 44242=IFO 5375=LSHB Ac.19=NCTC 598=NRLL 447=NRRL 692=QM6735=WB 447=C. Thom 113-L.A. Underkofler 22.

After the above cross-index card systems have been checked, the MAG CARDSELECTIVE TYPEWRITER (MCST) or WORD PROCESSOR, is employed to type the letters to solicit cultures. At the same time, a CULTURE REQUESTED CARD is created which includes the name of the culture, any strain designation(s), literature citation(s), the name and address of the principal investigator, and the date the culture was requested. The card is filed in the CULTURE REQUESTED CARD INDEX alphabetically in the following order: (a) name of genus, (b) name of species, (c) last name of depositor. If a culture arrives unsolicited, a CULTURE RECEIVED CARD is made at that time using the same format.

A data sheet called ACCESSION FORM (ATCC Form 1-F), to be completed and submitted by the contributor along with the culture, is included in the letter for every

culture requested. This data sheet requests information on: growth requirements, characteristics, reference(s) citing the culture, its nomenclature and classification, isolation, history, and proper handling of the culture with regard to pathogenicity. Importation of living fungi into the United States requires a permit from the Plant Germplasm Quarantine Center of the U. S. Department of Agriculture. To insure the safe arrival of cultures from foreign countries, USDA shipping labels (PPQ FORM 570) are enclosed with all foreign requests.

Responses to requests for cultures. During the last five years approximatley 50 percent of the cultures requested were sent to us for consideration for incorporation into the collection. One reason for this low response rate may be the retrieval systems of abstract services or *Current Contents*. It takes months for scientific papers to be published and more months for them to find their way into abstract services and retrieval systems. Therefore ATCC requests for cultures may be made a year or more after an investigator completed the research. By that time the cultures used might be discarded or lost.

If letters of solicitation are referred by the recipient to co-investigators, colleagues or other culture collections, the process is repeated until the desired cultures are received. Abstracts alone do not tell us the availability and usefulness of the cultures reported in the literature; reprints or publications are needed. Responses to requests for reprints of publications cited in the abstract services or *Current Contents* are very low, usually less than 30% of the total requests. Therefore, we rely heavily on the journals and other series subscribed to by the ATCC in the requisition of cultures.

MANAGEMENT SYSTEM IN DOCUMENT CREATION

Although the binomial established the taxonomic and nomenclatural position of a fungus species that identifies a group of strains from a variety of sources, there are

no nomenclatural rules regarding the naming of individual strains. In culture collections, the most practical way of establishing the identity of a strain is to use a combination of an institutional acronym followed by a pure-number. Because strains can show variations in any species characteristic, a considerable amount of documentation must be maintained and processed for each strain in the collection. Strain data result from repeated examinations by the original investigator, the culture collection, and users of the cultures. Thus creation of strain-specific data files is a necessary part of the accessioning procedure, and the degree of documentation in the fields represents the quality of the collection.

Record keeping when cultures arrive. When a culture arrives, a temporary number (also called working number) is assigned to the culture until it is accessioned. The name of the culture, the name and address of the depositor, depositor's strain number, date received, and the laboratory person assigned to handle the culture are logged in the TEMPORARY LOG BOOK.

The CULTURE REQUESTED CARD is converted to the CULTURE RECEIVED CARD by stamping the card with the date received. A postcard is sent to the depositor to acknowledge receipt of the culture.

The following pre-printed forms are initiated by the Secretary: (1) TEMPORARY WORK FORM filled in with the temporary number, depositor's number, name of the culture, and the date received; (2) PRESERVATION CARD filled in with the name of the culture, depositor's strain number, and the temporary number; and (3) a form for REQUEST OF ATCC ACCESSION NUMBER (ATCC Form 85) filled in with the culture name, depositor's name and address, strain designation(s), use of the culture and its literature citation(s), and name of the person handling the culture. The culture and all the data forms, reprints, completed ATCC ACCESSION FORM and copies of relevant correspondence are given to the person assigned to further process the culture.

If correspondence is received before the culture arrives (usually notifying that the culture is being grown or checked, etc.), the CULTURE REQUESTED CARD is converted to the CULTURE EXPECTED CARD by making a notation on the card, and all the correspondence is moved forward to the CULTURES EXPECTED FILE. If correspondence is received that the culture requested is not available, the CULTURE REQUESTED CARD is appropriately marked and all related correspondence is removed from the CULTURES REQUESTED FILE.

System of records in accessioning cultures. Before a new fungus culture is accessioned and catalogued, it is subjected to the following series of tests: viability, purity, identity, preferred temperature and media for growth and/or sporulation, and methods of preservation. Its potential usefulness in science is evaluated by reviewing the information sent by the depositor and the literature dealing with the culture. Based on this information, it is determined whether the new acquisition is to be accessioned. REQUEST FOR ATCC NUMBER is submitted to the Professional Services Department. The strain index of the ATCC cultures, catalogued or "in progress", is checked to avoid duplication in assigning ATCC numbers to the same strain. If there is no duplication, a new number is assigned and a TEMPORARY MASTER CARD (green in color), a NUMERICAL ACCESSION CARD and STRAIN CARD(S) are prepared.

ATCC numbers are assigned in numerical order to each strain accessioned. Upon receipt each new accession is entered into the Mycology Department ACCESSION BOOK which records ATCC number, binomial name, whether a type culture or not, depositor's number, name and address, use of the culture, date accessioned, and person handling the culture. Later notations are entered to indicate whether ATCC preparations of the culture have been returned to the depositor for verification, when the depositor has been notified of the accession number, and when the strain

data file has been turned over to the Professional Services Department for cataloguing at which time, the CULTURE RECEIVED CARD is converted into the CULTURE CATALOGUED CARD by typing the ATCC number on the card and moving it forward to the culture catalogued index file.

Maintenance of preservation data. The person working on the culture completes the PRESERVATION CARD and files it in the Mycology Laboratory. This record includes binomial name, ATCC number, other strain designations, date preserved, culture age and medium used, cryoprotective solution, material preserved such as sexual or asexual spores or mycelia, methods used, results of viability check, the inventory for seed and distribution stocks, and any special growth requirements for the culture. To minimize mutation, deterioration, selection and adaptation of variant clones and contamination, ATCC fungi are preserved by freeze drying (lyophilization), and/or storage in liquid nitrogen. A set of index cards with reference to the date, method, and number of vials of each strain preserved and the position of storage is kept in the Manufacturing Department. The inventory data are entered into a computer in the Professional Services Department for use in order processing and inventory control. To prevent loss of the materials in the event of extensive damage to the ATCC facility, two ampoules of each freeze-dried strain and one of each frozen strain are placed in safe deposit repositories established by the ATCC at the Virginia Polytechnic Institute and State University in Blacksburg, Virginia, and the Frederick Cancer Research Facility, Frederick, Maryland, respectively. Inventory of cultures, thus stored, is kept on preservation data cards.

The MEDIA INDEX CARDS which contain the formulation of each medium used at the ATCC are stored numerically by media numbers in the Media Unit. Each new formulation is reviewed against pertinent references before an ATCC medium number is assigned.

Establishment of strain-specific files. When the laboratory has completed processing each strain, the person handling the culture records the growth conditions, the preservation method used at the ATCC, and the code for type of packing used for the culture on the TEMPORARY WORK FORM. The entire strain data folder is then turned over to the Curator who completes the TEMPORARY WORK FORM with catalogue information which he extracts from the completed ACCESSION FORM, correspondence, reprints, or any other available sources. The binomial name used in the catalogue is determined by the Curator and added to the Form. If the culture received forms the teleomorphic (perfect) state, the name of the teleomorphic state is used. The name of the anamorphic (imperfect) state, if available, is also designated following the teleomorphic name. However, some of the fungi isolated from the teleomophic state in nature have never produced teleomorphic states in culture. Therefore, if a culture is received under the teleomorphic name, but forms an anamorphic state only, a statement "imperfect state" is noted on the TEMPORARY WORK FORM. For the name of a culture to be accurate and complete, it is necessary to cite the name of the authors who published the first description and bestowed the name. ATCC does not use any abbreviations of author's names for data files or for the catalogue.

The Curator also updates the current edition of the ATCC catalogue with nomenclatural changes. A Routing Slip (Form 75) is used for all changes or correction in information concerning a culture. The entire data folder including originals of all correspondence, references, lab reports, etc., is then sent to the Professional Services Department and processed for the catalogue, computer and strain index updating. The data file is microfilmed. For security, duplicates of all microfilmed data are stored in a bank safety deposit box located offsite.

Upon receipt of the entire data folder of each accession, the Information Scientist completes the TEMPORARY MASTER CARD by summarizing all essential information on the culture. A card index is updated with new uses, if applicable, for the next catalogue. A final master card and updated index cards for the strain and numerical files are typed from the handwritten TEMPORARY MASTER CARD. All the typed cards are proofread by the Information Scientist before they are filed. The MASTER CARD includes the scientific name, ATCC number, dates received and accessioned, designation, history, host or substrate, other strain designations, proper packaging requirement for safe shipment, availability (in freeze-dried, frozen, or living state), source of species description for the type culture, and special applications (e.g., uses in bioassays and for the production of antibiotics, mycotoxins, enzymes, vitamins, etc.) with the pertinent reference citations, other comments as required (e. g. mixed culture, non-sporulating culture or imperfect state, genotypes), and recommended medium and conditions for growth.

The master cards, strain cards and numerical cards are manually stored in a drum file for easy access.

When noting name changes or new uses involving ATCC fungi while reviewing the literature, the Curator completes the Routing Slip (Form 75) mentioned previously for each strain cited in the literature. It is sent to the Professional Services Department where the master cards, catalogue manuscript, index cards, and computer are updated accordingly.

The appropriate data on all potential plant pathogens accessioned are forwarded to the U. S. Department of Agricutlure for review and designation of those strains that require a permit for interstate shipment. The strains are so noted in the strain and computer files as well as in the ATCC catalogue.

All reprints concerning the ATCC fungi are included in the ATCC reprint collection which is indexed by author(s) and subject(s), and cross referenced for easy retrieval. A numerical "M" code identification number is assigned, upon indexing, to each reprint dealing with fungi. The reprints have been an invaluable aid in directing the ATCC to useful additions to the strain-specific files. At present the ATCC library contains more than 27,000 mycological reprints, from publication sources worldwide, and with many countries and languages represented. All reprints are microfilmed for convenient storage.

COMPUTER MANIPULATION OF CULTURE DATA FILES

In recent years electronic data processing has become important in managing data. In our case it includes the full range of activities including acquisition, accessioning, inventory, cataloguing, accounting, order processing, and shipping are involved. The computer facilities at the ATCC include a Waurax-Centurion minicomputer, one Jacquard 100 multifunction office word processor, one AM Comp/Edit 5810 combined word processor and phototypesetter, two computer-communicating terminal stations (Trendata 4000, 30 CPS, and an IBM 15 CPS with a communicating Mag Card 1) and two portable terminals (CDI and a DECwriter Correspondent).

Multifunction word processor. The Jacquard 100 Videocomputer consists of a central processor and memory, three disc storage drives, two remote CRT's plus the central station, and two Diablo 1650 character printers. The computer is programmed with the Jacquard software system for an Account-Rite Business Program (accounts payable), a Type-Rite Word Processing Program, and a telecommunications package. The character printer prints approximately 35 to 55 characters per second and gives a print quality similar to an electric typewriter. The multifunction office word processor is used for editing, correcting, storing and updating reports, manuscripts,

procedure manuals and lists of ATCC fungi with special applications as well as for solicitation of new cultures.

For example, the folllowing lists of ATCC fungi are stored in this comptuer and updated periodically.

1) Special applications of ATCC fungi -- Patent cultures
2) Special applications of ATCC fungi -- Antibiotics
3) Special applications of ATCC fungi -- Enzymes
4) Special applications of ATCC fungi -- Bioassays
5) Special applications of ATCC fungi -- Organic acids
6) Special applications of ATCC fungi -- Citric acid
7) Special applications of ATCC fungi -- Wine yeasts
8) Special applications of ATCC fungi -- Fermented foods and beverages
9) Mycorrhizae-forming fungi
10) Edible mushrooms
11) Mycotoxin-producing fungi
12) Entomopathogenic fungi
13) Fish pathogenic fungi
14) Mycoparasites
15) Fungi pathogenic for man and animals

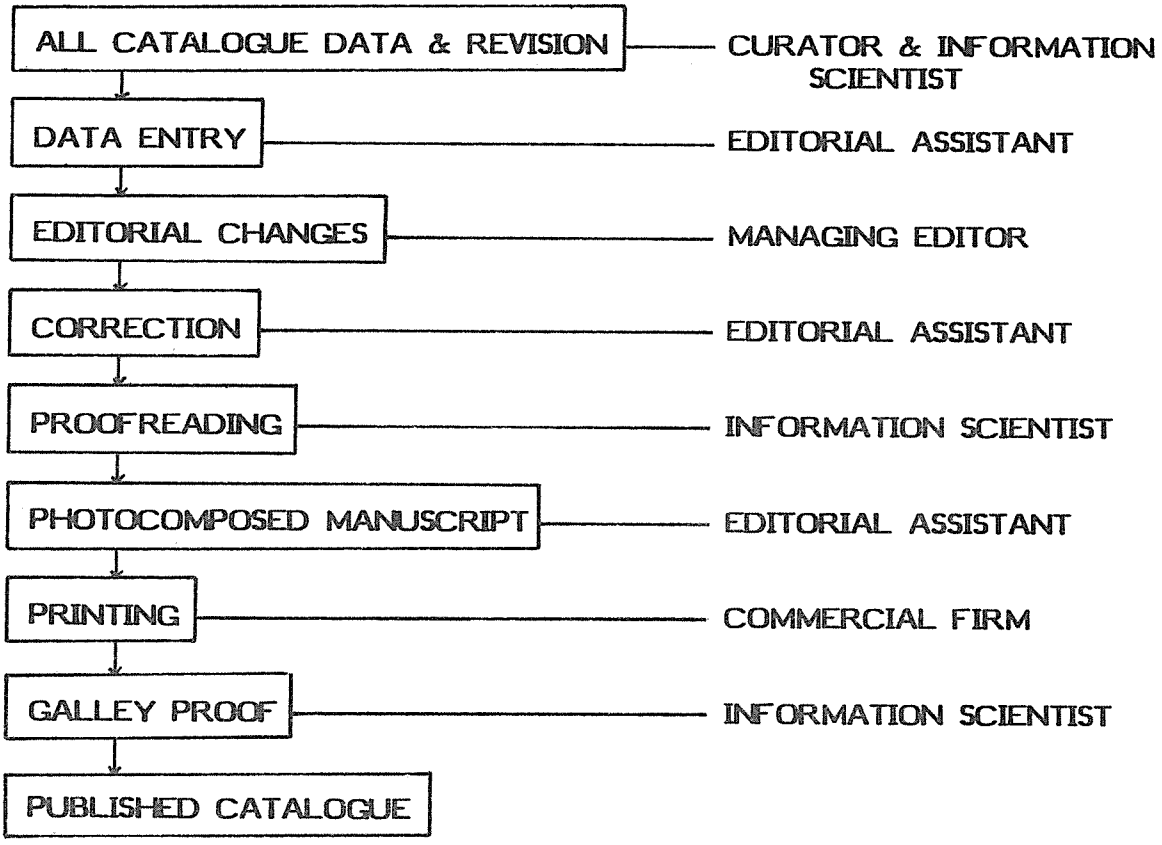
Minicomputer for order processing/inventory control. The Waurax-Centurion minicomputer has 64K memory and 40 megabyte disc storage, four disc drives, four remote CRT's and two Centronics printers (one medium 180 characters per second and one high speed 600 line per minute). It is programmed with custom software for order processing, inventory control, accounting records (accounts receivable and past due accounts), history files and management reports. All orders are entered into the computer and inventory reductions are automatically made. Upon notification of shipment each order is coded in the comptuer and invoices are generated on a daily basis. The culture master file programmed in the computer is only searchable by the ATCC accession number. It contains scientific name, alternate name, classification used for packing and shipping, preservation method and storage

location, status of availability, date the culture was prepared, delay for shipping, recommended medium and temperature for growth, export license and permit requirements, and inventory and distribution data. Therefore, the system offers on-line customer inquiry, on-line inventory and culture inquiries and order status information. It also produces management reports for sales analysis by cultures or customer, open order status, general inventory, critical items (low in stock), backorders, culture history reports, and distribution by number of cultures per category and dollar values. The system can be generated as a functional mailing list operation for 50,000-60,000 names and addresses for distribution of ATCC Quarterly Newsletters and other information.

Combined word processor and phytotypesetter. All ATCC catalogue data are stored and processed on an AM Comp/Edit 5810 phototypesetter which contains a keyboard, video display, 80K all-RAM memory, dual magnetic disc storage/retrieval and photo unit. It is a combined word processor and phototypesetter system that allows text-editing, data management and typographic control for easy updating of manuscripts in the production of photocomposed typeset copy for use by the printer. The content of each fungus catalogue entry is taken from the handwritten master card of each new accession and entered into the typesetter by the Editorial Assistant. The first document is proofread by the Managing Editor and Information Scientist who also make editorial changes for style. Corrections are made and again proofread by the Information Scientist before the final production of photocomposed manuscript for use by the commercial printer (Figure 2).

The catalogue consists of annotated lists of strains arranged alphabetically by species name, information on packaging and shipping of cultures, over 1100 complete culture media formulations, special applications indexes to strains used in assays, quality control, testing, and

FIGURE 2. FLOW CHART FOR PRODUCTION OF CATALOGUE



process fermentations, and an ATCC numerical index. Under each name the ATCC strain numbers are listed in numerical order. The components and arrangement of information in the entry for each strain follow:

1) Strain histroy includes the name of the depositor followed chronologically by the individuals who have maintained the culture since isolation, their strain designations, scientific names other than the current one. Replaced cultures are noted.
2) Additional strain numbers of other culture collections or individuals follow, enclosed by parenthesis.
3) Source of isolation, or derivation in the case of mutants.

4) Significance includes special applications, taxonomic data, genotype, pertinent facts concerning the strain and, if available, a verified reference for each.
5) Permits or other shipping requirements for distribution follow and include the U. S. government application form numbers.
6) Media and growth conditions are last and include the ATCC medium number assigned to the formulation found satisfactory for growth and maintenance of the strain.

This computerized text-editing system, which allows easy updates and produces photocomposed copy for use by the commercial printer, results in significant reductions in labor and costs associated with catalogue production. Since the format of the catalogue includes the comprehensive strain documentation, and the information is triple-checked, scientists find the ATCC catalogue of strains a very useful reference manual.

Communicating terminals. There are two communicating terminal stations and two portable communicating terminals available for data processing and text-editing. The system also allows information retrieval by allowing the operator to search, locate, and extract or abstract specific characters, words or phrases. Input codes and format permit the generation and retrieval of certain indexes. The terminals are also used in telecommunications access to computer databases of MEDLARS and Dialog systems for retrieval of bibliographic information.

The communicating terminals may also be connected to mainframes or minicomputers for the manipulation of large databases. The ATCC, under contract with the Food and Drug Administration, has developed programs for database management, report generation, microbial identification using probability matrices, and statistical analysis. These programs reside at the National Institutes of Health (NIH) Computer Center, on either the IBM 370/168 multiprocessor or the PDP-10 research computer.

The ATCC has developed internally and in collaboration with NIH, modified computer programs in three areas of importance to the Collection, namely:

1) QUERY — This program enables data files to be retrieved, searched and reformed to then generate reports and tables.
2) IDD — This program and modifications of it perform identifications of strains on the basis of characterization data. Fishers Maximum Likelihood theory and Bayes' theorem of probabilities are used as the basis of the identification algorithm.
3) TAXON — This program enables phenetic data to be subjected to cluster analysis whereby those strains which are most similar to one another on the basis of their characterization data, are placed together in taxonomic groupings.

CONCLUSIONS

A resource collection of fungus cultures has an obligation not only to preserve carefully selected cultures in substantially unchanged form, but to distribute those cultures on request, together with detailed information on their nature. Since the quality of each culture held depends on accurate culture data, there is a vast amount of paperwork generated in the day-to-day data management activities such as acquisition, accessioning, inventory, cataloguing, order processing, and shipping.

In recent years the increased demands of scientists in both academia and industry, and new or changing regulations have created a need for accessible and up-to-date documentation files relating to the cultures. At the same time, the rapid growth of the literature involving fungus cultures has also placed ever increasing burdens on culture collections for upgrading their data. With access to a wide variety of automation systems now available, it is time to look toward electronic data processing for inventory and information control in managing culture collections.

A resource collection may attempt to maintain a large number of strains of a few species, or a few strains of each species, or a large number of strains of all known species in culture. Consequently the system of data management will vary accordingly. In addition, there is a diversity in both software and hardware systems. Thus there are substantial variations in how a data management system can be structured and function. A system that works well for one collection may not work for another. Since computerized systems demand more precise and consistent forms and contents of data than manual systems, it is important that a system analysis of the existing manual documentation and collection activities be performed prior to implementing automation. With continued advances in computer technology, the ATCC data management system presented here is being reviewed by a data processing consulting firm for further improvement.

CURRENT STATUS OF CULTURE PRESERVATION AND TECHNOLOGY

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ABSTRACT

Preservation of microorganisms has progressed from continuous growth to methods that reduce metabolism to as low a level as possible. It is accepted that freeze drying and cryopreservation are most effective, but other less sophisticated methods have shown good results. The choice of preservation technique can therefore be made according to the requirements and facilities available. Biology and microbiology are going through a period of rapid development with an increased requirement for culture preservation to a high level of stability and viability. This is especially so when handling biochemically and genetically important strains in order to retain their activity during the research and during their retention for further use. Research on fungal culture preservation has led to improved technique but no fundamentally new method has appeared since liquid nitrogen storage in the 1960's. Technology has produced better refrigerators; low temperature (-80 to -120°C) mechanical freezers are now available. Cryopreservation is vulnerable and freeze drying harsh. However, despite shortcomings of present methods, cultures have been successfully maintained since the beginning of the century.

INTRODUCTION

"Fungus cultures used as vital tools, standards and measures must remain pure, viable and unaltered in form or function during storage and distribution. This requires continued research in materials and methods to

preserve cultures." This is one of Dr. Batra's statements in his pre-IMC3 Conference introductory paper and sums up the position of culture preservation relevant to technology. First it stresses the need to keep the cultures, then the need to keep them in good condition and finally the need to research into methods of keeping them.

Culture collections are an integral part of many biotechnological programmes and it is essential to maintain the organisms used in good condition and for future use. It is also important to keep and maintain isolates for teaching, research, standard strains for testing and production, industrial strains, genetically manipulated strains, pathogens of plants, man and animals, and various other reference isolates including types of species and genera. The large scale preservation of this reference material is the work of the National - International Resource Collections (NIRC) and the big industrial culture collections. However, a far greater number of cultures are kept by individual workers in Universities and elsewhere for research and teaching. The large collections can afford the most expensive and sophisticated forms of preservation but individual collections must often look for something simpler. Thus there is still a need, in small collections or for very sensitive organisms, for the older and simpler methods of maintenance. However, it is generally agreed that freeze drying, lyodrying and cryopreservation are the most satisfactory methods and these are used by the major NIRC, industrial collections and elsewhere whenever possible.

Preservation methods used at the Commonwealth Mycological Institute (CMI). At the CMI we hold some 11000 isolates of fungi and needless to say many different preservation techniques have been examined for the feasibility of keeping such a collection (Table 1). Almost the whole collection has been stored under mineral oil with up to 32 years survival for some isolates. The risk of variation and contamination led to experimentation with other techniques. Soil storage has been used

TABLE 1. Preservation techniques, the range of fungi surviving, longevity and their genetic stability

Preservation Technique	Genetic Stability	Longevity	Range of Fungi Surviving
Growth 18-20°C	Variable	1-6 months	All
4-7°C	Variable	6-12 months	Most, some are sensitive
Oil storage	Poor	1-32 years	All
Water storage	Moderate	2-5 years	Most
Soil storage	Moderate/low	5-20 years	Most
Silica gel storage	Good	5-11 years	Robust spore types
Freeze drying	Good	4-40 years*	Sporulators except the Mastigomycotina
Liquid nitrogen	Good	14 years*	All
Deep freeze (-17 to -23°C)	Moderate	4-5 years	Most, some are sensitive

*Death point undetermined — storage still continuing.

for the preservation of *Fusarium* and related genera for at least 10 and up to 20 years and water storage has allowed the maintenance of *Phytophthora* and *Pythium* for 2 years or more without subculturing. However, these techniques allow periods of growth during which variation may occur. Alternative techniques which do not allow growth are preferable. Silica gel storage has been in use for many years to keep genetic stocks of fungi and has been shown at CMI to keep sporulating fungi viable

for up to 11 years. However, yeasts usually fail to survive or have low viability using this technique. A similar range of fungi survive freeze drying but some appendaged and thin-walled spores which are damaged in silica gel may freeze dry well. Over 8000 isolates from our collection have been preserved successfully by freeze drying though many others have failed (Smith 1983a), some, because they had deteriorated during long term storage by other techniques previous to processing. Although they were in good condition others failed the technique because they were mainly non-sporulating strains. By far the most successful technique used at CMI is storage of frozen material in or above liquid nitrogen. Approximately 95% of all fungi tested (some 3500) have survived for up to 14 years. The only group of fungi that does not respond well to freezing is the Mastigomycotina although 50% of those tested at CMI have survived. It is difficult to predict whether an organism will survive a technique as in many cases survival has been shown to differ for isolates of the same species. From the work at CMI it is felt that the simple techniques can be useful but stability of cultures can be best achieved by the use of storage by freeze drying, in silica gel or in liquid nitrogen. Table 1 compares the techniques used at CMI with the range of fungi surviving, their longevities and genetic stability.

Research and growth preservation methods. A literature review has shown that research is improving techniques but no fundamentally new methods have been introduced since the work with liquid nitrogen in the 1960's (Hwang 1960, 1968). Since then technology has provided better refrigerators with easier access, automatic filling and alarm procedures. Mechanical refrigerators capable of providing ultra low temperatures of -80°C or even -120°C are now available. These are to be seen in many laboratories in Japan. They have liquid nitrogen or carbon dioxide back up. The original models were very small and present ones are still very expensive. Better plastic

ampoules, straws and storage units have been developed with improved storage capacity. Empirical work is being undertaken on cryoprotectants and freezing protocol. The use of mixtures of cryoprotectants at the CMI (Smith 1983b) showed best viabilities with 8% glycerol plus 10% dimethyl sulphoxide, though for practical purposes 10% glycerol proved satisfactory for routine work. Various workers have tried to replace ampoules with plastic straws, tin foil or cellophane packets to save space and achieve more rapid cooling. Mechanisms of preservation and research into death points have been carried out extensively for bacteria and yeasts but work on fungi is limited. This lack of interest in fungi might be due to the very great success of freeze drying and cryopreservation for the majority of fungal species coupled with the difficulty in assessing the degree of revival as it is not always possible to make viability counts especially when dealing with mycelial cultures.

In the UK a cooperative program to use electron microscopy to examine freezing damage is being undertaken. Freeze driers are now available in which the progress of cooling and drying can be monitored. Such an instrument is being used at CMI to devise routines for the preservation of the very sensitive *Phytophthora*, *Pythium*, and mycelial Basidiomycetes. The work is preliminary but the results are promising. An indication as to the stage of the freeze-drying process causing damage and death is being obtained by using freezing protocol which has proved successful in liquid nitrogen storage. This is followed by controlled variation of the warming and drying. Overdrying of fungi to residual moisture contents below 1% caused damage, variation or death and warming stages during the process have been shown to have some effect on the final viability of the fungus. By finding the critical points in the process it is hoped to improve the technique enormously. Freeze drying has been reported to induce mutation in microorganisms but at the CMI very

few have been observed in fungi and those seen have been associated with overdrying.

Culture collections are growing rapidly to store the increasing number of isolates required to support the developing biotechnology industry. Not only is this likely to create a problem with the amount of storage space necessary but also with the vast number of records needed. Already the large collections are using computers for records and stock control. We believe that some isolates were sent up in "space lab" but storage on a satellite although a fascinating idea does not at present seem a practical solution. Several laboratories, in particular the Deutsche Sammlung von Mikrooganismen (DSM), are experimenting with miniaturized preservation methods.

Future prospects. Is the research into freeze drying and cryopreservation even on a miniaturized scale enough? The latter may reduce the cost and space problem but cryopreservation is very vulnerable both to failure of supplies of liquid nitrogen and refrigeration while freeze drying is very harsh.

It has been suggested that the geneticists and cell biologists may have the answer. Will they really be able to reduce organisms to a stable form consisting of pure DNA or its components? If so it is suggested we could build organisms as required from the component parts. DNA and other cell components are fantastically complex so it seems likely to be a long time before we progress to this sophistication. Genetically manipulated material appears to present the same or more problems of stability and viability on preservation. Survival depends heavily on the care and expertise of the curator.

The eventual development of such techniques are not entirely a dream and research is progressing rapidly. The ATCC has built a large bank of cell lines and a National Animal Cell Culture Collection is being started at Porton, UK. The Upjohn company and other industrial firms maintain their genetically interesting organisms successfully

so preservation of genetically manipulated material is being undertaken despite the difficulties.

CONCLUSIONS

It is necessary to maintain banks of representative standard and important organisms. The work has already started. Some isolates have been maintained since the beginning of the century mostly by frequent transfer and the loving care of the curators. It is a matter of persisting with this work while developing, using and applying new biotechnology as it becomes available.

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LEGAL OBLIGATIONS OF CULTURE COLLECTIONS CONCERNING PATENT STRAINS AND THE SHIPMENT OF PATHOGENS

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ABSTRACT

The legal obligations of culture collections concern two areas: safety and patent cultures. Microbiological patent laws generally require the deposition of the subject strain in an appropriate culture collection. Patent deposits may be made under national laws, the European Patent Convention, or the Patent Cooperation Treaty. In order to facilitate patenting the same invention in more than one country, the Budapest Treaty allows a single culture deposit to satisfy the patent application disclosure requirements of all treaty countries. The saftey aspects of culture collections concern laboratory containment and safe shipping of potential pathogens. Shipping is governed by international, national, and local laws.

INTRODUCTION

When one thinks of the many responsibilities of culture collections, their legal obligations are not always readily apparent. However, these legal obligations, governed by international, national, and local laws, as

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[1]The mention of firm names or trade products does not imply that they are endorsed or recommended by the U. S. Department of Agriculture over other firms or similar products not mentioned.

well as by professional ethics and good judgment, primarily concern two areas — safety of the society at large and patent cultures. The scientific and popular literature are replete with accounts of the introduction of plant, animal, and human pathogens into new regions and the concomitant disastrous results. Although the shipment of such pathogens from culture collections has not been implicated as the cause of disease outbreaks, the possibility exists and must be guarded against.

Patent cultures, on the other hand, present an entirely different set of responsibilities. The patent system was introduced to provide legal protection to inventors so that they might have exclusive rights to commercially exploit their discoveries for a given period of time. The alternative to this system, which is also practiced, is the trade secret in which exclusivity is maintained by denying competitors knowledge of the process. As microbiological process patents increased in number, it became apparent that a representative culture of the subject microorganism would need to be maintained. The reasoning was that for chemical, electrical, or mechanical patents, a diagram or formula can sufficiently describe the invention, whereas in a microbiological patent, illustrations and narrative descriptions are generally inadequate to sufficiently define the microorganism used. For this reason, culture collections have assumed a role that significantly affects world commerce.

PATENT CULTURES

In 1949, the United States Patent and Trademark Office implemented its new requirement that cultures be deposited in conjunction with patent applications concerning microbiological inventions. The Patent Office initially asked the American Type Culture Collections (ATCC) to serve as depository for patent strains, but at that time, the ATCC was not prepared to assume this function. The other major collection in the U. S. was the Agricultural Research Service Culture Collection located at the Northern

Regional Research Laboratory (NRRL) (now Northern Regional Research Center). The NRRL collection had been established by the U. S. Department of Agriculture to provide cultures and microbiological expertise for programs in agricultural research. The important contributions of this collection in commercialization of penicillin, riboflavin, and numerous other products and processes have been well documented (Hesseltine *et al. 1970*). The ARS Culture Collection accepted the invitation of the Patent Office and became the first U. S. culture collection (and apparently the first in the world) to accession a patent strain. That culture was *Streptomyces aureofaciens* NRRL 2209, the strain deposited by the American Cyanamid Company of the United States for Aureomycin production. Shortly thereafter, the American Type Culture Collection also began accepting patent strains.

In the U. S., microbiological and genetic engineering patents fall within four statutory classes: (1) machine, (2) manufacture, (3) composition of matter, and (4) process. Saliwanchik (1982) has listed examples of these classes and has discussed recent legal decisions concerning them. Means for genetically modifying naturally occurring microorganisms through cell fusion or recombinant DNA techniques have been well developed in recent years. Because these organisms are not found in nature, some have suggested that they represent novel germplasm which is in itself patentable. Arguments for both sides of this issue have been summarized by Cooper (1982), Saliwanchik (1982), and Luckern and Hesseltine (1979).

In keeping with U. S. Patent Law, the subject strain of the patent application must be deposited with a culture collection which is to maintain this strain for at least the 17-year life of the patent. The depositor has the option of making the patent culture freely available from the date of deposit or requesting that it not be distributed until issuance of the patent at which time the culture has to be freely available. The depositor is

obligated to resupply the culture should the collection find its stocks changed or nonviable. Other industrial countries have implemented similar requirements and many have also established a national patent culture depository. Culture collections are obligated to maintain detailed accession records on their patent cultures and to keep records of all distributions.

Although the patent laws of most nations have the same basic aims, requirements for filing can differ considerably. In the event that an applicant wishes to apply for foreign patents, the filing, translation, and attorney fees can be costly. During the late 1970's, several international agreements were reached which allow a single application to be recognized in a number of countries.

Patent Cooperation Treaty (PCT). This treaty became effective in 1970 and was amended in 1978 and 1979, and has been signed by all industrially important countries. The treaty allows an applicant to file a single application in a standard format through the applicant's national patent office and have the application recognized as a valid filing in as many PCT countries as selected. This procedure results in extra costs, but these are much less than for the collective cost of numerous individual filings. One disadvantage of this system is loss of secrecy. In the U. S., the application is kept secret until approved. If rejected by the Patent Office or abandoned by the applicant, the procedure can still be practiced as a trade secret. In most other countries, patent applications are published, thus disallowing the trade secret option. The countries that have ratified or acceded to the Patent Cooperation Treaty are Australia, Austria, Belgium, Brazil, Cameroons, Central African Republic, Chad, Congo, Democratic People's Republic of Korea, Denmark, Federal Republic of Germany, Finland, France, Gabon, Hong Kong, Hungary, Japan, Liechtenstein, Luxembourg, Madagascar, Malawi, Monaco, The Netherlands, Norway, Romania, Senegal,

Soviet Union, Sri Lanka, Sweden, Switzerland, Togo, United Kingdom, and United States of America, (*Industrial Property*, April 1983).

European Patent Convention (EPC). This treaty, which is restricted to European countries, became effective on October 7, 1977. Applications may be filed with the European Patent Office in either the Hague [P. B. 5818, The Patentlaan 2, 2280-HV-RIJSWIJK(ZH), The Netherlands] or in Munich (Motorama-Haus, Rosenheimer Str. 30, D-8000 Munchen 80, Federal Republic of Germany). The official languages recognized by this treaty are English, French, and German. Applicants who file under the Patent Cooperation Treaty may list the combined EPC countries as a "selected country." Members of the European Patent Organization are Austria, Belgium, Federal Republic of Germany, France, Italy, Liechtenstein, Luxembourg, The Netherlands, Sweden, Switzerland, and United Kingdom.

The protocol for a deposit under the EPC requires that the depositor state in the letter accompanying the culture that the strain is being deposited under EPC Rule 28. Once the strain number is received, the depositor then completes the patent application with the European Patent Office (EPO). In order to receive a restricted culture under the EPC, the request must include the culture name and strain number as well as noting that the deposit is under the EPC. The culture collection then sends a form which the requestor must complete and forward to the EPO. The collection is then notified by the EPO if the strain is to be released.

Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. This treaty was signed on April 28, 1977, and provides that a single deposit in an approved culture collection satisfies the patent application disclosure requirements of all member countries of the Union established by the treaty. The major advantage of this treaty is that a single culture deposit in an approved

collection will satisfy all countries selected in multi-country filings under the PCT or the EPC. Additionally, applicants filing from a country with an approved collection may deposit there and not be concerned whether import permits might be required for deposit in another country.

The requirements for a culture collection to be designated an International Depositary Authority are given in Table I. At present, there are 11 international depositary authorities (Table II). Information concerning the Budapest Treaty may be obtained from the World Intellectual Property Organization, 32 chemin des Columbettes, 1211 Geneva 20, Switzerland.

TABLE I

Requirements for a culture collection to be designated an International Depositary Authority under the Budapest Treaty

Located on territory of contracting/member state
Continuous existence with provision to relocate cultures in event of cessation of activities
Impartial policy concerning all depositors
Appropriate staff and facilities for both scientific and administrative duties
Accept specified types of microorganisms or cell lines
Determine viability of deposit, and issue any required viability statements
Store deposited strains for at least 30 years
Provide sufficient safety measures to minimize loss
Maintain secrecy of deposit as required under the Treaty
Furnish samples in an appropriate and timely manner as required by the Treaty

TABLE II

Culture collections accepted as International Depositary Authorities under the Budapest Treaty[1]

U.S.A.

Agricultural Research Service Culture Collection (NRRL), Peoria, Illinois

American Type Culture Collection (ATCC), Rockville, Maryland

In Vitro International, Inc. (IVI), Ann Arbor, Michigan

United Kingdom

Commonwealth Mycological Institute (CMI), Kew, Surrey

Culture Centre of Algae and Protozoa (CCAP), Cambridge

National Collection of Industrial Bacteria (NCIB), Aberdeen

National Collection of Type Cultures (NCTC), London

National Collection of Yeast Cultures (NCYC), Nutfield, Surrey

The Netherlands

Centraalbureau voor Schimmelcultures (CBS), Baarn

Japan

Fermentation Research Institute (FERM, FERM-P), Chiba

Federal Republic of Germany

Deutsche Sammlung von Mikroorganismen (DSM), Göttingen

[1]Fees are listed in the January, March, September, and November 1983 issues of *Industrial Property* (Budapest).

The basic protocol for a deposit under the Budapest Treaty, which automatically comes under the conditions of the European Patent Convention, is as follows: The depositor states in a letter with the culture that the strain is being deposited under the Budapest Treaty. The depositor must complete the culture collection's accession form, and the collection must perform viability tests on the culture, if viable, then fill out a Budapest Treaty form that includes the number of the newly acces-

sioned strain. This is returned to the depositor who is responsible for completing the filing. The depositor may make the culture freely available at the time of deposit or request that it be restricted until publication of the patent or patent application as required under the various national laws.

Regulations concerning distribution of cultures are specified under Rule 11 of the Budapest Treaty. The depositor may receive a culture of the deposit at any time. The depositor may also authorize distribution to a third party. Any industrial property office (patent office) to which the Treaty applies may receive a culture if necessary in the patent procedure. Other persons may obtain a culture if an industrial property office to which the Treaty applies certifies that, under the applicable law, that person has the right to the subject culture. In practice, outside parties, usually make their requests directly to the depositary authority. When this happens, the requestor is sent a Treaty culture request form which must then be forwarded to the Treaty Office in Geneva. Following processing, the depositary authority is notified by the appropriate industrial property office concerning availability of the culture. Decisions on release of cultures under both the Budapest Treaty and the European Patent Convention are made by industrial property offices and not by the depositary authorities.

In addition to the preceding international treaties, governments may select certain foreign culture collections as suitable for their national deposits. In that event, the collection must be knowledgeable of those particular foreign laws. Several references are available and quite helpful in explaining the various patent laws: Saliwanchik, 1982; Cooper, 1982; and Hüni and Buss, 1982. The journal *Industrial Property* is also an excellent source of information on contemporary patent law. Copies of the various international treaties may be obtained from the offices listed in the preceding text.

SAFETY

The containment and shipment of microbial cultures subject culture collections, as well as individual scientists, to a complex set of laws that have been designed to protect humans, plants, and animals from disaster through accidental release or introduction of infectious agents. Most countries have laws concerning both the import and the export of microbial cultures, and frequently the states and provinces of these countries also regulate movement of pathogenic materials. Accordingly, those who ship or import cultures need to ensure compliance with these laws and regulations.

In the U. S., the Public Health Service and the Department of Agriculture represent the agencies concerned with the importation and transportation of pathogenic materials, although it is the prerogative of the U. S. Customs Service to first inspect and pass on imported materials. The shipment of cultures to other countries is regulated by the Department of Commerce and an export license is usually required.

In addition to the U. S. Public Health Service and the U. S. Department of Agriculture, shipment of cultures is also regulated by the U. S. Department of Transportation, the Civil Aeronautics Board, and the International Air Transport Association. The American Type Culture Collection has provided a concise summary of regulations in their catalog. Additional information may be found in the NIH publication Laboratory Safety Monograph (USDHEW 1979).

On the basis of guidelines established by the U. S. Public Health Service, and the U. S. Department of Agriculture, microorganisms have been assigned to four classes according to biological hazard. These classes with examples and recommended shipping containers are given in Table III.

TABLE III

Classification of microorganisms according to biological hazard and their shipping requirements.

Class I	Agents of no recognized hazard under ordinary conditions
Examples	*Saccharomyces cerevisiae, Trichoderma reesei, Lactobacillus casei*
Shipping	Culture tube in fiberboard or other container. Permits as required
Class II	Agents of ordinary potential hazard
Examples	*Aspergillus fumigatus, Candida albicans, Cryptococcus neoformans, Staphylococcus aureus*
Shipping	Culture tube wrapped in absorbent material, placed in metal screwcap can, placed in fiberboard container. Permits as required. Etiologic agent warning label necessary
Class III	Pathogens involving special hazard
Examples	*Coccidioides immitis, Histoplasma capsulatum, Bacillus anthracis, Yersinia pestis*
Shipment	Culture tube heat sealed in plastic, wrapped in absorbent material, placed in hermetically sealed can, placed in sturdy cardboard box. Permits as required. Etiologic agent warning label necessary
Class IV	Pathogens of extreme hazard
Examples	*Arthroderma simii, Pasteurella multocoida,* certain animal/plant viruses
Shipment	Culture tube heat sealed in plastic, wrapped in absorbent material, placed in hermetically sealed can, placed in sturdy cardboard box. Required permits. Etiologic agent warning label necessary

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CRITICAL PROBLEMS IN CULTURE COLLECTION RELATED TO BIOENGINEERING

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Roles and functions of a culture collection are to collect valuable species and strains of microorganisms of importance in science and technology, to preserve them and to provide these authenticated subcultures to researchers all over the world with their taxonomical characteristics unaltered. Culture collections are intentionally organized to maintain a wide range of microorganisms and to ensure availability of taxonomically authenticated strains which can be used as reference standards.

To accomplish these roles, a culture collection must have enough experienced staffs who are able to keep their collection in a reliable state, that is, they have been checking the characteristics of the strains and are able to guarantee the presence of characteristics claimed. A culture collection must be equipped with modern facilities and it must have ability to perform both scientific and administrative tasks in accordance with the pace of development in both fields. Culture collections serve as active research centers for taxonomy, because they have a number of reference strains whose historical background is clearly documented. Basic study areas, such as taxonomy, biochemistry and genetics are necessary research units comprising a culture collection. But these ideal situations are hard to realize.

Patent Cultures. With the rapid development of applied microbiology, and rapidly expanding importance of micro-organisms in industry, culture collections have to play another important role i.e. to participate in patent

procedures (See Kurtzman, this symposium). They have to act as depository organizations of patent strains. This new role places additional responsibilities on collection staff to accelerate the development of methods or researches for sustaining the physiological characteristics of cultures entrusted to them.

Obligations of a culture collection related to patent procedures are to accept the patent strains, preserve them for more than 30 years to cover the life of the patent, and to assure dispatching the deposited strains to the requesters who have a right to obtain it. Culture collections have accumulated much basic information and established various methods to preserve strains with a high survival rate for a long time without losing the taxonomic characteristics. However, they have little experience to preserve such characteristics, as the production of antibiotics, production of enzymes and other biologically useful substances, which are often not directly related to the taxonomic position of a microorganism, nor so important to taxonomy. It can be expected that accessions of microorganisms having such productive properties ('productivity') will increase with the development of applied microbiology, not only in patent procedures but also in the fundamental field of microbiology.

Bioengineering and responsibilities of culture collections. I would like to note here some of the critical problems to be solved in the future related to bioengineering. As I mentioned earlier some culture collections must accept patent strains and must preserve non-taxonomic characteristics which generally tend to be lost during serial transfers. A few problems will be solved partially by applying available methods of long term preservation.

Methods for preserving microorganisms have been investigated and a number of efficient and satisfactory methods for preservation have been established (see Onions and Smith, this symposium). For example, preservation of

microorganisms by freeze drying or lyophilized sample, preservation under an ultra low temperature, such as in liquid nitrogen or -80°C deep-freezer, and L-drying methods ['L' stands for drying from the liquid state — a reviewer] are wide-spread techniques utilized in culture collections. By these methods culture collections acquired practical but tentative strategy to face up to the new circumstances.

Dr. Maruyama (1), Research Laboratory of Japan-Roche Co., examined the ability to produce β-lactam antibiotics in a number of *Streptomyces* which had been preserved by lyophilized sample or other methods. He found that the ability to produce β-lactam antibiotics (for example clavulanic acid, cephamycin and penicillin) were well preserved in a number of *Streptomyces* strains which were preserved by lyophilization. Some of the tested strains, for example *Streptomyces* sp. 372A was well preserved; however, its productivity on its original medium had been lost. In this case he changed the medium and growth conditions and found that the strain still retained its productivity. He emphasized that there was no general method to maintain productivity but that you will find the most suitable method for maintaining productivity among the suitable conditions for maintaining their viability. In particular cases the most suitable method for maintaining productivity was not the most suitable method for getting high survival rates!

Dr. Hamada (2) in the Institute of Microbial Chemistry in Tokyo, has determined the conditions for the preservation of *Streptomyces* with antibiotics productivity. After long-term preservation, she examined some of the characteristics to produce antibiotics such as novobiocin, chloramphenicol, kasugamycin and some of the enzyme inhibitors. She found that almost all of the productivities were well preserved in lyophilized samples or paraffin-sealed slant cultures under frozen state. The most remarkable result was that strains with high level

of production were found to retain the high productivity after the preservation. She also found that some of the strains can be rejuvenated by the change of media, and which retained their productivity.

Dr. Kusaka (3) at our Institute, i. e. IFO, compared the differences of productivity between samples transferred on agar slants and lyophilized samples. Among 185 ISP (International *Streptomyces* Project) strains of *Streptomyces*, about 140 strains (76%) showed equally or nearly equal characteristics of antibiotic production in both cases.

These results show that most productivities can be preserved by methods hitherto used for preserving viability, although some particular cases need some tricks.

The second problem to be solved, related to bio-engineering would be a preservation of characteristics which are unfamiliar to staffs of a culture collection, for example, microorganisms with plasmids or recombinant DNA, or productivity of a particular substance such as a growth hormone. Some of these strains seem to be unstable and need special care to hold the plasmid or recombinant DNA, and furthermore an assay of the produced substance requires specialized facilities and various techniques. In most cases, culture collections do not have enough staff to check the abilities to produce or check the authenticity of a particular product.

A possible solution to this problem would be a checking system by a special organization or a committee. The ISP Committee in Japan for confirmation of standard descriptions is an example of a successful program.

International *Streptomyces* Project aims for international cooperation for standardization of descriptions and deposition of type cultures of *Streptomyces* by the following method. A number of authentic strains are deposited with four culture collections including the Institute for Fermentation, Osaka. The Society for Actinomycetes of Japan organized this committee for the confirmation of

these ISP strains deposited in IFO, and 12 members of the committee have checked these strains every 4 years. According to this arrangement, IFO has the responsibility to maintain and to distribute these strains, and the committee has the responsibility to check the characteristics of ISP strains in IFO. This system can be applied to other special strains, such as standard strains used for antibiotics assay, for bioassay, or for mutagen testing. Some of these strains have been checked by another committee when a standard characteristic seemed to have changed.

Similar systems could be applied to check a particular enzyme or special substance. However, it is preferable that these areas be the responsibility of each depositor.

Methods of preservation of plasmids are being investigated and preliminary results are encouraging. We are hopeful to maintain plasmids using some of these methods.

With the development of microbial industries, achievements of bioengineering will spread to new fields of animal and plant cell lines. The demands of deposits of cell lines to culture collections are increasing. But there would be more serious problems in maintaining cell cultures, than maintaining microorganisms, such as fungi and bacteria. These may not only include technical but also financial problems.

Therefore, the third and most serious problem would be a shortage of financial support to culture collections.

Financial aspects. Every scientist understands the important role of culture collections in promoting science and technology, and of preserving properties of life itself. These properties are irreplacable accumulations of long history of evolution. A number of famous culture collections are already threatened by shortage of financial support. Some are general culture collections and some are specialized collections of particular species in research organizations such as

universities. Most of these culture collections have been supported by grants from government, scientific societies and other organizations. If support were sufficient, they could fulfill their services without charge. However, even if the culture collection charged for distributing cultures, the resulted income would not cover the whole expenses of the culture collection, unless the cost of a culture was tremendously high. As many of you may know, the income from distributional charge in ATCC was about one-half of the total revenue in 1982 (4), and in IFO that was 20% of the annual revenue in 1982.

A culture collection is not a profitable business, but it is similar to a museum. Most museums charge little or no admission to the public. The admission does not cover all the expenses of the museum. Similarly users of culture collections are charged reasonable rates, because unbalanced budgets must be covered by other kinds of support.

I regret to say that I do not have solutions for solving the financial problems. I only feel that it might be a problem of choice between two alternatives, that is whether users prefer to pay a fee directly to the culture collection, or prefer to pay tax to the government or fund for culture collection, through a foundation.

In conclusion, a culture collection has various problems to solve when it plays its important roles in science and technology. Some of these can be solved by cooperation among culture collections or cooperation among culture collections and other organizations.

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FUNDING PROBLEMS OF CULTURE COLLECTIONS NATIONAL AND INTERNATIONAL

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Reflexes and instinct are inherent properties of all life observed throughout the animal kingdom. One does not think about breathing or blinking, birds build highly species specific nests without plans or training. Likewise scientists collect things and usually don't think of this activity apart from their ordinary course of work.

Collecting is so taken for granted that usually no category of funds, levels of effort nor even significant attention is directed to collection activity when support is sought from granting agencies.

After years of work and accumulation of extensive reference collections, scientists are often dismayed to find that their university or their granting agency is surprised to be asked to provide a home for their material and reluctant to agree.

Administrators should know of this collecting propensity of scientists only if they are scientists themselves — most are not therein lies the first level of funding problems.

The second level funding problem has to do with the exchange of "courtesy" or gratis cultures between scientists.

This form of professional courtesy, a benevolent generosity, can also be defined as charity — the rich giving to the poor. Although this practice is considered a virtue in most cultures — establishing a type of moral savings account — it can have side effects that are pernicious.

We have a saying — "Dont' look a gift horse in the mouth" —. In this case of gift cultures you are foolish not to do so because what you get may not be a horse but a dragon. Unless you independently verify it or have it done for you by a competent authority, years of work and lots of money can be wasted studying the wrong thing.

This traffic is most active in newly described material. The investigator originating the material is flattered by the attention and often spends considerable resources to comply with the requests. The recipient, feeling he has the means to be *au courant* and ahead of the pack is apt to overlook tests for identity and purity that would really define what he has.

This service can also be a financial burden bootlegged out of the donor's grant funds and certainly is a distraction from his ordinary research.

Customarily no charge is levied for this courtesy and there seems to be a conspiracy of silence to shield this fact from administrative people who pay the bills. Somehow this must be changed and the practice legitimized.

This is a normal cost of doing research and should be recognized as such.

The third level of problem is the financial support of reference collections and national repositories. Microbial collections are not unqiue in having these problems but zoological and botanical collections can pander to the public's curiosity and financial support by exhibiting their specimens and provide a form of cultural entertainment. Monkeys are cute and we see our foibles reflected in them, but, try as we may, it is hard for humans to identify with a *Mucor* or *Penicillium.*

We have not done a good job of informing the public and its political representatives of the importance and need for microbial collections and until we do whatever public funds we obtain will be small in amount and capricious in coming. Keep in mind that such requests also carry the obigation to circumscribe the limits of collections.

Bigger is not necessarily better and a well culled selection of representative strains is better than many duplicates varying only in place and time of isolation.

A collection requires a constituency of users to develop support and it is incumbent upon curators to provide useful screened material, not a garbage dump.

The fourth problem level is the lack of internationl attention to these collections and the devising of means to assure uniform quality and ready world wide access to the materials. There is a tendency for national collections to be isolated because of low funding levels. This inhibits conferences and working committees from meeting and working out procedural specifics, developing strain data bank networks, and establishing programs to develop and exchange collections of scope and depth.

What paltry resources are available are now being spread very thin and in my opinion a lot of duplication of effort is squandering money better spent on organized collaboration.

It is often said that defining the problem is more than half of the solution to it.

There is also an obligation for the critic to suggest ways to improve what is found deficient.

The first level problem — recognizing the collecting process as integral to the pursuit of scientific knowledge is obvious but not trivial.

Educational processes must be invoked and include chapters in textbooks, informative articles in scientifc and lay media and exposure of scientific administrators to the concept that collections are libraries and deserve similar support.

The second level problem — exchange of "free" cultures with their attendant risks could be minimized by more timely and aggressive accessioning of new material by curators. Prompt and thorough characterization by experts before wide distribution would insure the subsequent investments and would relieve investigators of nuisance and financial burdens.

The third level problem — institutional support for collection activities is primarily a political one. Public funds are usually made available if widespread support for an institution is perceived by government. To this end scientists through their scientific societies need to define and promote the usefulness of these libraries and the economic sense of sharing these resources rather than having to maintain multiple small efforts or to depend upon continual re-isolation of material.

The value of national or international collections depends upon reference collections developed by peer specialists and those in turn depend upon the individual scientist's creativity and initiative. This process continuence needs to be defined, explained and rationalized to a much larger audience than heretofore done.

The fourth level problem — better international cooperation and use of resources is appropriately addressed in a forum such as an international congress. Representatives of major collections and individual scientists capable of contributing to these efforts are here. First, a culture collection committee as a standing committee needs to be appointed and charged with responsibility to inventory and evaluate collection activities. National and regional collections and federations of collections need to be involved in this process. Summaries and recommendations for improvement of international exchanges and standards need to be developed and forwarded to appropriate individuals and institutions for policy and financial support.

Coming from an internationally recognized organization of scientific peers such a statement would have an effect on policy decisions.

The preceding recommendations distill the thoughts and opinions of a number of conferences, advisory panels and individuals. I claim no credit for unique insights but feel very passionately that no progress will be made until we collectively act in a positive way to improve matters.

COLLECTIONS OF THE WORLD - UNITE.

APPENDIX

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