

1
Ag 849h

(5)

U.S. DEPT. OF AGRICULTURE
NATIONAL LIBRARY OF MEDICINE

Sources and Management of Micro-Organisms For the Development of a Fermentation Industry

Agriculture Handbook No. 440

**Agricultural Research Service
U.S. DEPARTMENT OF AGRICULTURE**

PREFACE

THIS REVIEW of micro-organisms provides a background for the manufacture of chemicals by fermentation. It was prepared at the Northern Regional Research Laboratory of the North Central Region, Agricultural Research Service, U.S. Department of Agriculture, Peoria, Ill. At this Laboratory, investigations are being conducted on the industrial utilization of cereal grains, oilseeds, and agricultural wastes by fermentation. The ARS Culture Collection, maintained there, is one of the world's most complete collections of industrially important bacteria, molds, actinomycetes, and yeasts. It serves as a source of authentic micro-organisms for the fermentative production of organic acids, vitamins, antibiotics, enzymes, feeds, beverages, and foods.

Inasmuch as the key to success or failure in most fermentative processes is availability of the proper micro-organisms, the characteristics of suitable microbial strains are enumerated; the industrial microbial collections of the world—their locations, their general holdings, and the names of their directors—are listed. The attributes of a good culture collection are emphasized. Various fermentation processes in use throughout the world are listed, together with the specific micro-organisms needed to carry them out.

Of these processes, the ones most likely to be beneficial in developing countries are indicated. Because information is so frequently requested about micro-organisms: for example, how to maintain stable cultures, how small fermentation plants may acquire suitable microbial strains, and how micro-organisms can be shipped through international channels, this publication is being issued to answer the many requests received yearly by the U.S. Department of Agriculture.

Trade names are used in this publication solely for the purpose of providing specific information. Mention of a commercial product or company does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture or an endorsement by the Department over other products not mentioned.

**Sources and Management
of Micro-Organisms
For the Development of
a Fermentation Industry**

By C. W. HESSELTINE and W. C. HAYNES

Agriculture Handbook No. 440

**Agricultural Research Service
U.S. DEPARTMENT OF AGRICULTURE**

Washington, D.C.

Issued March 1974

For sale by the Superintendent of Documents, U.S. Government Printing Office
Washington, D.C. 20402—Price 60 cents
Stock Number 0100-02682

CONTENTS

	Page
Introduction	1
Sources of micro-organisms for industry	2
Characteristics of a good culture collection	21
Location of culture collections	23
Procedures for isolation and selection of micro-organisms from nature	24
Classification of micro-organisms used for production of fermentation products	26
Problems in maintaining stable industrial cultures	30
Problems of strain degeneration and loss	32
Contamination by other micro-organisms	33
Infestation by mites	33
Phage infestations	33
Natural selection and mutation	33
Untrained staff	34
Physical conditions affecting micro-organisms	34
Regulations regarding deposit of cultures for patent purposes	35
Procedures and policies for deposition of cultures for patent purposes in the ARS Culture Collection	35
Shipment of micro-organisms	36
Literature cited	38



Use Pesticides Safely
FOLLOW THE LABEL
U.S. DEPARTMENT OF AGRICULTURE

Sources and Management of Micro-Organisms For the Development of a Fermentation Industry

By C. W. HESSELTINE and W. C. HAYNES, *Northern Regional Research Laboratory
North Central Region, Agricultural Research Service,
U.S. Department of Agriculture, Peoria, Ill.*

INTRODUCTION

The micro-organism used in a fermentation is the key to the success or failure of the process. It is the catalyst that makes the fermentation work. A microbial culture must have certain general attributes if the process it generates is to be operable, regardless of the nature of the product and the simplicity or complexity of the engineering process:

1. The strain must be genetically stable. A culture that constantly and spontaneously produces one or more different forms is undesirable.
2. The strain must readily produce many vegetative cells, spores, or other reproductive units. Since Basidiomycetes produce only mycelium, they are rarely, if ever, used in industrial fermentation.
3. The strain should grow vigorously and rapidly after inoculation into seed tanks or other containers used to prepare large amounts of inoculum before an industrial fermentation.
4. The strain should be a pure culture, not only free of other microscopically visible micro-organisms, but also free of phages.
5. The strain should produce the required product within a short period of time, preferably in 3 days or less.
6. The strain should produce the desired product to the exclusion of all toxic substances. The desired product should be easily separated from all others.

7. The strain should be able to protect itself against contamination, if possible. Self-protection might take the form of lowering the pH, growing at high temperature, or rapidly elaborating a desirable microbial inhibitor.

8. The strain should be readily maintained for reasonably long periods of time.

9. The strain should be amenable to change by certain mutagenetic or group of mutagenetic agents. A mutation program may even be conducted with the object of developing strains that give enhanced yields of the product.

10. The strain must give a predictable amount of desired product in a given fermentation time.

Micro-organisms that meet these conditions may be either isolated from nature or obtained from a culture collection. Trained microbiologists are required to isolate, purify, screen, and test a culture from nature. Since, in developing nations, such trained people are often in shorter supply even than money, it seems to us that culture collections would be the best source of micro-organisms for setting up a fermentation industry.

Plenty of time and money would still not guarantee success. To obtain the proper culture, sometimes one must isolate the micro-organism from a special, ecological niche that may not even exist in a particular country. For

example, *Blakeslea trispora*, which produces large amounts of β -carotene, cannot be isolated in temperate regions of the United States, but rather one must seek wild strains growing in the tropics on flowers of certain higher plants. For such cultures, collections are almost always the only logical source.

Another source of cultures in the food industry, which should not be overlooked, is the micro-organisms selected through the centuries for preparing native fermented food products. The principal micro-organisms can be obtained with little difficulty. Since the micro-organisms have been used in a particular food fermentation for centuries, there has been a constant, purposeful selection of the best strains. The yeast strains used in the municipal Bantu beer breweries of South Africa were acquired in this fashion. One of the authors (C. W. Hesseltine) was told that the original strains were isolated

from the better native brews. After a number of strains were tested, the best were chosen and are now the ones used in an industry producing 150 million imperial gallons of the beverage yearly.

In this Handbook, we have tried to be realistic in our approach to the problem of obtaining the proper micro-organisms for use in industrial fermentations. Our views are based upon first-hand knowledge of the operation of a large industrial culture collection supported entirely by government funds; experience during several years of operating a culture collection in a large industrial fermentation company; an understanding of the problems faced by fermentologists in developing countries; contact with microbiologists working in our fermentation laboratory from developing countries; and an acquaintance with some of the primitive food fermentations of the world.

SOURCES OF MICRO-ORGANISMS FOR INDUSTRY

The ultimate sources of culture and micro-organisms for industry are soil; water; fresh, fermenting, and rotting vegetables; living plants and animals; sewage; fresh and spoiled food; frass and insect droppings; and the like.

The immediate sources of cultures, however, are permanent culture collections. Almost all large industrial firms dealing in fermentations have their own collections of micro-organisms secured from a continuous program of isolation. New isolates and variant substrains derived from concurrent mutation studies swell the numbers of strains so that many of the proprietary collections are quite large. However, most of their micro-organisms never get into general circulation, being intended solely for exploitation by the parent company.

A few cultures from proprietary industrial collections are in general and private collections in the United States. In 1949, the U.S. Patent Office took the position that a culture is an essential part of a patent process and that the

culture must be disclosed. Hence, it must be deposited in a recognized culture collection and be available to the public at the time the patent issues.

As a result of this practice, two U.S. collections—the American Type Culture Collection at Rockville, Md., and the ARS Culture Collection in Peoria, Ill.—are recognized as official depositories for cultures from industrial concerns, both domestic and foreign. As might be expected, the depositing companies do not advertise that particular strains have been placed in outside culture collections, and the named depositories agree not to reveal possession of patent cultures or to distribute them without authorization by the depositor, if this is his wish, until the U.S. patent issues.

The holdings of the companies are supplemented also by accessions from public and private culture collections whose culture distributions are not so rigidly controlled.

Private collections do not have as a principal purpose of existence the distribution of cultures. They usually are specialist collections; that is, their scope is confined to one or a few taxa of special interest to the scientists who operate or control them. Generally, private collections are associated with a university or research institute. Although their curators decline to distribute cultures far and wide to anyone who asks, they nevertheless often send cultures to other investigators with like interests, or to research institutes and to industrial men who might continue research which they no longer can pursue or who might continue development of an industrial process. Private collections generally do not charge fees for their cultures. Like proprietary collections, they usually do not publish or distribute lists of their cultures.

Public collections have as one of their principal reasons for existence the accumulation of a diverse collection of salable micro-organisms. They send cultures anywhere in the world to any bona fide investigator who is willing to pay their price. As might be expected, they publish catalogs listing the micro-organisms that are for sale. They often provide other services, such as identification of micro-organisms and preservation of cultures by lyophilization or liquid-nitrogen refrigeration. Their diversity may be as wide as that of the American Type Culture Collection, which maintains actinomycetes, algae, bacteria, cell lines, molds, protozoa, viruses, and yeasts.

Among the specialized culture collections, some concentrate on industrially useful micro-organisms. Such micro-organisms are bacteria, yeasts, molds, actinomycetes, algae, and protozoa that are used in the food, pharmaceutical, and fermentation industries and in research and development laboratories to convert selected substrates to products of enhanced nutritional, medicinal, or industrial value or to reduce the biochemical oxygen demand (BOD) in sewage and industrial effluents. Such collections are of principal interest to the United

Nations Industrial Development Organization (UNIDO) and its adherent groups and members.

We concluded that a list of such collections, giving addresses, names of curators, and types of micro-organisms contained, would be useful (Table 1). We are indebted to S. M. Martin of the Division of Biosciences, National Research Council, Ottawa 7, Ontario, Canada, for most of the names of collections and information about them. Under the aegis of the World Federation of Culture Collections (WFCC, formerly the Section on Culture Collections) of the International Association of Microbiological Societies, Dr. Martin published a World Directory of Collections of Cultures of Micro-organisms (11),¹ in which most of the collections in the world are named and described.

Names and addresses of additional collections may be found in some of the larger culture collection catalogs listed at the end of this paper (2, 3, 4).

Fees for cultures vary from one collection to another. In the United States, the American Type Culture Collection charges \$30 per strain for all cultures to profit-making institutions. The cost is reduced to \$20 for nonprofit institutions (except for some special teaching strains, which are \$10). The Centraalbureau voor Schimmelcultures in The Netherlands charges 40 guilders for cultures that are to be used for industrial purposes. There is a reduction in cost if 10 strains or more are purchased in 1 year. This collection, like some others, does not guarantee the production of chemical substances by its cultures.

As a general rule, collections which advertise their cultures in printed catalogs charge a fee for their strains. Some collections, such as the one with which we are associated, do not issue a catalog, do not charge a fee, but do exert considerable restraint on the number of strains sent at any one time to any individual or institution.

¹ Italic numbers in parentheses refer to Literature Cited, p. 38.

TABLE 1.—*Collections containing industrially useful micro-organisms*
(Includes main collections and those containing at least 500 strains)

Collection, parent organization, address, and person in charge	Contents
ARGENTINA	
Centro de Micología Facultad de Ciencias Médicas Universidad de Buenos Aires Paraguay 2155, 11°, Buenos Aires Prof. Dr. P. Negroni	Fungi Yeasts
Colección Cátedra Microbiología Agrícola (FAV, Bs. As.) Facultad Agronomía y Veterinaria Universidad de Buenos Aires Avenida San Martín 4453 (Suc. 17), Buenos Aires Prof. Ing. Agr. R. E. Halbinger	Bacteria Fungi Yeasts
Colección de Cultivos Microbianos Facultad de Farmacia y Bioquímica Universidad de Buenos Aires Junin 956 Piso. 8°, Buenos Aires Prof. Dr. R. A. Margni	Bacteria Fungi Yeasts
Instituto de Microbiología e Industrias Agropecuarias Instituto Nacional de Tecnología Agropecuaria Villa Udaondo, Castelar FCDFS, Buenos Aires Ing. Agr. E. Schiel	Bacteria Fungi Yeasts
Instituto de Patología Vegetal Instituto Nacional de Tecnología Agropecuaria Villa Udaondo, Castelar FCDFS, Buenos Aires Ing. Agr. C. J. M. Carrera	Bacteria Fungi
Cátedra de Microbiología Facultad de Ciencias Agrarias Universidad Nacional de Cuyo Almirante Brown 500, Chacras de Coria, Mendoza Ing. Agr. N. J. Palleroni	Bacteria Yeasts
AUSTRALIA	
Rhizobium Strain Collection Division of Tropical Pastures Commonwealth Scientific and Industrial Research Organization Mill Road, St. Lucia, Brisbane, Queensland, 4067 Dr. D. O. Norris	Bacteria
Culture Collection The Australian Wine Research Institute Private Bag No. 1, Glen Osmond, P.O., South Australia, 5064 W. W. Forest	Yeasts

TABLE 1.—*Collections containing industrially useful micro-organisms*—Continued

Collection, parent organization, address, and person in charge	Contents
AUSTRALIA—Continued	
Soil Microbiology Culture Collection Division of Soils Commonwealth Scientific and Industrial Research Organization Private Bag No. 1, Glen Osmond, South Australia, 5064 Dr. E. G. Hallsworth	Bacteria
Institute of Agriculture University of Western Australia Nedlands, Western Australia, 6009 Dr. C. A. Parker	Bacteria
Division of Wood Technology Forestry Commission of New South Wales 96 Harrington Street, Sydney, New South Wales, 2000 E. B. Huddleston	Fungi
AUSTRIA	
Milchwirtschaftliche Bakterienkulturen Rotholz/Post Jenbach, Tirol Dipl.-Ing. S. Winkler	Bacteria
BELGIUM	
Collection of the Laboratory for Microbiology Laboratorium voor Microbiologie, Fac. Wetenschappen Rijksuniversiteitn Ledeganckstraat, Gent Prof. J. DeLey	Bacteria
Laboratoire de Mycologie Systématique et Appliquée Université Catholique de Louvain 92, Avenue Cardinal Mercier, B-3030-Heverlee Prof. Dr. Ing. G. L. Hennebert	Fungi
BRAZIL	
Coleção de Culturas Instituto Adolfo Lutz Av. Dr. Arnaldo 355, C.P. 7027, São Paulo F. de B. M. Jordão	Bacteria Fungi Protozoa Animal Viruses
Culture Collection, Instituto Zimotécnico (IZ) Escola Superior de Agricultura "Luis de Queiroz" C.P., 56, Piracicaba, São Paulo Prof. J. Leme, Jr.	Bacteria Fungi Yeasts

TABLE 1.—*Collections containing industrially useful micro-organisms*—Continued

Collection, parent organization, address, and person in charge	Contents
BULGARIA	
Bulgarian Type Culture Collection Institute for State Control of Medical Preparations Ministry of Health Vladimir Zaimov No. 26, Sofia Prof. R. Ovtcharov/Dr. M. Zheleva	Bacteria Fungi Yeasts
BURMA	
Type Culture Collection Burma Pharmaceutical Industry (B.P.I.) Industrial Development Corp., Ministry of Industry BPI Road, Gyogon, Rangoon Dr. Ko Gyi Ko	Bacteria Fungi
CANADA	
Mold Herbarium and Culture Collection (UAMH) University of Alberta Edmonton, Alberta Dr. J. W. Carmichael	Fungi Yeasts
Atlantic Regional Laboratory National Research Council of Canada 1411 Oxford Street, Halifax, Nova Scotia Dr. A. C. Neish	Bacteria Fungi Yeasts
University of Western Ontario Culture Collection (UWO) Botany Department University of Western Ontario London, Ontario Dr. J. C. Hickman	Bacteria Fungi Yeasts Algae
Biosciences Division National Research Council of Canada Sussex Drive Ottawa 2, Ontario Dr. G. C. Butler	Bacteria Fungi Yeasts
University of Windsor Culture Collection Department of Biology University of Windsor Windsor, Ontario R. J. Doyle	Bacteria Fungi Yeasts Animal Viruses
Macdonald College Collection Department of Microbiology, Macdonald College McGill University Ste. Anne de Bellevue, Quebec Dr. R. Knowles	Bacteria Fungi Yeasts

TABLE 1.—*Collections containing industrially useful micro-organisms*—Continued

Collection, parent organization, address, and person in charge	Contents
CANADA—Continued	
Prairie Regional Laboratory National Research Council of Canada Saskatoon, Saskatchewan Dr. R. H. Haskins	Bacteria Fungi
CEYLON	
Department of Biological Sciences (Microbiology) Vidyodaya University Gangodawila	Bacteria
CHILE	
Collection Bactéries Institut Centre National de Microbiologie Bactériologique Avenida Maratón 1000, Santiago Prof. E. Dussert	Bacteria
CZECHOSLOVAKIA	
(All but one (*) of these collections are part of the Czechoslovak Collections of Micro-organisms)	
Research Institute for Viticulture and Enology (RIVE) Matušková ul. 97, Bratislava Dr. Ing. A. Vereš, C.Sc.	Fungi Yeasts
Collection of Cultures of Wood-Rotting Fungi Laboratory for Anatomy and Physiology of Plants (LAPP) J. E. Purkyně University Kotlářská 2, Brno Prof. Dr. V. Rypáček, Dr. Sc.	Fungi
Czechoslovak Collection of Microorganisms (CCM) J. E. Purkyně University tr. Obránců Míru 10, Brno Prof. Dr. T. Martinec, Dr. Sc.	Bacteria
Collection of Rhizobium and Other Soil Microorganisms Central Research Institute of Plant Production (CRIPP) Ruzyně, Prague Dr. E. Hamatová-Hlaváčková, C.Sc.	Bacteria
Culture Collection of Entomogenous Bacteria (CCEB) Department of Insect Pathology Institute of Entomology, Czechoslovak Academy of Sciences Na cvičišti 2, Prague 6 Dr. O. Lysenko, C.Sc.	Bacteria

TABLE 1.—*Collections containing industrially useful micro-organisms*—Continued

Collection, parent organization, address, and person in charge	Contents
CZECHOSLOVAKIA—Continued	
Culture Collection of Fungi (CCF) Department of Botany, Faculty of Sciences Charles University Benátská 2, Prague 2 Dr. O. Fassatiova, C.Sc.	Fungi
* Czechoslovak National Collection of Type Cultures Institute of Epidemiology and Microbiology Šrobarova 48, Prague 10 Dr. J. Šourek, C.Sc.	Bacteria Fungi Yeasts Animal Viruses Bacteriophages
Research Laboratories of the Dairies Association for the Production of Pure Milk Cultures Laktoflora (LAKTOFLORA) Ke dvoru 2, Prague 6 Ing. Dr. M. Teplý	Bacteria Fungi Yeasts
DENMARK	
Bacillus Collection Institute of Hygiene University of Aarhus DK-8000, Aarhus Prof. G. J. Bonde	Bacteria
Bacteriological Department Lövens kemiske Fabrik 2750 Ballerup L. Tybring	Unspecified
Antibiotics Department Dumex, Ltd. Prags Boulevard 37, 2300 Copenhagen S Dr. K. Andersen	Unspecified
Bacteriological Department H. Lundbeck and Co. Ottiliaveg 7-8, 2500 Copenhagen Valby L. Szabo	Unspecified
Statens Forsøgsmejeri 3400 Hillerød A. M. Madsen	Unspecified
Department of Technical Biochemistry Danmarks tekniske Højskole Bygning 223, 2800 Lyngby Dr. M. Jensen	Unspecified

TABLE 1.—*Collections containing industrially useful micro-organisms*—Continued

Collection, parent organization, address, and person in charge	Contents
DENMARK—Continued	
Laboratory for Microbiology Danmarks tekniske Højskole Bygning 221, 2800 Lyngby Prof. Dr. J. Hedegaard	Unspecified
FINLAND	
Culture Collection Department of Microbiology University of Helsinki Helsinki 71 Prof. U. J. Vartiovaara	Bacteria Fungi Yeasts
FRANCE	
Centre de Collection de Types Microbiens Centre d'Études et de Recherches Technologiques des Industries Alimentaires 62 Boulevard Maréchal Vaillant, Lille Dr. H. Beerens	Bacteria Yeasts Animal Viruses Bacteriophages
Institut Pasteur de Lyon: IPL Rue Pasteur, Lyon 69 M. Carraz	Bacteria
Collection de Microorganismes Associes Aux Invertebres Station de Recherches Cytopathologiques I.N.R.A.-C.N.R.S. Montpellier 30 Saint-Christol Prof. C. Vago	Bacteria Yeasts Animal Viruses
Laboratoire de Cryptogamie Museum National d'Histoire Naturelle Paris Dr. J. Nicot	Fungi
Laboratoire des Fermentations Institut Pasteur de Paris 28 Rue du Docteur Roux, Paris 15 ^e P. Bréchet	Yeasts
Service des Anaérobies Institut Pasteur de Paris 25 Rue du Docteur Roux, Paris 15 ^e Dr. Rouyer	Bacteria

TABLE 1.—*Collections containing industrially useful micro-organisms*—Continued

Collection, parent organization, address, and person in charge	Contents
GERMANY, EAST (DEMOCRATIC REPUBLIC)	
Fachgebiet Allgemeine Botanik und Pflanzenphysiologie Ernst-Moritz-Arndt-Universität Grimmerstrasse 86/88, DDR-22 Greifswald Prof. Dr. H. Borriß	Bacteria Fungi Yeasts Algae Bacteriophages Cell lines
Kultursammlungen Zentral Institut für Mikrobiologie und Experimentelle Therapie (IMET) Deutsche Akademie der Wissenschaften zu Berlin Beuthenbergstrasse 11, Jena 69 H. Prauser	Bacteria Fungi Yeasts Animal Viruses Bacteriophages Protozoa Actinomycetes
Kultursammlung Institut für Mikrobiologie Humboldt-Universität 1532 Kleinmachnow, Max-Reimannstrasse 16, Kleinmachnow bei Berlin Prof. Dr. Jentzsch	Bacteria Fungi Yeasts
Botanisches Institut Mykologie Weimar Friedrich-Schiller Universität Jena Frh.-v-Stein-Allee 2, Weimar Prof. Dr. R. Tröger	Fungi Yeasts
GERMANY, WEST (FEDERAL REPUBLIC)	
Mikroorganismensammlung Institut für Garungsgewerbe und Stärkefabrikation Seestrasse 13, 1 Berlin 65 Prof. Dr. S. Windisch	Bacteria Fungi Yeasts
Instituts Staamsammlung, Biologische Bundesanstalt für Land und Fortwirtschaft Institut für biologische Schädlingsbekämpfung Kranichsteinerstrasse 61, Darmstadt Prof. Dr. J. M. Franz	Bacteria Fungi Animal Viruses
Sammlung von Algenkulturen Pflanzenphysiol. Institut Universität Göttingen Nikolausberger Weg 18, 34 Göttingen Prof. Dr. A. Pirson	Algae
Food Spoiling Molds Deutsche Forschungsanstalt für Lebensmittelchemie Leopoldstrasse 175, Munich, Bayern Prof. Dr. S. W. Souci	Fungi

TABLE 1.—*Collections containing industrially useful micro-organisms*—Continued

Collection, parent organization, address, and person in charge	Contents
GERMANY, WEST (FEDERAL REPUBLIC)—Continued	
Bayerische Landensanstalt für Wein-, Obst-u. Gartenbau Residenzpl. 3, 87 Würzburg, Bayern Dr. I. Benda	Yeasts
GHANA	
University Microbial Cultures, Kumasi (U.M.C.K.) Department of Biological Sciences University of Science and Technology Kumasi K. O. Nyako	Bacteria Fungi Yeasts
HUNGARY	
Culture Collection Pedological Inst. Hungarian Academy of Sciences Herman 0.15, Budapest II Dr. J. Szegi	Bacteria Actinomycetes
Diagnostical and Research Laboratory National Institute for Tuberculosis "Korányi" Pihenő ut 1, Budapest XII I. Szabó, M.D., D.Sc.	Bacteria Bacteriophages
INDIA	
BSM Culture Collection Botany Department University of Allahabad Allahabad Dr. B. S. Mehrotra	Fungi
Fermentation Technology Laboratory Indian Institute of Science Bangalore-3 Dr. J. V. Bhat	Bacteria Yeasts
Department of Microbiology Bose Institute 93/1 Acharya Prafulla, Chandra Road, Calcutta 9 Prof. P. Nandi	Bacteria Fungi Yeasts Actinomycetes Bacteriophages
D. R. L. (M) Kanpur Culture Collection Defense Research Laboratory (Materials) Research and Development Organization, Ministry of Defense P.B. 320 Kanpur, Uttar Pradesh Dr. J. N. Nanda	Bacteria Fungi Yeasts

TABLE 1.—*Collections containing industrially useful micro-organisms*—Continued

Collection, parent organization, address, and person in charge	Contents
INDIA—Continued	
National Collection of Industrial Microorganisms (NCIM) National Chemical Laboratory Council of Scientific and Industrial Research (CSIR) Poona-8 Dr. V. Jagannathan	Bacteria Fungi Yeasts
INDONESIA	
Culture Collection, Treub Laboratory National Biological Institute The Botanical Garden Bogor Dr. S. Saono	Bacteria Fungi Yeasts Actinomycetes
IRAN	
Razi Culture Collection State Razi Institute-Hessarak, Ministry of Agriculture P.O. Box 656, Karadj, Tehran Dr. F. Entessar	Bacteria Animal Viruses Protozoa
IRELAND	
Department of Industrial Microbiology University College Ardmore, Stillorgan Rd., Dublin, 4 Prof. M. J. Geoghegan	Fungi Actinomycetes
Guinness (Dublin) Culture Collection A. Guinness Son and Co. (Dublin) Ltd. St. James's Gate, Dublin, 8 Dr. C. E. Dalglish	Bacteria Yeasts
Johnstown Castle Collection Soil Laboratory The Agricultural Institute Wexford C. L. Masterson	Bacteria
ITALY	
Centro di Studio dei Microorganismi Autotrofi Istituto di Microbiologia Agraria e Tecnica Università di Firenze Piazzale della Cascine, 27 Firenze Prof. G. Florenzano	Bacteria Fungi Yeasts Algae

TABLE 1.—*Collections containing industrially useful micro-organisms*—Continued

Collection, parent organization, address, and person in charge	Contents
ITALY—Continued	
Istituto di Patologia Vegetale Università di Milano Via Celoria 2, Milano 20133 Dr. E. Baldacci	Actinomycetes Fungi
Lepetit S.p.A. Via Durando 38, Milano 20158 Prof. P. Sensi	Bacteria Fungi Yeasts Actinomycetes Protozoa
Collezione dei Lieviti Vinari Istituto di Microbiologia Agraria e Tecnica Università di Perugia Bg. XX Guigno, Perugia 06100 Prof. T. Castelli	Bacteria Yeasts
Collezione Microbica Agraria, Marina e Industriale (COMAMI) Istituto Microbiologia Agraria e Tecnica E. de Nicola, Sassari Prof. A. Capriotti	Bacteria Yeasts
JAMAICA	
Department of Microbiology University of West Indies Mona St., Kingston 7 Prof. L. S. Grant	Bacteria Animal Viruses
JAPAN	
Department of Fermentation Technology (HUT) Faculty of Engineering Hiroshima University 3-Chôme, Senda-machi Hiroshima Dr. T. Nehira	Bacteria Fungi Yeasts
Culture Collection (IFO) Institute for Fermentation Juso Nishinocho 4-54, Higashi-yodogawa-ku, Osaka Dr. T. Hasegawa	Bacteria Fungi Yeasts Bacteriophages
Department of Fermentation Technology (OUT) Faculty of Engineering Osaka University 9-Chôme Higashinoda-machi, Miyakoshima-Ru, Osaka Dr. G. Terui	Bacteria Fungi Yeasts

TABLE 1.—*Collections containing industrially useful micro-organisms*—Continued

Collection, parent organization, address, and person in charge	Contents
JAPAN—Continued	
ATU Culture Collection Department of Agricultural Chemistry Faculty of Agriculture University of Tokyo 1-Chôme, Yayoi, Bunkyô-ku, Tokyo Dr. K. Arima	Bacteria Fungi Yeasts Actinomycetes
Institute of Applied Microbiology (IAM) University of Tokyo 1-Chôme, Yayoi, Bunkyô-ku, Tokyo Prof. Dr. H. Iizuka	Bacteria Fungi Yeasts Algae Actinomycetes
Nagao Institute (NI) 96, Kinuta-machi, Setagaya-ku, Tokyo K. Nagao	Fungi Yeasts Algae
The Research Institute of Fermentation Yamanashi University 1 Kitashin-machi, Kofu, Yamanashi Prof. Dr. I. Yokotsuka	Bacteria Fungi Yeasts
KOREA	
Korean Federation of Culture Collections of Microorganisms Ministry of Science and Technology 2nd Chongro, Seoul Prof. Dr. Lee Zoo Shik	Bacteria Fungi Yeasts Algae Protozoa Plant Viruses Animal Viruses Bacteriophages
MALAYSIA	
Pathology Division Rubber Research Institute of Malaya P.O. Box 150, Kuala Lumpur, Selangor Mr. B. S. Rao	Bacteria Fungi Yeasts
NETHERLANDS	
Centra lbureau voor Schimmelcultures (CBS) Oosterstaat 1 Baarn Dr. J. A. Von Arx	Fungi Yeasts Actinomycetes

TABLE 1.—*Collections containing industrially useful micro-organisms*—Continued

Collection, parent organization, address, and person in charge	Contents
NETHERLANDS—Continued	
Culture Collection Laboratory of Microbiology Technical University Julianalaan 67A, Delft Prof. Dr. T. O. Wiken	Bacteria
NEW ZEALAND	
Culture Collection Plant Diseases Division Department of Scientific and Industrial Research Private Bag, Auckland Mr. J. D. Atkinson	Bacteria Fungi Yeasts Plant Viruses
NIGERIA	
Fungus Culture Collection University of Lagos University Road, Yaba, Lagos Prof. S. H. Z. Naqvi	Fungi Yeasts
NORWAY	
Norges Tekniske Hagskoles Collection (NTHC) Department of Biochemistry Technical University of Norway Trondheim MTH Dr. K. Eimhjellen	Bacteria Fungi Yeasts
Department of Microbiology Agricultural College of Norway Box 40, Vollbekk Dr. G. G. Lindeberg	Fungi
PHILIPPINES	
<i>Rhizobium japonicum</i> and <i>Rhizobium</i> spp. from other tropical legumes College of Agriculture University of the Philippines College, Laguna M. E. Raymundo	Bacteria
POLAND	
Special Centre of Industrial Cultures Department of Industrial Microbiology Technical University of Łódź Wólczajska 171/173, Łódź Prof. dr. J. Jakubowska	Bacteria Fungi Yeasts

TABLE 1.—*Collections containing industrially useful micro-organisms—Continued*

Collection, parent organization, address, and person in charge	Contents
POLAND—Continued	
Culture Collection of Industrial Microorganisms Institute of the Fermentation Industry Rakowiecka 36, Warsaw Dr. J.N.Z.T. Golébiewski	Bacteria Fungi Yeasts
Central Centre of Microorganisms Collections Microbiologic Committee Polish Academy of Sciences Commission of Taxonomy and Storage of Microorganisms ul. Chalubinskiego 4, Wroclaw Prof. dr. S. Slopek	Bacteria
PORTUGAL	
Collecao de Culturas de Fungos Micologia, Faculdade de Ciências Lisbon Prof. J. Pinto-Lopes	Fungi
Institute Gulbenkian Ciência Laboratório de Microbiologia Centro de Biologia Rua da Quinta Grande, Oeiras Dr. N. van Uden	Yeasts
RHODESIA	
Grasslands Rhizobium Collection Grasslands Research Station P.B. 701, Marandellas T. C. D. Kennan	Bacteria
ROMANIA	
Microbiological Laboratory Biosynthesis Department Institutu de Cercetări Chicico-Farmaceutice 112, Soseaua Vitan, Bucharest Mr. T. Slave	Bacteria Fungi
SOUTH AFRICA	
Microbiology Research Group South African Council for Scientific and Industrial Research Pretoria Dr. J. P. van der Walt	Bacteria Fungi Yeasts

TABLE 1.—*Collections containing industrially useful micro-organisms*—Continued

Collection, parent organization, address, and person in charge	Contents
SWITZERLAND	
Culture Collection of Microorganisms J. R. Geigy S. A. Schwarzwaldallee, CH 4021 Basel Dr. W. A. Vischer	Bacteria Fungi Yeasts Protozoa
Botanische Sammlungen der Eidg. Technische Hochschule Institut für spezielle Botanik Universitätstrasse 2, 8006 Zürich Dr. E. Müller	Fungi
Mikrobiologisches Institut der Eidg. Technische Hochschule Swiss Federal Institute of Technology Universitätstrasse 2, CH 8032 Zürich Prof. Dr. L. Ettliger	Bacteria Fungi Yeasts Actinomycetes
UNITED ARAB REPUBLIC	
Agricultural Microbiology Division (A.M.D.) Ministry of Agriculture Soils Department Building University Street, Orman, Giza Mr. Abou El-Fadl	Bacteria Fungi Yeasts
Microbic Collection College of Agriculture Ain Shams University Shoubra El-Khaima, Kalubia Dr. S. M. Taha	Bacteria Fungi Yeasts Algae
UNITED KINGDOM	
The Wellcome Bacterial Collection Wellcome Foundation Ltd. Langley Court, Beckenham, Kent, England Dr. J. Cameron	Bacteria
Culture Collection of Algae and Protozoa 36 Storey's Way Cambridge University Cambridge CB3 0DT England Dr. E. A. George	Algae Protozoa
Glaxo Research Ltd. Greenford, Middlesex, England Dr. P. W. Muggleton	Bacteria Fungi Yeasts Protozoa

TABLE 1.—*Collections containing industrially useful micro-organisms*—Continued

Collection, parent organization, address, and person in charge	Contents
UNITED KINGDOM—Continued	
The Commonwealth Mycological Institute (CMI) The Ministry of Technology Ferry Lane, Kew, Surrey, England Mr. A. Johnston	Fungi
Akers Culture Collection Pharmaceuticals Division Imperial Chemical Industries Ltd. P.O. Box 25, Alderley Park, Macclesfield, Cheshire, England Dr. D. Broadbent	Bacteria Fungi Yeasts
British National Collection of Yeast Cultures Brewing Industry Research Foundation Nutfield, Surrey, England Dr. A. H. Cook	Yeasts
National Collection of Dairy Organisms National Institute for Research in Dairying Shinfield, Reading, Berkshire, England Dr. L. A. Mabbitt	Bacteria Bacteriophages
National Collection of Industrial Bacteria Ministry of Technology Torry Research Station P.O. Box 31, 135 Abbey Road, Aberdeen AB9 8DG, Scotland Dr. J. M. Shewan	Bacteria Bacteriophages
National Collection of Marine Bacteria Ministry of Technology Torry Research Station P.O. Box 31, 135 Abbey Road, Aberdeen AB9 BDG, Scotland Dr. M. Shewan	Bacteria Yeasts
UNITED STATES OF AMERICA	
Chas. Pfizer & Co., Inc. Groton, Connecticut 06340 Dr. J. Routien	Bacteria Fungi Actinomycetes
IMC Culture Collection Growth Sciences Center International Minerals & Chemical Corporation P.O. Box 192, Libertyville, Illinois 60048 Dr. M. H. Rogoff	Bacteria Fungi Bacteriophages

TABLE 1.—*Collections containing industrially useful micro-organisms*—Continued

Collection, parent organization, address, and person in charge	Contents
UNITED STATES OF AMERICA—Continued	
ARS Culture Collection (NRRL) Northern Regional Research Laboratory Agricultural Research Service U.S. Department of Agriculture 1815 N. University St., Peoria, Illinois 61604 Dr. T. G. Pridham	Bacteria Fungi Yeasts Actinomycetes
The Culture Collection of Algae Department of Botany Indiana University Bloomington, Indiana 47401 Dr. R. C. Starr	Algae
Microbiology Research Eli Lilly and Company 307 East McCarty St., Indianapolis, Indiana 46206 Dr. D. H. Lively	Bacteria Fungi Actinomycetes
Grain Processing Corporation 1600 Oregon St., Muscatine, Iowa 52761 Mr. C. Smith	Bacteria Fungi Yeasts Algae
American Type Culture Collection (ATCC) 12301 Parklawn Drive, Rockville, Maryland 20852 Dr. R. Donovanick	Bacteria Fungi Yeasts Actinomycetes Algae Protozoa Animal Viruses Bacteriophages Cell lines
Culture Collection of the U.S. Army Material Command U.S. Army Natick Laboratories Natick, Massachusetts 01762 Dr. E. G. Simmons	Bacteria Fungi Yeasts
The Upjohn Stock Culture Collection The Upjohn Company 301 Henrietta Street, Kalamazoo, Michigan 49001 Dr. G. B. Whitfield, Jr.	Bacteria Fungi Yeasts Algae Actinomycetes Protozoa
Culture Collection Squibb Institute for Medical Research E. R. Squibb & Sons, Inc. Georges Road, New Brunswick, New Jersey 08901 Mrs. F. Arnow	Bacteria Fungi Yeasts Actinomycetes

TABLE 1.—*Collections containing industrially useful micro-organisms*—Continued

Collection, parent organization, address, and person in charge	Contents
UNITED STATES OF AMERICA—Continued	
IMRU Collection Institute of Microbiology Rutgers—The State University New Brunswick, New Jersey 08903 Dr. R. E. Gordon	Bacteria Actinomycetes
Merck Sharp & Dohme Research Laboratories Culture Collection Merck & Co., Inc. Rahway, New Jersey 07065 Dr. T. H. Stoudt	Bacteria Fungi Yeasts Algae Actinomycetes
Lederle Microbiology Research Collection Lederle Laboratories Division American Cyanamid Company No. Middletown Road, Pearl River, New York 10965 Dr. E. J. Backus	Bacteria Fungi Yeasts Algae Actinomycetes
Bristol Laboratories P.O. Box 657, Syracuse, New York 13201 Dr. C. A. Claridge	Bacteria Fungi
Actinoplanaceae (Actinomycetales) Botanical Department University of North Carolina Coker Hall, Chapel Hill, North Carolina 27514 Dr. V. A. Greulach	Actinomycetes Bacteriophages
Wyeth Laboratories Collection Wyeth Laboratories P.O. Box 8299, Philadelphia, Pennsylvania 19101 Dr. G. H. Warren	Bacteria Fungi Yeasts
Department of Plant Pathology and Botany Agricultural Experiment Station P.O. Box H, Río Piedras, Puerto Rico 00928 Dr. J. E. Pérez	Bacteria
Department of Bacteriology College of Agricultural and Life Sciences University of Wisconsin Madison, Wisconsin 53706 Dr. K. B. Raper	Fungi

TABLE 1.—*Collections containing industrially useful micro-organisms*—Continued

Collection, parent organization, address, and person in charge	Contents
UNION OF SOVIET SOCIALIST REPUBLICS	
All-Union Collection of Type Cultures Institute of Microbiology U.S.S.R. Academy of Sciences Profsovnaya Str., 7a, Moscow, B-133 Prof. V. Kudriavtsev	Bacteria Fungi Yeasts Actinomycetes
Institute for Research of New Antibiotics U.S.S.R. Academy of Medical Sciences Bolshaia Pirogovskaia 11, Moscow Dr. G. F. Gauze	Actinomycetes
VENEZUELA	
Centro de Microscopía Electronica Edficio Central Universidad de los Andes Apartado 163, Calle Vargas, Merida Dr. J. A. Serrano	Bacteria Fungi

CHARACTERISTICS OF A GOOD CULTURE COLLECTION

Although much has been spoken and written about culture collections, to our knowledge no one has ever laid down the characteristics of a good applied or industrial culture collection. Many of the following points apply equally well to other types of collections:

1. The collection must be part of, or closely related to, a fermentation research laboratory or to a fermentation plant, or both. At the Northern Regional Research Laboratory, the ARS Culture Collection is the responsibility of one of the four research units in the Fermentation Laboratory.

Interactions between fermentologists and culture-collection staff work to the mutual benefit of both. The fermentologists, being aware of general trends in fermentation research, are able to anticipate future areas of interest and to provide guidance as to what micro-organisms the culture collection should accession to meet future needs. The microbiologists, with their knowledge of the relationships among genera and the physiological requirements of various micro-organisms, can

make valuable suggestions regarding screening programs. In their detailed studies on individual strains, they may make observations leading to new fermentation products or higher yields of known products. Their ready recognition of contamination or degeneration of the culture being used in the development of a process acts as a type of quality control.

2. A culture collection must be well funded, and this funding must be at a relatively uniform level each year. In many other research operations, a program may be increased or decreased readily with changes in the amount of budgeted money. Personnel can be shifted easily from one project to another. On the other hand, a culture collection is a continuing operation which must be sustained without great fluctuations in budget or people from year to year. Many of the culture-collection projects become long-term studies of a genus or family, and years are required to assemble wild cultures and known type materials in order to do a first-rate job.

3. A culture collection must have adequate

facilities and equipment, including transfer rooms, refrigerator space, incubators, microscopic and photographic equipment, autoclaves, and lyophilizers. Usually these facilities should be separate from those of other research groups.

4. Library facilities are necessary so that personnel may have access to the taxonomic and fermentative literature being published, not only in the region or country of location, but also in the world.

5. The collection should have an active and continuous program of isolating new strains of micro-organisms from nature. This goal will lead to the discovery of new products and reactions. New material will also add to the understanding of the classification of special groups of micro-organisms. New material makes it possible to discover species and genera new to science.

6. The collection must have an adequate staff to support the curators. By this requirement, we mean technical help to prepare media, sterilize glassware, and perform routine techniques; secretarial help to keep the voluminous records and to handle correspondence; and shops to construct special apparatus. At the Northern Laboratory, our glassblower devised an automatic machine to make lyophil tubes. Reliable sources of supplies are also necessary. We have had experience in setting up a lyophil apparatus in a developing nation. Although the lyophil equipment was readily made to our specifications, not a culture could be processed for 2 years because there was no dry ice.

Optimally, each curator should have a careful, intelligent, and dedicated assistant with some microbiological training. In our experience, technicians need not be specialized because they always must be trained in the special techniques required for the collection. These assistants should handle periodic transfers, lyophilization and associated records, inoculation of cultures for study by the curator, seeding of flask cultures for preliminary surveys for new products, and making and recording routine observations on all cultures.

7. The curator(s) must do research, as well as maintain the collection. Each must have an active research program either in taxonomy or

genetics, with preference to the former. Thus a curator will have an intimate knowledge of the strains he is maintaining and will develop a reputation as an expert in his field. Consequently, important material will be sent to him for safekeeping, for identification, and for other purposes. Other microbiologists will know from whom they may get expert advice, cultures, and information. This point is an important one that we have stressed before (7, 9).

8. College-trained members of a culture-collection staff must be aware of the field of applied microbiology, appreciate the work being done in fermentation research and development, and understand the operation of fermentation plants. They must comprehend the problems of geneticists, fermentologists, engineers, biochemists, and organic chemists. Probably the most difficult job from an administrator's standpoint is indoctrinating the curators of a culture collection. They must understand fully the point of view of other scientists and must realize that they are part of a team. They must be made aware of the needs of other research people. Anyone in a culture collection who does not appreciate other areas of work should never be in the position of decisionmaking.

In turn, the members of a culture collection should be informed of developments in associated fermentation research areas and of problems in a fermentation plant. Currently, all reports and papers from our Fermentation Laboratory are circulated to all the other senior scientists. Before papers and reports are given at scientific meetings, the authors present them before other members of the Fermentation Laboratory for review and criticism. By this means errors are detected, speaking time is adjusted, and lastly, the staff is kept informed on progress in related areas.

9. Although the training of curators should be in taxonomy, the overall background of the staff should have balance. If the collection has more than one senior man, then the broader the interests of the group, the better. It does no good to have three specialists on bacteria and yet have no mycologists, or vice versa.

In the ARS Culture Collection, we currently

have a zymologist, two bacteriologists, two mycologists, a plant pathologist, and a biochemist. Although a geneticist is not part of the Collection, a microbial geneticist works closely with the Collection members.

10. At least in larger collections, young people with new ideas and knowledge of new techniques should be brought into the group periodically. This means of rejuvenation may be supplemented with postdoctoral fellows and exchange of personnel from other institutions. They should not necessarily be people from other collections. In turn, the resident staff needs periodically to travel or study in other laboratories.

11. Members of a culture collection should be like playmakers in a basketball game. They should spot profitable new ideas of fermentation research. They should be the originators of new processes and products. On the other hand, once a profitable research area has been discovered, they should become advisors to other groups who are responsible for developmental research. They should not do developmental research beyond this point.

12. Culture-collection people should not only be engaged in research, but they should be actively reporting their research in the form of papers published in scientific journals, giving lectures, and occasionally taking out initial patents.

LOCATION OF CULTURE COLLECTIONS

The questions of how many kinds of culture collections (agricultural, general, medical, or reference) should be sponsored and where they should be located are under study by the WFCC. Our concern, which overlaps theirs, is the narrower one of where collections of industrial micro-organisms should be situated so that they will be accessible to, and do the most good for, people in developing nations. Should each nation have its own collection? Will the existing ones suffice? We think the answer to both questions is "No."

It seems unrealistic to support the idea of national collections of any sort because scientists trained in culture-collection science in developing countries are scarce. It seems to us that the source of cultures of micro-organisms should be limited to a few well-equipped collections located at various places around the world. They need to be adequately staffed and financially supported on a long-term basis. A culture collection in each country would be wholly unrealistic and unworkable. Money spread over so many places would be utterly wasted. On the other hand, public collections that distribute industrial micro-organisms are rare and often are located too far from the emerging nations to furnish the expert assistance that is needed in handling the cultures. Also, the cost of the cultures is prohibitive be-

cause hard currency is difficult to obtain in many developing areas. These are our conclusions based upon practical experience and on frank discussions with a number of knowledgeable people from various countries.

We think that it would be good if regional collections could be set up in strategic places where governmental stability would allow proper development of a collection and where political considerations would not restrict the free flow of cultures and information to fermentologists in the service areas. Certainly, the existing ones must suffice for now, and in certain regions they can provide satisfactory service. Thus culture collections in existence in the United States, U.S.S.R., United Kingdom, Netherlands, Japan, Canada, and South Africa serve many areas. The excellent Dutch collection would, and does, fill the needs of Central Europe. The U.S.S.R. All-Union Collection can adequately meet the requirements of the U.S.S.R., Poland, Romania, and Bulgaria. Well-staffed and financed collections need to be established in South America (perhaps in Brazil or Argentina), India, Central Africa, and the Middle East and North Africa. We do not mean to imply that none exist in these regions, but rather we suggest that those that do should be enlarged in size, better equipped, and more adequately staffed. We believe that if these pro-

posed collections were established, the fermentation industry in these areas would be adequately backstopped with sources of cultures, culture information, and technical expertise.

Existing collections, which have gained stature over the years, are associated with either a research institute or with a university that is famous for its fermentation studies. For example, the University of Tokyo collection is housed in the Departments of Agricultural Chemistry and Applied Microbiology. The famous Dutch collection of yeasts is housed with the Institute of Microbiology at Delft. Governments or organizations establishing new collections should bear this kind of location in mind. A culture collection of fermentation micro-organisms not placed in close proximity with an active institution of fermentation and applied microbiological research would be like planting a seed on a rock.

The question can then be justly asked, "What should one do with a small plant producing a

given product, say a fermented food destined for human consumption?" In this instance, the developmental work should be done in some central research laboratory. To ensure reliability, inoculum should be prepared and supplied in a dry, stable form which the workers in the plant can use to seed the fermentation to the degree that the process will reach completion despite contaminants. For example, in the Bantu beer process, even the larger plants do not keep cultures or prepare inoculum. Instead, it is supplied to them in 1-pound packages which a technician uses to inoculate a given quantity of media in full confidence that he can depend on obtaining a certain type of product at a certain time. Little or no formal microbiology needs to be known by the plant operator. The original starter culture can be kept in a central culture collection and supplied to a company that makes, packages, and distributes the inoculum.

PROCEDURES FOR ISOLATION AND SELECTION OF MICRO-ORGANISMS FROM NATURE

Innumerable techniques for isolation of micro-organisms are described in the literature. No attempt will be made here to give specific details because they vary from group to group, and sometimes even a special technique is required for a single species. Information can usually be found in textbooks on microbiology or taxonomic monographs. Currently, the Mycological Society of America has a project involving the preparation of a manual on methods for the isolation and study of all the groups of fungi. An expert on each fungus family or genus has prepared a section on how to isolate and to study his group. The material was written more than 2 years ago and now is being evaluated by graduate students to discover which parts are workable and which must be revised. The entire text will be edited for uniform style. Eventually, the whole will be published as a source book of information.

Techniques for isolating micro-organisms, which can grow free of other living things, can be classified into several general categories.

Micro-organisms are here interpreted to include bacteria, fungi, algae, and protozoa. One of the oldest techniques is culture enrichment. It involves the transfer of soil, sewage, or some other material with a large and diverse population of micro-organisms into a selective medium, followed by incubation of the culture under such conditions of temperature, aeration, and pH, among others, that the growth of desired forms is favored. A small amount from the initial culture is transferred to a second culture that is established in the same manner as the first, and this procedure is continued until the desired flora predominates and can be isolated in pure culture.

If a strain of *Clostridium* that would produce acetone on cornmeal is wanted, a sterile corn mash would be inoculated with soil, sewage, and other material containing large bacterial populations. The corn mash would be kept under anaerobic conditions at a desirable fermentation temperature, for example, 37° C. A large number of flasks would be started. Certain ones

that gave the appearance of vigorous anaerobic growth and that had a solvent odor would be used to inoculate new flasks. These, in turn, would be incubated until a culture of exceptional solvent-producing ability emerged. Finally, the selected *Clostridium* would be plated out on a cornmeal medium under anaerobic conditions, and many strains would be isolated as pure cultures. Each would then be tested in the proposed industrial fermentation for which cornmeal is the basic ingredient. Assays of the solvent yields would be determined, and the best strain selected for evaluation in the scale-up of the fermentation.

Sometimes, the isolation of pure cultures is not even necessary. In the fermentation of cucumbers for the manufacture of pickles, conditions are established in the fermentation tanks that favor part of the natural microbial flora on the cucumbers. Certain bacteria grow to the practical exclusion of all other microorganisms, and the fermentation goes to completion without resort to a pure culture. The efficacy of pure culture starters in picklemaking is being studied.

In the native African fermentation of corn, called magou, conditions of anaerobiosis and temperature are so adjusted that a high-temperature, lactic acid fermentation occurs without the use of pure culture starter. Part of a previous batch is used as starter for each new batch.

Another general approach is to isolate a microbial strain from the natural flora of a choice sample of material and to use it to make a uniformly good product. For example, during the past 12 years in the United States a culture of *Pediococcus* has been used to inoculate commercial sausage. According to information supplied by the manufacturer, a search was made for samples of superior quality sausage. From these sausages, a strain was selected that produced the desired fermentation; that also could be grown under conventional fermentation conditions and then be preserved by freeze-drying without undue loss of vitality; and that could initiate growth rapidly in fresh sausage despite competing bacteria.

However, these various approaches cannot always be used because we do not know where

to look for suitable strains or because the actual nature of the product desired is not known. The search for antibiotics is an example. In this instance, initial searches indicated that *Streptomyces* had excellent possibilities. From earlier studies on soil microbiology, it was known that soil, particularly grassland soil, had a great number and variety of species. The method used in this research was to get many different samples of soil from various geographical and ecological areas. The soils were plated out on media suitable for the growth of *Streptomyces* and other bacteria, and myriads of strains of actinomycetes were isolated. Selection of colonies for making pure cultures was often influenced by observation of their inhibition of adjacent bacterial or mold colonies. A multitude of strains so selected were then tested on plates against the target pathogen or, more often, against a harmless micro-organism known to be closely related to the pathogen.

Ultimately, the antibiotic from a particular culture had to be tested against strains of the pathogen either in vitro or in vivo. As an aside, a search of this sort for new antibiotics now appears to be a useless activity except by possibly the most highly skilled and experienced researchers in industrial laboratories. A better approach is the modification of known antibiotic compounds.

The plating technique can be used to isolate single cells or spores of practically all microorganisms that grow in laboratory media. This technique involves the dilution and separation of propagules of the micro-organism. These then grow into colonies of sufficient size to be seen with the naked eye or under a dissecting microscope on, or in, the agar medium. Using aseptic techniques, one can pick some or all of a colony off the substrate and establish a pure culture. However, with many fungi, a more rapid and efficient technique is the isolation of a few spores from one fructification.

Routinely in our laboratory, mold growth that has fruiting heads on the substrate (this growth may be on medium in petri dishes) is used to start pure cultures. This technique can readily be done by placing the material on which the mold is growing under a dissecting microscope and selecting a well-isolated fruiting

head containing mature spores. A transfer needle with a fine straight wire (a filament from an electric light bulb mounted in a holder works very well) is flame-sterilized, cooled, and moistened in sterile agar. The tip is carefully brought into contact with the fruiting head. The adhering spores may then be transferred to suitable nutrient agar and a pure culture established in a matter of a few seconds. This technique requires considerable hand dexterity and practice. It works well for all fungi that produce spores on stalks. We use it routinely for the isolation of Mucorales, *Aspergillus*, *Penicillium*, and the Fungi Imperfecti—including genera such as *Alternaria*, *Cladosporium*, and *Gliocladium*.

A modification of this technique is the use of a micromanipulator to isolate single spores from the surface of agar. It is particularly useful when all the ascospores in an ascus or the basidiospores on a basidium are to be isolated for genetic studies. In some yeasts and fungi, spores are forcibly discharged, and these can be isolated from an agar surface placed above growing colonies.

For some fungi, especially those of the class Basidiomycetes, spores are only produced in, or on, large macroscopic fruiting structures. Basidiospores may be allowed to discharge on nonnutrient agar; then a few are transferred

to nutrient agar, and cultures become established. However, one often encounters basidiospores that fail to germinate. Frequently cultures can be made, if the fruiting body is large enough, by carefully dissecting a small fragment of tissue from inside the sterile fruiting body. The tissue is transferred to an appropriate medium. Since colonies will produce mycelium but no fruiting structure, extreme care must be taken to ensure that one is not isolating mold contaminants. There is no way of positively identifying a culture that produces sterile mycelium.

One last technique, often combined with one or more of the general methods described, is the use of specific inhibitors in either liquid or solid medium to eliminate other groups of microorganisms. Routinely, in making yeast and mold counts of cereal products, tetracycline is incorporated into the medium to inhibit almost all bacteria. It has no adverse effect on the growth of molds or yeasts. On the other hand, actidione, an antifungi antibiotic, can be incorporated into nutrient media to inhibit both yeasts and fungi without affecting the growth of the bacteria. We are not aware of any combination of materials that will inhibit all bacteria and fungi but will permit the exclusive growth of actinomycetes.

CLASSIFICATION OF MICRO-ORGANISMS USED FOR PRODUCTION OF FERMENTATION PRODUCTS

Since this topic is reviewed in great detail in various texts on fermentation (11, 12, 13), no attempt will be made to discuss the microorganisms involved except to summarize the information in tabular form (table 2). Even this summary cannot be complete because some fermentation products were, or are, made on a limited custom basis and are not regular articles of commerce. When one considers all the types of fermented food made all over the world,

some of which are exclusively local, it becomes quite impossible to enumerate even a small portion of them. Also, some products once made by fermentation are now made by chemical synthesis, especially from petrochemicals. Thus ethanol for industrial uses is made exclusively via this method in the United States. Other countries with a shortage of oil, but with enormous amounts of molasses, still make ethanol by fermentation.

TABLE 2.—Alphabetical listing of commercial products by genus and kind of micro-organisms

Product	Genus	Kind of micro-organism
AMINO ACIDS		
Aspartic acid	<i>Pseudomonas</i>	Bacteria.
Glutamic acid (monosodium glutamate)	<i>Bacillus</i>	Do.
Do	<i>Brevibacterium</i>	Do.
Do	<i>Micrococcus</i>	Do.
Isoleucine	<i>Pseudomonas</i>	Do.
Lysine	<i>Micrococcus</i>	Do.
Phenylalanine	do	Do.
Threonine	<i>Bacillus</i>	Do.
Valine	<i>Micrococcus</i>	Do.
ANTIBIOTICS		
Various products	<i>Aspergillus</i>	Mold.
Do	<i>Bacillus</i>	Bacteria.
Do	<i>Cephalosporium</i>	Mold.
Do	<i>Fusidium</i>	Do.
Do	<i>Micromonospora</i>	Actinomycetes.
Do	<i>Nocardia</i>	Do.
Do	<i>Penicillium</i>	Mold.
Do	<i>Streptomyces</i>	Actinomycetes.
BEVERAGES		
Beer	<i>Saccharomyces</i>	Yeast.
Distilled spirits	do	Do.
Sake	<i>Aspergillus</i>	Mold.
Do	<i>Saccharomyces</i>	Yeast.
Wine	do	Do.
ENZYMES		
Amylase (EC 3.2.1.1) ¹	<i>Aspergillus</i>	Mold.
Do	<i>Bacillus</i>	Bacteria.
Do	<i>Endomycopsis</i>	Yeast.
Do	<i>Rhizopus</i>	Mold.
Amyloglucosidase (see Glucoamylase) ²		
Asparaginase (EC 3.5.1.1)	<i>Erwinia</i>	Bacteria.
Do	<i>Escherichia</i>	Do.
Catalase (EC 1.11.1.6)	<i>Aspergillus</i>	Mold.
Do	<i>Penicillium</i>	Do.
Cellulase (EC 3.2.1.4)	<i>Aspergillus</i>	Do.
Do	<i>Myrothecium</i>	Do.
Do	<i>Trichoderma</i>	Do.
Dextranase (EC 3.2.1.11)	<i>Penicillium</i>	Do.
β -Fructofuranosidase (EC 3.2.1.26)	<i>Saccharomyces</i>	Yeast.
β -Galactosidase (EC 3.2.1.23)	do	Do.
Glucoamylase (EC 3.2.1.3)	<i>Aspergillus</i>	Mold.
Do	<i>Endomycopsis</i>	Yeast.
Do	<i>Rhizopus</i>	Mold.

See footnotes at end of table.

TABLE 2.—*Alphabetical listing of commercial products by genus and kind of micro-organisms—Continued*

Product	Genus	Kind of micro-organism
ENZYMES—Continued		
Glucose isomerase (<i>see</i> Xylose isomerase)		
Glucose oxidase (EC 1.1.3.4)	<i>Aspergillus</i>	Mold.
Do	<i>Penicillium</i>	Do.
α -Glucosidase (EC 3.2.1.20)	<i>Aspergillus</i>	Do.
β -Glucosidase (EC 3.2.1.21)	do	Do.
Hemicellulase (<i>see</i> Xylanase)		
Invertase (<i>see</i> β -Fructofuranosidase)		
Lactase (<i>see</i> β -Galactosidase)		
Laundry enzymes (<i>see</i> Proteases, alkaline)		
Lipase (EC 3.1.1.3)	<i>Aspergillus</i>	Mold.
Do	<i>Candida</i>	Yeast.
Do	<i>Mucor</i>	Mold.
Milk-clotting enzymes (<i>see</i> Rennin)		
Pectinase (<i>see</i> Polygalacturonase)		
Penicillinase (EC 3.5.2.6)	<i>Bacillus</i>	Bacteria.
Penicillin amidase (EC 3.5.1.11)	Many micro-organisms	
Pentosanase (<i>see</i> Xylanase)		
Plasmin (EC 3.4.4.14)	<i>Streptococcus</i>	Bacteria.
Polygalacturonase (EC 3.2.1.15)	<i>Aspergillus</i>	Mold.
Proteases (EC 3.4.4)	do	Do.
Do	<i>Conidiobolus</i>	Do.
Do	<i>Mucor</i>	Do.
Do	<i>Streptomyces</i>	Actinomycetes.
Proteases, alkaline	<i>Bacillus</i>	Bacteria.
Rennin (EC 3.4.4.3)	<i>Endothia</i>	Mold.
Do	<i>Mucor</i>	Do.
Streptokinase (<i>see</i> Plasmin)		
Xylanase (EC 3.2.1.8)	Many micro-organisms	
Xylose isomerase (EC 5.3.1.5)	<i>Streptomyces</i>	Actinomycetes.
FOODS		
Ang-kak	<i>Monascus</i>	Mold.
Bacterial starters (fermented dairy products and sausage)	<i>Lactobacillus</i>	Bacteria.
Do	<i>Leuconostoc</i>	Do.
Do	<i>Pediococcus</i>	Do.
Do	<i>Propionibacterium</i>	Do.
Do	<i>Streptococcus</i>	Do.
Bantu beer	<i>Lactobacillus</i>	Do.
Do	<i>Saccharomyces</i>	Yeast.
Blue-cheese flavor	<i>Penicillium</i>	Mold.
Bread (bakers' yeast)	<i>Saccharomyces</i>	Yeast.
Cheese and fermented dairy products	<i>Lactobacillus</i>	Bacteria.
Do	<i>Penicillium</i>	Mold.
Do	<i>Propionibacterium</i>	Bacteria.
Do	<i>Streptococcus</i>	Do.
Chinese yeast	<i>Chlamydomucor</i>	Mold.
Do	<i>Hansenula</i>	Yeast.
Do	<i>Rhizopus</i>	Mold.
Do	<i>Saccharomyces</i>	Yeast.

TABLE 2.—*Alphabetical listing of commercial products by genus and kind of micro-organisms—Continued*

Product	Genus	Kind of micro-organism
FOODS—Continued		
Fermented fish	<i>Aspergillus</i>	Mold.
Do	Unidentified	Halophilic bacteria.
Hamanatto	<i>Aspergillus</i>	Mold.
Koji	do	Do.
Do	<i>Rhizopus</i>	Do.
Magou	<i>Lactobacillus</i>	Bacteria.
Miso	<i>Aspergillus</i>	Mold.
Do	<i>Saccharomyces</i>	Yeast.
Nata	<i>Acetobacter</i>	Bacteria.
Natto	<i>Bacillus</i>	Do.
Ontjom	<i>Neurospora</i>	Mold.
Pickles and sauerkraut	<i>Lactobacillus</i>	Bacteria.
Do	<i>Streptococcus</i>	Do.
Shoyu	<i>Aspergillus</i>	Mold.
Do	<i>Saccharomyces</i>	Yeast.
Do	<i>Torulopsis</i>	Do.
Sufu	<i>Actinomucor</i>	Mold.
Do	<i>Mucor</i>	Do.
Tempeh	<i>Rhizopus</i>	Do.
Yeast	<i>Candida</i>	Yeast.
Do	<i>Saccharomyces</i>	Do.
INDUSTRIAL SOLVENTS		
Acetone	<i>Clostridium</i>	Bacteria.
Butanol	do	Do.
2,3-Butanediol	<i>Aerobacter</i>	Do.
Do	<i>Bacillus</i>	Do.
Dihydroxy acetone	<i>Acetobacter</i>	Do.
Ethanol	<i>Clostridium</i>	Do.
Do	<i>Saccharomyces</i>	Yeast.
Glycerol	do	Do.
Do	<i>Torulopsis</i>	Do.
MISCELLANEOUS		
Alkaloids	<i>Claviceps</i>	Mold.
Bioinsecticides	<i>Bacillus</i>	Bacteria.
Dextran	<i>Leuconostoc</i>	Do.
Gibberellin	<i>Gibberella (Fusarium)</i>	Mold.
Inosinic acid	<i>Bacillus</i>	Bacteria.
Do	(Several genera)	Yeast.
Nucleotides	<i>Brevibacterium</i>	Bacteria.
Do	<i>Candida</i>	Yeast.
<i>Rhizobium</i> culture	<i>Rhizobium</i>	Bacteria.
Silage	<i>Lactobacillus</i>	Do.
Sorbose	<i>Acetobacter</i>	Do.
Steroid transformations	<i>Aspergillus</i>	Mold.
Do	<i>Corynebacterium</i>	Bacteria.
Do	<i>Curvularia</i>	Mold.
Do	<i>Rhizopus</i>	Do.
Do	<i>Streptomyces</i>	Actinomycetes.

TABLE 2.—*Alphabetical listing of commercial products by genus and kind of micro-organisms*
—Cont.

Product	Genus	Kind of micro-organism
ORGANIC ACIDS		
Acetic	<i>Acetobacter</i>	Bacteria.
Citric	<i>Aspergillus</i>	Mold.
Erythorbic	<i>Penicillium</i>	Do.
Fumaric	<i>Rhizopus</i>	Do.
Gluconic	<i>Aspergillus</i>	Do.
Itaconic	do	Do.
Itatartaric	do	Do.
2-Ketogluconic	<i>Pseudomonas</i>	Bacteria.
5-Ketogluconic	<i>Acetobacter</i>	Do.
α -Ketoglutamic	<i>Pseudomonas</i>	Do.
Kojic	<i>Aspergillus</i>	Mold.
Lactic	<i>Lactobacillus</i>	Bacteria.
PROTEIN		
Various products	<i>Chlorella</i>	Algae.
Do	<i>Saccharomyces</i>	Yeast.
Do	<i>Torula</i>	Do.
VITAMINS		
Ascorbic acid (vitamin C) (in part)	<i>Acetobacter</i>	Bacteria.
B ₁₂	<i>Bacillus</i>	Do.
Do	<i>Propionibacterium</i>	Do.
Do	<i>Streptomyces</i>	Actinomycetes.
β -Carotene	<i>Blakeslea</i>	Mold.
Riboflavin	<i>Ashbya</i>	Yeast.
Do	<i>Eremothecium</i>	Do.

¹ Enzymes are identified by their code numbers, the key to classification as assigned by the Commission on Enzymes, International Union of Biochemistry (6).

² Included in this alphabetical list of enzymes are those names familiar to microbiologists but no longer recommended by the Enzyme Commission for use. Recommended and familiar names are cross-referenced.

PROBLEMS IN MAINTAINING STABLE INDUSTRIAL CULTURES

From years of experience in handling cultures of all kinds, we believe that certain steps should be taken as soon as a micro-organism is isolated in pure culture to ensure it remains in a stable state. These steps need to be taken as soon as the culture is brought into the laboratory or isolated, long before its potential or lack of potential is known.

1. The culture is examined under a dissecting microscope to determine: (a) That the culture is growing uniformly; (b) that it is free from other micro-organisms; (c) that if it is a mold, mature spores are present; and (d) that

the culture appears to be the genus and perhaps species isolated or named when received. This examination will then determine the next step to be taken.

2. If the culture appears to be pure, shows vigorous and uniform growth, and has mature spores, three to five ampules of the micro-organism should be lyophilized immediately.

3. If the strain is known to be a member of a species or genus in which lyophilization is always successful, no viability check is needed. However, it is good practice, even with such strains, to sacrifice one lyophil tube and to di-

lute or streak the culture out onto a suitable growth medium. The check on viability will show three things: (a) If the propagules have survived in large numbers; (b) if the lyophil preparations are free of other micro-organisms; and (c) if the regenerated culture is still uniform in growth and sporulation. If the strain being preserved has not been lyophilized before, the viability check is a must because some micro-organisms fail to survive this process. In case of failure, an alternative method must be found while the first generation culture is still available and in a healthy state.

4. If the culture is recovered successfully from lyophil or from a preparation preserved by another method, such as freezing in liquid nitrogen, records should be kept of the proper medium for growth and sporulation, as well as for any special requirements, such as temperature of incubation, length of incubation, or pH. For example, the mold *Blakeslea trispora* will sporulate rapidly (3 to 4 days at 25° C.), but often within 10 days the spores will germinate in place. Lyophilization subsequent to this occurrence will be a complete failure. The process works beautifully if the spores are processed when they have just reached maturity.

5. The lyophil tubes should be stored at 4° to 10° C. and, perhaps, checked for viability at the end of each 10 years of storage.

6. At the time of lyophilization, the culture should be examined in the appropriate way to determine its identity. Sometimes this examination may lead to species and variety identification, but other times it leads only to the approximate species and genus. The records should certainly show its approximate identity because (a) it allows the person reviving the culture, perhaps years later, to know what was preserved and (b) it makes the records more complete and, therefore, more useful. In some collections of fungi, a microphotograph is made of the fungus at the time of identification. This is an excellent type of record.

7. At the same time that lyophil preparations are made, records should be completed showing the following items: (a) The name of the organism; (b) where obtained—whether it was isolated in a laboratory or received from another microbiologist and, if the latter, his

name and address; (c) accession number assigned and any other designation given to it, such as a temporary number or other laboratory or collection number; (d) location and original source of the material (where was the organism found in nature); (e) special requirements—medium of maintenance, optimum temperature, and other conditions; (f) products or unique properties and approximate yields; (g) number of cultures made; and (h) references if strain is cited in a paper or patent. Rarely can all this information be assembled. With time, additional information will be needed for the record. The data can be placed on cards, which should be cross-indexed so that one can find a culture by number, source, product, and name. Some collections are being indexed for computer sorting. With a large collection, such as ours (35,000+ strains), to put this data onto cards now would be a Herculean task.

Records should be kept showing who uses a given strain in the laboratory and to whom it is sent in laboratories at other institutions. This information will be useful if it should become necessary to obtain a replacement of a culture which died or degenerated, but which may still be in the original state in someone else's laboratory. Written and dated records are also very essential for industrial strains that may become involved in patent and legal cases. For this reason, we ask anyone requesting cultures from our collection to put his request in writing. When the culture is sent, a letter to the requester is prepared by the appropriate curator as a matter of written record.

8. If the culture does not meet the requirements set forth in item 2, the following steps are taken: If the culture is pure, but shows sectoring, then the nonuniform culture is lyophilized. Also, the various forms are isolated separately, and each type is lyophilized individually. Sometimes a heterogeneous culture cannot be separated into its components. The philosophy of lyophilizing the sectoring culture is to try to preserve all the component parts because typically, at this stage, you do not know which part you actually may need later.

If the culture is impure, methods must be used to rid the culture of its contaminants either by dilution and picking an isolated col-

ony or by picking one or a few spores from a fruiting head. Occasionally, two or more organisms are associated (mixed culture) as, for instance, in koji starters, where it may be necessary to lyophilize the total starter as well as its components.

9. Frequently, two different methods of preservation should be used at the same time. For instance, we still carry some of our fungus cultures on agar slants with periodic transfers, as well as in lyophil. Oiled and soil cultures are other possibilities. The former consists of agar slant cultures covered with sterile mineral oil. In the latter, spores are placed in sterile soil or sand and allowed to dry. Details of preservation techniques require too much space to describe them here.

10. If a culture that has degenerated is received, certain steps must be carried out to obtain a better one. Perhaps the ability to sporulate is deteriorating; often a series of dilution plates will produce some colonies that grow more vigorously or sporulate more heavily in a natural manner. In some cultures, especially molds, the isolation of spores from individual heads may lead to better cultures. In others, the fault may be the medium or the growth conditions. Many *Aspergilli* and *Penicillia* grow normally and vigorously on a synthetic medium. However, even though some cultures grow and fruit on a synthetic medium, they will do much better if the medium contains organic nitrogen and growth factors in the form of malt or yeast extract. This response may represent a better form of nitrogen, or it may reflect a partial vitamin deficiency that has been overcome.

We believe that it is appropriate to list a number of principles which we consider important for the cultivation of micro-organisms in order to ensure vigorous, healthy, and stable starters:

1. For the maintenance of stock cultures, a chemically undefined, but reproducible, stock medium is better than a synthetic one. A micro-organism, as it occurs in nature, is almost al-

ways growing on an undefined substrate. A defined medium will more likely select a certain part of the genetic population. The result may well lower the yield of the desired product.

2. In general, a stock culture medium should be no more nutritionally rich than is required to perpetuate the culture without change. Thus glucose (or other sugar) is customarily excluded, or if glucose is essential (as it is for lactic acid bacteria), a buffer is incorporated to control the pH. If the pH were allowed to drop, the longevity of cells might be endangered. Worse yet, the population imbalance mentioned in number 1 might reduce or destroy the usefulness of the culture. Appropriate media for use with a variety of micro-organisms are given in a paper entitled "Maintenance of cultures of industrially important micro-organisms" (8).

3. Stock cultures are usually subjected to two different sets of conditions. First, they are encouraged to grow rapidly and vigorously for a relatively short time by incubating them at or near their optimum temperatures and, if they are aerobic, allowing them free access to air. Then they are induced to slow down metabolically by storing them for a comparatively long time in a refrigerator and sometimes also by limiting their access to air by stoppering test tubes and flasks and sealing petri dishes. Stoppering also hinders loss of moisture from the culture. These variations are not too much different from those they encounter in nature. Regardless, they seem not to harm the micro-organisms.

The pH of the medium is also important. Generally, bacteria are grown in neutral media; molds are grown in media that have a pH between 6 and 7; and yeasts, in the vicinity of pH 6.

4. When new cultures are started, inoculum is taken from a mature culture. It consists of a small amount of growth of yeasts or bacteria or, for molds, a few spores without mycelium.

PROBLEMS OF STRAIN DEGENERATION AND LOSS

In looking back on our experiences involving the loss of pure cultures, five causes come to mind: (1) Contamination by other micro-

organisms, (2) infestation by mites, (3) phage infestations, (4) natural selection and mutation, and (5) untrained staff.

Contamination By Other Micro-organisms

We have encountered many cultures reputedly pure, but they carried a second micro-organism never separated from the original culture at the time of isolation. This situation is particularly true when colonies are picked from dilution plates in which an inhibitor was placed in the medium to control bacterial growth. Often colonies growing on the surface of the agar plates with tetracycline as a bacterial inhibitor appear to be well isolated and pure. When the colony is picked off, however, dormant bacterial cells are removed with it. When placed on a medium free of the inhibitor, they grow again. A bacterial culture producing a thin growth may be obscured by the more luxuriant growth of a mold.

A common cause of contamination is the storage of cotton-plugged, agar slant cultures in refrigerators. Often in the summer the air is warm and moist. When the refrigerator door is opened, moist air enters and upon cooling condenses on labels and cotton plugs. Some *Penicillia* can grow on moist cotton and the labels at 4° to 5° C. in a few weeks. When they do, the conidia present on the tubes make pure culture transfers all but impossible. If sufficient time is allowed, mycelial growth of the *Penicillia* will penetrate the cotton plug where they sporulate, and conidia will then drop onto the surface of the agar and develop new colonies.

Infestation By Mites

Certain species of mites feed on fungus spores. These mites are extremely small and barely can be seen with the naked eye. They occur in nature in decaying plant material and are worldwide in distribution. When an active program of isolating fungi from soil, humus, or moldy plant material is going on, these animals are often present as adults or eggs. If care is not taken, the mites will travel from the contaminated material into petri dishes and test-tube cultures. They invariably carry various mold spores on them, and they appear to be attracted by the odor of certain mold species.

Even though cotton plugs are a good barrier to mold spores, the fungus mites traverse the

cotton plugs into pure cultures unless the cotton plugs are poisoned. Besides contaminating a culture, they will lay eggs that hatch in a few days, and the young will migrate into new cultures. In a short time, hundreds of cultures are contaminated, and a whole collection may be lost. If infestation has not spread too far, the contaminated cultures may be destroyed, but many other cultures that appear to be pure will contain mites, and the contamination will reoccur.

Once mites are introduced from natural material or have been introduced from cultures deposited in the collection, they are difficult to control. The best solution is to prevent mites from becoming free in the laboratory by quarantining all suspected, contaminated material in a location away from stock cultures. The same precaution should be taken with respect to cultures received from outside the culture collection. The further precaution of poisoning all cotton plugs of stock cultures should be taken.

Phage Infestations

Some strains of bacteria and actinomycetes carry phage in one form or another. Generally, these are difficult to detect, and it is even harder to free the culture from them. At one time, yeasts and mold species were believed never to be infected with phage, but this belief is now known to be false.

Natural Selection And Mutation

Changes in the genetic population in a culture will occur in all micro-organisms. Our personal belief is that some of these changes may be prevented by the use of more natural media. For example, in our collection of *Mucorales*, no change appears to occur if the cultures are maintained on a potato-dextrose-salts medium. Some of the *Mucorales* will develop sectoring and sterile growth if cultures are carried on a synthetic medium.

Culture rundown frequently occurs in some species of *Penicillium* and *Aspergillus*. Once this process has gone to a certain phase, it is impossible to regain the original form. For

example, some three culture lines of the type strain of *A. parasiticus* NRRL 502 are poor aflatoxin producers, but the same strain carried under different cultural conditions in two other laboratories over many years is still a good producer of the mycotoxin.

Untrained Staff

Probably as serious as any cause of culture failure is the handling of cultures by untrained persons. Often the media are improperly sterilized by people who do not comprehend that some complex media require more heat than others and that the larger the amount of medium, the more sterilization is needed. Some-

times inadequately trained people just do not know how to transfer cultures to avoid contamination. Often they transfer a large mass of spores and mycelium of a mold causing the whole work area in the transfer room to be filled with spores suspended in the air and on the table tops. Only a few spores attached to sterile agar on a transfer needle are needed to start new stock cultures. Some microbiologists do not recognize even the micro-organisms they are working with. One prominent microbiologist has estimated that from one-third to one-half of all the work published on bacterial physiology has been done with contaminated cultures or with the wrong species!

PHYSICAL CONDITIONS AFFECTING MICRO-ORGANISMS

The physical conditions that affect the growth and longevity of micro-organisms are the same as those that influence other forms of life; viz., pH, temperature, light, humidity, pressure, oxidation/reduction potential, surface tension, and radiations. In the context of this discussion, we are interested in the effect of these factors on the survival of microbes in culture collections and while the organisms are *in transit*.

In most modern culture collections, stock strains are carried as lyophilized (freeze-dried) cultures. Essentially to lyophilize a culture, microbial cells, spores, or, sometimes, portions of mycelium are suspended in a protective colloid—such as blood serum or skim milk—quickly frozen at about -40° C., and dehydrated by allowing sublimation of moisture *in vacuo*. The dried preparation is sealed under vacuum and stored, usually in a refrigerator at 5° to 10° C.

In the lyophilized state, microbes take on some of the properties of bacterial endospores becoming less susceptible to extremes of temperature, dryness, and radiations. They are safe from contamination, changes in pressure, pH, humidity, oxidation/reduction potential, and surface tension. They can be shipped by land, sea, and air in temperate, tropical, or frigid climates without loss of life or change in

character. (See reference 5 for a review of methods.)

Some micro-organisms cannot withstand lyophilization and must be maintained by other, less convenient, means. One that has come into use in recent years, and that has some of the advantages of lyophilization, is preservation by freezing and storage in and over liquid nitrogen (-165° to -195° C.). The full range of microbial types that can be preserved in this manner is not yet known, but many fastidious forms that fail to survive lyophilization have remained viable for long periods in liquid-nitrogen refrigerators. For instance, some fungus cultures are reported still viable and apparently unchanged after 5 years in a liquid-nitrogen refrigerator (10).

Ultra-low-temperature frozen cultures are sealed in glass vials or ampules so that they have essentially the same protection as lyophilized preparations against contamination and changes in the physical environment. However, they must be shipped in special trucks, freight cars, or in portable, liquid-nitrogen refrigerators because it is only while they are kept at -165° to -195° C. that they are guarded against damage. Although this method is less convenient than lyophilization, it is becoming more common because it is still better than alternative methods.

Use of alternative methods is still inescapable for microbial cells that cannot be preserved by either of the two techniques already discussed. These alternatives have been in use for many years although more time consuming and subject to hazards that are minimal or absent in the other two methods. Basically, there is a single technique but with modifications. It is the serial transfer method by which some growth (vegetative cells, spores, mycelium, tissue) is transferred from one culture (agar slant, agar stab, agar plate, broth, tissue culture) to fresh medium, allowing the new culture to grow under optimum conditions to maturity, storing the new stock culture for a time, and then repeating the cycle.

Storage usually is in a refrigerator (5° to 10° C.), but sometimes it is at room temperature. The length of time between transfers

varies, depending upon the nature of the strain, from one or a few days to several months or even years. Often the interval may be lengthened by preventing dehydration of stock cultures by covering them with mineral oil (oiled cultures) or by closing the cultures with rubber stoppers, corks, or by impregnating the cotton plugs with paraffin. The rate of growth is slowed by refrigerating the cultures. This step minimizes changes in pH and in oxidation/reduction potential, and it also reduces the danger that predominance in the population will be gained by the progeny of cells mutated by stray radiations (cosmic rays).

These are the principal methods of maintaining and preserving cultures, and they all succeed to some degree in minimizing damage to, or loss of, life of cultures by inimical physical conditions.

REGULATIONS REGARDING DEPOSIT OF CULTURES FOR PATENT PURPOSES

One activity in which many of the larger culture collections become involved is the handling of cultures of micro-organisms deposited in connection with patent applications. In some countries it is desirable to deposit a culture, not necessarily a high producer of a product, with a recognized culture collection. This deposit is to ensure that a process being patented is fully disclosed to the public. In other words, a fermentation process is not considered fully

operable until a culture is available for use in the process.

Over the years, the Northern Regional Research Laboratory (NRRL) has developed guidelines regarding this culture-collection activity. They are based on considerable experience and also on consultations with inventors, companies, and patent lawyers. These guidelines are updated from time to time and are not to be construed as being final. The latest revision was on November 9, 1971.

PROCEDURES AND POLICIES FOR DEPOSITION OF CULTURES FOR PATENT PURPOSES IN THE ARS CULTURE COLLECTION

The ARS Culture Collection serves as a depository for cultures that are involved in fermentation patents and, therefore, will be glad to receive such a culture in connection with a patent application. When such a culture is received, it is assigned a number in the collection and is maintained thereafter in a living state. Immediately after receipt, a letter is written to the depositor advising of the number assigned and including the following statement:

“... Furthermore, insofar as is practicable in carrying out the business of the Department of Agriculture, we shall refrain from distributing this culture pending the issuance of the United States patent to your company, with the exception, however, that access to this culture by other parties will be granted upon receipt of written authorization from your company specifying the name and the

ARS Culture Collection designation (NRRL number) of the culture and identifying the party who is to receive it."

More recently, some depositors have requested replacement of the paragraph above by a simple statement such as:

"As of this date, the subject culture(s) will be made available to anyone who requests the same."

It is suggested that advice be sought from an attorney as to which type of statement should be used. Either one of these statements will be written, depending upon the wishes of the depositor. The ARS Culture Collection letter then can be attached to the patent application for the Patent Examiner.

Curators in the ARS Culture Collection do not attempt to make an identification or to name any organism that has been deposited in connection with a patent application, nor do they carry out research work with such deposits until a U.S. patent issues or cultures are otherwise released. It is not necessary, of course, to provide a precise identification, but the microbiologist concerned should at least state to what genus the micro-organism belongs. Also, if special media are required for its maintenance, the curators need to know this. Ordinarily, one or two agar slant cultures, one lyophilized preparation, or both, are received from depositors. Depositors also are responsible for resupplying material should the need ever arise.

The depositor has the option of sending cultures for deposit in the ARS Culture Collection in three ways:

1. Thirty lyophilized preparations, clearly labeled with the depositor's original strain designation and in tubes no longer than 2 inches. One of these is checked for viability, the

NRRL designation is placed on each tube, and the supply of tubes is stored at 3° to 5° C. Bona fide letter requests for the culture would be shipped from this stock.

2. One lyophilized preparation, clearly labeled with the depositor's original strain designation. On receipt, the micro-organism is cultivated on appropriate agar media and 30 lyophilized preparations made. One of these is checked for viability, the remainder handled as in option 1.

3. One, or preferably two, agar slant cultures of the micro-organism growing on an appropriate medium. Sufficient material is prepared by our curators to make 30 lyophilized preparations; one is checked for viability, and the remainder are handled as in options 1 and 2. When the initial agar slant cultures deposited appear suitable, lyophilizations often are made from that material.

There is no charge for the deposit or maintenance of cultures.

Cultures deposited in connection with patent applications may be obtained, free of charge, by letter request stating the name of the micro-organism and the ARS Culture Collection strain designation (NRRL number).

The ARS Culture Collection does not issue a catalog or list. It has no regulations imposing restrictions on the use of such cultures deposited for patent purposes. Such materials are distributed according to the depositor's wishes which, in turn, generally are based on his interpretation of Patent Office requirements. Use of such materials, once distributed, are the responsibility of the requestor. Cultures are automatically removed from any restrictive category, once a U.S. patent issues in which the particular micro-organism is involved.

SHIPMENT OF MICRO-ORGANISMS

Living cultures of micro-organisms are items of international commerce. Every year many thousands of strains are transported by land, sea, and air to scientists on every continent, with the possible exception of Antarctica. Most cultures are dispatched from large culture col-

lections, such as the American Type Culture Collection in the United States, the various national collections in England, and the Institute for Fermentation in Japan. Many strains are also distributed by small, specialized collections and by individual scientists who maintain

a few micro-organisms, primarily for their own research. In the course of time, the large collections have learned to solve the problems associated with packaging and shipping living cultures so that they arrive at their destinations whole, alive, and unchanged.

Except for micro-organisms used in the pharmaceutical industry to produce vaccines and antisera, very few of the microbes used in the food, feed, and fermentation industries and carried in the mails endanger public health or agriculture. In the United States, and presumably in other nations, nonpathogenic cultures are virtually immune from legal restrictions on their movement from laboratory to laboratory. However, in the United States and very likely in other countries, a number of laws apply to the import, export, and internal transport of "etiologic agents and vectors." These are discussed in a brochure published in 1970 (1) by the American Type Culture Collection.

Six Departments of the U.S. Government—Agriculture; Commerce; Health, Education, and Welfare; Transportation; Treasury; and the U.S. Postal Service are listed as those concerned with regulating potentially dangerous micro-organisms.

In the U.S. Department of Agriculture, the Animal Plant Health and Inspection Service's (APHIS) Animal Health Programs are charged with responsibility to see that "no organisms (which may introduce or disseminate any contagious or infectious diseases of animals, including poultry) . . . shall be imported into the United States or transported from one State . . . to another State . . . without a permit issued by the Secretary and in compliance with the terms thereof." APHIS' quarantine inspection programs require a permit for the movement of any plant pest into or through the United States or any of its territories and possessions. "Plant pest," as defined in the Federal Plant Pest Act, includes microbial cultures which can directly or indirectly injure or cause disease or damage in plants.

The primary concerns of the Department of Commerce, as it relates to distribution of living cultures, are safeguarding our national security and furthering our foreign policies. It has published a "general license" that authorizes

the export of living cultures to most destinations. Special "validated" licenses are required for the export of living cultures to a few nations.

The Public Health Service of the U.S. Department of Health, Education, and Welfare, through its Foreign Quarantine Program (Center for Disease Control), promulgates and enforces regulations to "prevent the introduction and spread of communicable disease from foreign countries into the United States . . . , or from one state to another." It operates under a law which states, "A person shall not import into any place under the control of the United States, nor distribute after importation, any etiologic agent . . . of human disease . . . unless accompanied by a permit issued by the Surgeon General."

Shipments of living cultures are free of customs duty. The principal reasons that the Bureau of Customs of the United States Department of the Treasury is involved are:

1. To determine from documentation and inspection at ports and airports of entry if shipments are controlled by Federal law and regulations enforced by other Government agencies.

2. To select required samples for referral to enforcement agencies.

3. To withhold release of shipments during examination of referred samples and pending issuance of permits or licenses that are required as a condition of release.

Regulations of the U.S. Postal Service and the Department of Transportation specify how pathogenic micro-organisms shall be packaged. The intent of these instructions is the same as those governing permits and licenses; that is, to ensure that etiologic agents do not escape and endanger public health and agriculture.

The reasons we have detailed the United States agencies and their requirements are that we suspect other nations either have similar regulations or will ultimately pattern theirs after those of the United States. It behooves culture collection curators to be cognizant of such laws, if they wish to avoid difficulty and delay in receiving and sending cultures.

In perusing culture catalogs from several of the larger collections, we found only three that

make any mention of possible need for licenses and permits. One is the Catalogue of Strains of the American Type Culture Collection (2). Another is the Catalogue of the Culture Collection of the Commonwealth Mycological Institute (4). The third is the List of Cultures of the Centraalbureau voor Schimmelcultures (3).

Inasmuch as no mention is made in catalogs of other collections (Argentina, Czechoslovakia, England, Germany, India, Indonesia, Japan, Netherlands, Scotland, U.S.S.R.) about the need for licenses, permits, or customs arrangements, we suspect that either such requirements in countries other than the United States and Canada do not exist or else enforcement is ineffective. If true, curators need concern them-

selves only with safe packaging and labeling of cultures destined for most nations.

Problems to be overcome in packaging are the selection of a sturdy mailing tube or carton that will remain intact, despite rough handling and possible exposure to moisture, and that will protect the enclosed cultures from breakage. Additionally, the container must be so made that, if despite all precautions breakage does occur, the released micro-organisms cannot escape to the outside.

Although the requirements delineated may seem formidable to the uninitiated, curators ordinarily have little or no trouble in obtaining cultures from anywhere in the world or in sending cultures to anyone who has a legitimate need for them.

LITERATURE CITED

- (1) ALEXANDER, M. T., and BRANDON, B. A.
1970. THE PACKAGING AND SHIPPING OF LIVING REFERENCE CULTURES. ATCC Pub. No. 1, 26 pp., American Type Culture Collection, Rockville, Md. 20852.
- (2) AMERICAN TYPE CULTURE COLLECTION.
1970. CATALOGUE OF STRAINS. Ed. 9, 243 pp. Rockville, Md. 20852.
- (3) CENTRAALBUREAU VOOR SCHIMMELCULTURES.
1968. LIST OF CULTURES. Ed. 27, 262 pp. Baarn, Netherlands.
- (4) COMMONWEALTH MYCOLOGICAL INSTITUTE.
1968. CATALOGUE OF THE CULTURE COLLECTION OF THE COMMONWEALTH MYCOLOGICAL INSTITUTE. Ed. 5, 162 pp. Kew, Surrey, England.
- (5) FENNELL, D. I.
1960. CONSERVATION OF FUNGUS CULTURES. Bot. Rev. 26: 79-141.
- (6) FLORKIN, M., and STOTZ, E. H., eds.
1965. ENZYME NOMENCLATURE. Ed. 2, vol. 13. In *Comprehensive Biochemistry*. New York.
- (7) HAYNES, W. C.
1963. DISCUSSION II. (on "The organization of a type culture collection," by Shewan, J. M., Torry Research Station, Aberdeen.) In Martin, S. M. (ed.), *Culture Collections: Perspectives and Problems*. Proc. Specialists' Conf. on Cult. Collect., August 1962, pp. 36-39, Ottawa.
- (8) HAYNES, W. C., WICKERHAM, L. J., and HESSELTINE, C. W.
1955. MAINTENANCE OF CULTURES OF INDUSTRIALLY IMPORTANT MICROORGANISMS. *Appl. Microbiol.* 3: 361-368.
- (9) HESSELTINE, C. W., HAYNES, W. C., WICKERHAM, L. J., and ELLIS, J. J.
1970. HISTORY, POLICY, AND SIGNIFICANCE OF THE ARS CULTURE COLLECTION. In Iizuka, H. I., and Hasegawa, T. (eds.), *Culture Collections of Microorganisms*. Proc. Internatl. Conf. on Cult. Collect. October 7-12, 1968, pp. 21-38. University of Tokyo Press, Tokyo.
- (10) HWANG, S. H.
1966. LONG-TERM PRESERVATION OF FUNGUS CULTURES WITH LIQUID NITROGEN REFRIGERATION. *Appl. Microbiol.* 14: 784-788.
- (11) MARTIN, S. M., ed.
1972. WORLD DIRECTORY OF COLLECTIONS OF CULTURES OF MICROORGANISMS. 560 pp. New York.
- (12) PEPLER, H. J., ed.
1967. MICROBIAL TECHNOLOGY. 454 pp. New York.
- (13) PRESCOTT, S. C., and DUNN, C. G.
1959. INDUSTRIAL MICROBIOLOGY. Ed. 3, 945 pp. New York.
- (14) SMITH, G.
1969. AN INTRODUCTION TO INDUSTRIAL MYCOLOGY. Ed. 6, 390 pp. New York.