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Manufacture of Chemicals by Fermentation
Vienna, 1 - 5 December 1969

MICROORGANISMS AND THEIR ROLE IN FERMENTATION [1]

by

C.W. Hesseltine and W.C. Haynes
Northern Regional Research Laboratory [2]
Peoria, Illinois
United States of America

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SUMMARY

MICROORGANISMS AND THEIR ROLE IN FERMENTATION [1]

by

C.W. Hesseltine and W.C. Haynes
Northern Regional Research Laboratory
Peoria, Illinois
United States of America

The paper emphasizes the use of microorganisms in fermentations in the developing countries. Inasmuch as the key to success or failure in most fermentation processes is availability of the proper microorganisms, the characteristics of suitable microbial strains are enumerated, and some 80 pages are devoted to listing the industrial collections of the world, their locations, their general holdings and the names of their directors. The attributes of a good culture collection are given. The source of new strains of microorganisms for fermentations are the isolation of new wild

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strains and isolates from culture collections. Various fermentations in use throughout the world are listed, together with the specific microorganisms used to carry them out. Those processes most likely to be useful in developing nations are stressed. The means by which small fermentation plants may acquire suitable microbial strains is discussed as also are the problems of maintaining stable cultures. Considerable space is devoted to the question of shipment of microorganisms in international channels and also to legal problems relating to patents involving microorganisms.

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Introduction

The microorganism 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 extremely 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 microorganisms, but also free of phages.

5. The strain should produce the required product within a short period of time, preferably in 5 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. Thus a mutation program may 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.

Microorganisms that meet these conditions must be either isolated from nature or obtained from a culture collection. Since this Working Group deals with the problems of fermentation in developing nations, it seems to us the latter source of cultures should be used. To isolate, purify, screen, and test a culture from nature requires trained microbiologists who are in shorter supply even than money. But plenty of time and money still is no guarantee of success. To obtain the proper culture, sometimes one must isolate the microorganism from a special, ecological niche that may not even exist in a particular country. For example, Blakeslea trispora, which produces large amounts of beta-carotene, cannot be isolated in temperate regions of the United States, but rather one must seek wild strains in the tropics growing 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 microorganisms selected through the centuries for preparing native fermented food products. The principal microorganisms can be obtained with little difficulty. Since the microorganisms 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. We were told 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 food yearly.

In the preparation of this paper, we have tried to be realistic in our approach to the problem of obtaining the proper microorganisms for use in industrial fermentations. Our views are based upon firsthand knowledge of the operation of a large industrial culture collection supported entirely by government funds; experience of several years 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 Microorganisms for Industry

The ultimate sources of cultures of microorganisms 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 microorganisms secured from a continuous program of isolation. New isolates and variant substrains derived from laboratory mutation studies swell the numbers of strains so that many of

the proprietary collections are quite large. However, most of their microorganisms 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 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. Two U.S. collections that have been recipients of cultures from industrial concerns both domestic and foreign as a result of this practice are the American Type Culture Collection at Rockville, Maryland, and the ARS Culture Collection in Peoria, Illinois. As might be expected, the depositing companies do not advertise the fact that particular strains are 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, by which is meant 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 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 microorganisms. They send cultures to any bonafide investigator anywhere in the world who is willing to pay their price. As might be expected, they publish catalogs listing the microorganisms that are for sale. They also often provide other services such as identification of microorganisms 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 microorganisms. Such microorganisms 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 BOD in sewage and industrial effluents. Such collections are of principal interest to UNIDO and its adherent groups and members. Therefore, we concluded that a list of such collections giving addresses, names of curators, and types of

microorganisms contained would be useful (Table 1). We are indebted to Dr. 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 Section on Culture Collections of the International Association of Microbiological Societies, Dr. Martin is preparing and will soon publish a World Survey of Culture Collections in which most of the collections in the world will be named and described.

Additional collections and addresses may be found in some of the larger culture collection catalogs listed at the end of this paper.

Fees for cultures vary from one collection to another. In the United States the American Type Culture Collection charges $20 per strain for all cultures to commercial and noneducational institutions. The cost is reduced to $15 for educational institutions. A handling charge of percent to a maximum of $25 is added to each invoice. The Centraalbureau voor Schimmelcultures in The Netherlands charges 10 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, as 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 we are associated with, do not issue a catalog and 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.

Table 1. Collections Contributing Industrial Microorganisms

Nation	Collection and Parent Organization	Address	Dept. Head or Curator	Sponsors[a]	Contents[b]
Argentina	Instituto de Microbiologia e Industrias Agropecuarias, Instituto Nacional de Tecnologia Agropecuaria	Villa Udaondo, Castelar FCDFS, Buenos Aires	Ing. Agr. E. Ohiel	G	BFY
	Instituto de Patologia Vegetal, Instituto Nacional de Tecnologia Agropecuaria	Villa Udaondo, Castelar FCDFS, Buenos Aires	Ing. Agr. N. J. M. Carrera	G	BF
	Coleccion Cátedra Microbiologia Agricola (FAV, Bs.As.), Facultad Agronomia y Veterinaria, Universidad de Buenos Aires	Avenida San Martín 4453 (Suc. 17), Buenos Aires	Prof. Ing. Agr. R. E. Balbinger	U	BFYAc
	Colección de Cultivos Microbianos, Facultad de Farmacia y Bioquimica, Universidad de Buenos Aires	Junín 956, 8° piso, Buenos Aires	Prof. Dr. R. A. Margni	U	BFI
	Centro de Micologia, Facultad de Medicina, Universidad de Buenos Aires	Paraguay 2155, piso 11, Buenos Aires	Prof. Dr. F. Negroni	U	F
	Cátedra de Microbiologia, Facultad de Ciencias Agrarias, Universidad de Cuyo	Almirante Brown 500, Chacras de Coria, Mendoza	Ing. Agr. N. J. Palleroni	U	BY

continued—

Table 1.--Collections Containing Industrially Useful Microorganisms--continued

Nation	Collection and Parent Organization	Address	Dept. Head or Curator	Sponsors[c]	Contents[*]
Australia	Division of Food Preservation, CSIRO	Delhi Road, North Ryde, New South Wales	Mr. M. V. Tracey	G	B
	Soil Microbiology Culture Collection, Division of Soils, CSIRO	Private Bag No. 1, Glen Osmond, 5064, South Australia	Dr. E. G. Hallsworth	G	B
	[illegible] Collection, Division of Tropical [illegible], CSIRO	Mill Road, St. Lucia, Brisbane, Queensland	Dr. D. O. Norris	G	B
	[illegible] Collection, Western Australia Dept. of [illegible]	Jarrah Road, South Perth, Western Australia	Dr. T. C. Dunne	G	BF
	[illegible] Technology, The [illegible] of NSW	664 Harrington Street, Sydney 2000, New South Wales	E. B. Huddleston	G	F
	[illegible] Institute of Dairy Technology, Dept. of [illegible]	Werribee, Victoria [illegible]	Mr. R. I. Howey	G	B
	[illegible] Microbiological Collection, University of Sydney	Sydney, New South Wales 2000	Prof. S. Smith-White	U	F
	Botany Bay Collection, Dept. of Agricultural Botany, University of Sydney	Sydney, New South Wales 2006	Prof. N. H. White	U	F

continued--

Nation		Address	Director	Sponsors?	Contents*
[illegible]	[illegible]	[illegible]	Dr. [illegible] A. [illegible]	[illegible]	B
	[illegible]	[illegible]	[illegible] M. [illegible]	I	Y
[illegible]	[illegible]	[illegible]	[illegible]. S. [illegible]	?	B
[illegible]	[illegible]	[illegible]	[illegible] Dr. Ing. [illegible]	J	F
	[illegible]	[illegible]	Prof. J. [illegible]	U	B
Brazil	[illegible]	Av. [illegible], 95[illegible], [illegible], São	P. de B. M. [illegible]	G	BPPVa
	[illegible]	[illegible], 56, [illegible], São	Prof. [illegible]	U	BPY

continued—

Table . -- Collections containing industrially useful microorganisms--continued

Nation	[illegible]	Address	[illegible]	Sponsors[3]	Contents[4]
Bulgaria	[illegible]	[illegible] 26, Sofia	Assoc. Prof. R. Dertcharov	G	BFY
Burma	[illegible]	[illegible], Burma	Dr. [illegible]	G	BF
Canada	[illegible]	Ottawa, Ontario	Dr. R. Hamilton	G	BFVb
	[illegible]	[illegible], Ottawa, Ontario	Dr. R. Hamilton	G	BF
	[illegible]	[illegible] Building, [illegible], Ontario	Dr. [illegible] Chan	G	F
	[illegible]	[illegible], Nova Scotia	Dr. D. Brewer	G	BFY
	[illegible] Bacteriology Section, National Research Council	Ottawa 2, Ontario	Dr. C. Gunsling	G	BFY

[illegible]--

Nation	Collection and Market Designation	Address	Dept. Head or Curator	Sponsors[G]	Contents[H]
Canada (cont.)	Horticultural Products Laboratory, Horticultural Research Institute (CDA and);	Victoria Avenue N., Vineland, Ontario	Dr. J. A. Archibald	G	BFY
	Mold Herbarium and Culture Collection (DAOM), University of Alberta	Edmonton, Alberta	Dr. J.W. Carmichael	U	FY
	Microbiology Department, University of Guelph	Guelph, Ontario	Dr. F. E. Chase	U	BFY
	Department of Microbiology, University of Manitoba	Winnepeg 19, Manitoba	Prof. H. Lees	U	BF
	Dept. of Plant Pathology, Macdonald College, McGill Univ.	Ste. Anne de Bellevue, Quebec	Prof. W. E. Sackston	U	FVP
	Macdonald College Collection, Dept. of Microbiology, Macdonald College, McGill Univ.	Ste. Anne de Bellevue, Quebec	Dr. A. C. Blackwood	UI	BFY
	Mycological Culture Collection, Department of Biology, Univ. of Waterloo	Waterloo, Ontario	Dr. H. B. N. Hynes	U	F
	Prairie Regional Laboratory	Saskatoon, Saskatchewan	Dr. R. H. Haskins	G	BF

continued--

Table 1.--Collections Containing Industrially Useful Microorganisms--continued

Nation	Collection and Parent Organization	Address	Dept. Head or Curator	Sponsors[o]	Contents[x]
Canada (cont.)	Dept. of Bacteriology and Immunology, Univ. of Western Ontario	London, Ontario	Dr. R. G. E. Murray	U	B
	University of Western Ontario Culture Collection, Botany Dept., Univ. of Western Ontario	London, Ontario	Dr. J. C. Hickman	U	BFYAl
	University of Windsor Culture Collection, Department of Biology, University of Windsor	Windsor, Ontario	R. J. Doyle	U	BFYVa
Ceylon	Department of Biological Sciences (Microbiology), Vidyouaya University	Gangodawila	Dr. N. N. DeSilva	U	B
Chile	Collection bacteries, Centre Microbiologie, Institut Bacteriologique	Avenida Maratón 1000, Santiago	Prof. E. Dussert J.	GU	B
Czechoslovakia	Yeast Collection, Research Institute for Viticulture and Enology, Czechoslovak Collection of Microorganisms	Matuskova 21, Bratislava	Doc. Ing. A. Veres, CSc	G	FY
	Culture Collection of Fungi, Botany Dept., Charles Univ., Czechoslovak Collection of Microorganisms	Benátska 2, Prague 2	RNDr. O. Fassatliova, CSc	GU	F

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Table 2.--Collections Containing Industrially Useful [illegible]

Nation	Collection and Parent Organization	Address	Dept. Head or Curator	Sponsor[b]	Contents[c]
Czechoslovakia (cont.)	Collection of Rhizobium and other Soil Microorganisms, Central Research Institute for Plant Production	Ruzyně, Prague	Dr. E. Hamatová	G	B
	Culture Collection of Entomogenous Bacteria (CCEB), Institute of Entomology, Czechoslovak Academy of Sciences	Flemingovo n. 2, Prague 6	Dr. O. Lysenko	G	B
	Czechoslovak National Collection of Type Cultures, Institute of Epidemiology and Microbiology	Šrobarova 48, Prague 10	Dr. J. Šourek CSc	G	BFIVaVb
	Czechoslovak Collection of Microorganisms, J. E. Purkyně University	Tř. Obránců míru 10, Brno	Prof. Dr. T. Martinec	U	B
	Collection of Cultures of Wood-Rotting Fungi, Research Laboratory of Plant Pathology and Anatomy, Faculty of Science, J. E. Purkyně University	Kotlářská 2, Brno	Prof. Dr. V. Rypáček, Dr. Sc.	U	F

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Table 1.—Collections Containing Industrially Useful Microorganisms—continued

Nation	Collection and Parent Organization	Address	Dept. Head or Curator	Sponsor[C]	Contents[A]
Czechoslovakia (cont.)	Institute of Chemistry, Slovak Academy of Sciences	Dubravská cesta, Bratislava	Dr. [illegible]vé-Arnošt[illegible]ová	G	Y
	Laktoflora, Research Laboratories of the Dairies Association for the Production of Dried Milk Products, Dairy Industry Direction	Ke Dvoru 2, Prague 6-Vokovice	Dr. Ing. M. Teply	I	BFY
Denmark	Bacillus Collection, Institute of Hygiene, University of Aarhus	DK-8000, Aarhus	Prof. C. J. Bonde	U	B
	Antibiotics Department, Leo Pharm. Lab.	Prags Boulevard 37 2300 Copenhagen S	Dr. K. Andersen	I	?
	Bacteriological Dept., Vitamin-Kemiske Fabrik	2730 Ballerup	L. Dybring	I	?
	Bacteriological Dept., H. Lundbeck and Co.	Ottiliavej 7-8, 2500 Copenhagen Valby	L. Saeto	I	?
	Laboratory for Microbiology, Danmarks tekniske Højskole	Bygning 221, 2800 Lyngby	Prof. Dr. J. Hedegaard	G	?
	Department of Technical Biochemistry, Danmarks tekniske Højskole	Bygning 223, 2800 Lyngby	Dr. M. Jensen	G	?
	[illegible]				

Table 1.--Collections Containing Industrially Useful Microorganisms--continued

Nation	Collection and Parent Organization	Address	Dept. Head or Curator	Sponsor[o]	Contents[x]
Deutsche Demokratische Republik (East Germany)	Kulturensammlungen, Institutes für Mikrobiologie und experimentelle Therapie (IMET), Deutsche Akademie der Wissenschaften zu Berlin	Beuthenbergstrasse 11, Jena	Prof. Dr. med. H. Knöll	G	BFYVa VoPAc
	Botanischen Instituts der Ernst-Moritz-Arndt, Universität Greifswald	Grimmerstrasse 86/88, Greifswald	Prof. Dr. H. Borriss	U	BFYAL VbC
	Inst. f. Forstbotanik, Humboldt-Universität	Schicklestr. 5, Eberswalde	Prof. Dr. H. Lyr	U	F
	Botanisches Institut, Mykologie Weimar, Friedrich-Schiller Universität	Frh.-v-Stein-Allee 2, Weimar	Prof. Dr. habil R. Träger	U	FY
	Kulturensammlung, Institut für Mikrobiologie, Humboldt-Universität	1532 Kleinmachnow, Max-Reimannstrasse 16, Kleinmachnow bei Berlin	Prof. Dr. Jentzsch	U	BFYAC

continued--

Table 1.--Collections Containing Industrially Useful Microorganisms--Continued

Nation	Collection and Parent Organization	Address	Dept. Head or Curator	Sponsor[c]	Content[d]
Bundesrepublik Deutschland (West Germany)	Instituts Stammsammlung, Biologische Bundesanstalt für Land und Fortswirtschaft, Institut für biologische Schädlingsbekampfung	Kranichsteinerstrasse 61, Darmstadt	Prof. Dr. J. M. Franz	G	BFVa
	Bakt. Inst. d. Süddt. Veraucha-u. Forschungsanstalt f. Milchwirtschaft	805 Freising, München, T. H.	Dr. H. Frauk	?	B
	Schering AG.	Tegeler Weg 28-33, Berlin-Charlottenburg	Dr. E. Olivar	I	B
	Sammlung von Algenkulturen, Pflanzenphysiol. Institut, Universität Göttingen	Nikolausberger Weg 16, 34 Göttingen	Prof. Dr. E. G. Pringsheim	IU	Al
	Botanisches Institut	6222 Geisenheim	Dr. H. Schanderl	?	BFY
	Food Spoiling Molds, Deutsche Forschungsanstalt für Lebensmittelchemie	Leopoldstrasse 175, Munich	Prof. Dr. S. W. Souci	G	F
	Bayerische Landesanstalt für Wein-, Obst-u. Gartenbau	Residenzpl. 3, 87 Würtzburg, Bayern	Dr. I. Benda	G	Y
	Mikroorganismensammlung, Institut für Gärungsgewerbe [illegible]	Seestrasse 13, 1 Berlin 65	Prof. Dr. S. [illegible]	I	BFY
	[illegible] Inst. f. angew. Botanik,	[illegible]	[illegible]	? continued--	F

Table 1.--Collections Containing Industrially Useful

Nation	Collection and Parent Organization	Address	Dept. Head or Curator	Sponsor[o]	Contents[x]
Finland	Culture Collection, Department of Microbiology, University of Helsinki	Helsinki 71	Prof. V. J. Vartiovaara	GU	BFY
France	Collection de Microorganismes Associés Aux Invertébrés, Station de Recherches Cytopathologiques I.N.R.A.-C.N.R.S.	Montpellier-Saint-Christol, Gard	Prof. C. Vago	GU	BFVa
	Service des Anaérobies, Institut Pasteur de Paris	Docteur Roux, 25, Paris 15°, Seine	Dr. Rouyer	P	B
	Laboratoire des Fermentations, Institut Pasteur	28 Rue du Docteur Roux, Paris 15e	P. Bréchot	P	Y
	Laboratoire de Cryptogamie, Muséum National d'Histoire Naturelle	Paris	Dr. J. Nicot	G	F
	Centre de Collection de Types Microbiens, Institut Pasteur	20, Boulevard Louis XIV, Lille	Prof. R. Buttiaux	P	BYVaVb
	Institut Pasteur de Lyon: IPL	Pasteur, Lyon 69	M. Carraz	P	B
Ghana	University Microbial Cultures, Kumasi (U.M.C.K.), Department of Biological Sciences, University of Science and Technology	Kumasi	K. O. Nyako	U	BFY

continued--

Table 1.--Collections Containing Industrially Useful Microorganisms--continued

Nation	Collection and Parent Organization	Address	Dept. Head or Curator	Sponsor[O]	Contents[M]
Hungary	Diagnostical and Research Laboratory, National Institute for Tuberculosis "Korányi"	Pihenö ut 1, Budapest. XII	I. Szabó, M.D., D.Sc.	G	BVo
	Culture Collection, Pedological Inst., Hungarian Academy of Sciences	Budapest 11. Herman O.15, Budapest	Dr. T. Szepi	G	BAc
India	D. R. L. (M) Kanpur Culture Collection, Defense Research Laboratory (Materials) P.B. 320, Research and Development Organization, Ministry of Defense	Kanpur, Utter Pradesh	Dr. J. N. Nanda	G	BFY
	National Collection of Industrial Microorganisms (NCIM), Council of Scientific and Industrial Research (CSIR)	Poona	Dr. V. Jagennathan	G	BFY
	IJIRA, Indian Jute Industries Research Association	17 Taratola Road, Calcutta 53	Dr. J. P. Bhattacharyya	I	F
	Department of Microbiology, Bose Institute	93/1 Acharya Prafulla Chandra Road, Calcutta 9	Prof. P. Nandi	P	BFYAcVb

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Nation	Collection and Parent Organization	Address	[illegible]	[illegible]	[illegible]
India (cont.)	Haffkine Institute	Parel, Bombay-12	[illegible]	[illegible]	[illegible]
	Fermentation Technology Laboratory, Indian Institute of Science	Bangalore-3	[illegible]	[illegible]	[illegible]
	Division of Mycology and Plant Pathology, Indian Agricultural Research Institute	New Delhi-12	Dr. R. S. [illegible]	[illegible]	[illegible]
	BSM Culture Collection, Botany Department, University of Allahabad	Allahabad	Dr. B. S. [illegible]	[illegible]	[illegible]
Indonesia	Culture Collection, Treub Laboratory, National Biological Institute, The Botanical Garden	Bogor	Dr. S. [illegible]	[illegible]	[illegible]
Iran	"Razi Culture Collection," State Razi Institute-Hessarak, Ministry of Agriculture	P.O. Box 656, Karadj Tehran	Dr. F. [illegible]	[illegible]	[illegible]

[illegible]

Table 1.--Collections Containing Industrially Useful [illegible]

Nation	Collection and Parent Organization	Address	Dept. Head or Curator	Sponsor[a]	[illegible][a]
Ireland	Johnstown Castle Collection, Soil Laboratory, The Agricultural Institute	Wexford	C. L. Masterson	[illegible]	[illegible]
	Guinness (Dublin) Culture Collection, A. Guinness, Son and Co. (Dublin) Ltd.	St. James's Gate, Dublin, 8	C. E. Dalgliesh	I	[illegible]
	Department of Industrial Microbiology, University College	Ardmore, Stillorgan Rd., Dublin, 4	Prof. Dr. E. Küster	U	[illegible]
Italy	Instituto di Patologia Vegetale, Università Cattolica del S. Cuore	S. Lazzaro, Piacenza	Prof. G. Fogliani	[illegible]	[illegible]
	Instituto di Patologia Vegetale, University Milano	Milano	Dr. E. Baldacci	G	[illegible]
	Centro di Studio dei Microorganismi Autotrofi, Instituto di Microbiologia Agraria e Tecnica, Università di Firenze	Via Barbacane 3, Firenze	Prof. G. Florensano	U	[illegible]
	Instituto Microbiologia Agraria e Tecnica, Università di Napoli	Piazzale della Sapienza, Portici	Prof. Dr. M. Formisano	U	[illegible]
	Collezione dei Lieviti Vinari, Instituto di Microbiologia Agraria e Tecnica, University of Perugia	Bg. XX Guigno, 06100 Perugia	Prof. T. Castelli	[illegible]	[illegible]

Table 1.--Collections Containing Industrially [illegible]

Nation	Collection and Parent Organization	Address	Dept. Head or Curator	Sponsor[2]	Contents[3]
Italy (cont.)	Instituto di Patologia Vegetale e di Microbiologia Generale e Agraria, Università di Pisa	Via J. Michele 6, Pisa	Prof. O. Verona	U	FY
	Collezione Microbica Agraria, Marina e Industriale (COMAMI), Instituto Microbiologia Agraria e Tecnica	E. de Nicola, Sassari	Prof. A. Capriotti	U	BFY
	FI, Research Institute-Farmitalia	Via de' Gracchi 35, Milano	Prof. A. Buogo	I	BFYVa Vp?
	Lepetit S.p.A.	Via Durando, 38, Milano 20159	Prof. P. Sensi	I	BFYAcP
Jamaica	Department of Microbiology University of West Indies	Mona St., Kingston 7	Prof. L. S. Grant	U	BVa
Japan	HUT, Department of Fermentation Technology, Faculty of Engineering, Hiroshima University	Senda-machi 3, Hiroshima	T. Nehira	G	BFY
	Institute of Applied Microbiology, University of Tokyo	Bunkyo-ku, Tokyo	Prof. Dr. H. Iizuka	U	BFYAlAc

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Table 1.-- Collections Containing Industrially Useful Microorganisms—continued

Nation	Collection and Parent Organization	Address	Dept. Head or Curator	Sponsorship	Contents
Japan (cont.)	The Research Institute of Fermentation, Yamanashi University	1 Kitashin-machi, Kofu	Prof. Dr. [illegible] Yokotsuka	G	BFY
	National Institute of Agricultural Sciences	Nishigahara, Kita-ku, Tokyo	S. Tanizawa	G	BFAc
	Department of Fermentation Technology, Faculty of Engineering, Osaka University	Higashinoda, Miyakozi-ma-ku, Osaka	Dr. G. Terui	U	BFY
	ATL-Culture Collection, Department of Agricultural Chemistry, Faculty of Agriculture, University of Tokyo	Yayoi-1-1, Bunkyo-ku, Tokyo	Prof. K. Arima	U	BFDAc
	Culture Collection, Institute for Fermentation (IFO)	[illegible]-54, Juso-nishinocho, [illegible]-ku, Osaka	Dr. T. Hasegawa	P	BFYD
	Nagao Institute	96, [illegible]-machi, Setagaya-ku, Tokyo	K. Nagao	P	FDL
Korea	Korean Federation of Culture Collections of Microorganisms, Ministry of Science and Technology	2nd Chongno, Seoul	Prof. Dr. Lee Zoo Shik	XII	BFDAl FPshp

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Nation	Collection and parent institution	Address	Curator/Contact person	Sponsor[a]	Contents[b]
Malaysia	Pathology Division, Rubber Research Institute of Malaya	P.O. Box 150, Kuala Lumpur	Dr. A. Seeman	I	BFY
Netherlands	Centraalbureau voor Schimmelcultures (CBS)	Baarn	Dr. J. A. von Arx	GI	FXAc
	Culture Collection, Laboratory of Microbiology, Technical University	Julianalaan 67A, Delft	Prof. Dr. T. O. Kluyver	U	B
New Zealand	Culture Collection, Plant Diseases Division, Department of Scientific and Industrial Research	Private Bag, Auckland	Dr. D. W. Chamberlain	G	BFYVp
Nigeria	Fungus Culture Collection, University of Lagos	University Road, Yaba, Lagos	Prof. S. H. Z. Naqvi	U	FY
Norway	Department of Microbiology, Agricultural College of Norway	Box 40, Vollbekk	Mrs. M. Lindeberg	U	F
	Norges Tekniske Høgskoles Collection (NTHC), Department of Biochemistry, Technical University of Norway	Trondheim NTH	Dr. K. Dimhjellen	U	BFY
Philippines	Rhizobium japonicum and Rhizobium spp. from other tropical legumes, College of Agriculture, University of the Philippines	College, Laguna	M. E. Raymundo	U	B

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Table 1.--Collections Containing Industrially Useful Microorganisms--continued

Nation	Collection and Parent Organization	Address	Dept. Head or Curator	Sponsors[O]	Contents[M]
Poland	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	G	B
	Special Centre of Industrial Cultures, Department of Industrial Microbiology, Technical University	Wólczańska 171/173, Lódz	Prof. dr. J. Jakubowska	U	BFY
	Laboratoire des Collections de Cultures, Chaire de Microbiologie technique	Kortowo bl. 43, Olsztyn	Prof. H. Karnicka	U	BFY
	Culture Collection of Industrial Microorganisms, Institute of the Fermentation Industry	Rakowiecka 36, Warsaw	Dr. J. N. Z. T. Golębiewski	I	BFY
Portugal	Coleccao de Culturas de Fungos, Microbiologia, Faculdade de Ciencias	Lisbon	Prof. J. Pinto-Lopes	G	F
	Instituto Gulbenkian Ciencia, Laboratorio de Microbiologia, Centro de Biologia	Rua da Quinta Grande, Oeiras	Dr. N. Van Uden	P	Y

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Table 1.--Collections Containing Industrially Useful Microorganisms--continued

Nation	Collection and Parent Organization	Address	Dept. Head or Curator	Sponsors[o]	Contents[x]
Rhodesia	Grasslands Rhizobium Collection, Grasslands Research Station	P.B. 701, Marandellas	W. P. L. Sandmann	G	B
	University College of Rhodesia	Mount Pleasant, Salisbury	Prof. H. Wild	U	BFY
Romania	Microbiological Laboratory, Biosynthesis Department, Institutal de Cercetari chimico-farmaceutice	112, Soseaua Vitan, Bucharest	Dr. G. Nicolae	G	BF
South Africa	Council for Scientific and Industrial Research	Pretoria	Dr. J. van der Walt	G	YFB
Switzerland	Botanische Sammlungen der Eidg. Technischen Hochschule, Institut für spezielle Botanik	Universitätstrasse 2, 8006 Zürich	Dr. E. Müller	G	F
	Mikrobiologisches Institut der Eidg. Technischen Hochschule, Swiss Federal Institute of Technology	Universitätstrasse 2, CH 8032 Zürich	Prof. Dr. L. Ettlinger	U	BFYAc
	Culture Collection of Microorganisms, J. R. Geigy A.G.	Schwarzwaldallee, CH 4021, Basel	Dr. W. A. Vischer	I	BFYP

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Table 1.--Collections Containing Industrially Useful Microorganisms--continued

Nation	Collection and Parent Organization	Address	Dept. Head or Curator	Sponsors[c]	Contents[d]
U.A.R.	Agricultural Microbiology Division, Ministry of Agriculture	Soils Department Building, University Street, Orman, Giza	M. Abou El-Fadl	G	BFY
	Microbic Collection, College of Agriculture, Ain Shams University	Shoubra El-Khaima	Dr. S. M. Taha	U	BFYAl
United Kingdom	The Commonwealth Mycological Institute (CMI), The Ministry of Technology	Ferry Lane, Kew, Surrey, England	Dr. A. H. S. Onions	G	F
	National Collection of Dairy Organisms, National Institute for Research in Dairying	Shinfield, Reading, Berkshire, England	Dr. L. A. Mabbitt	G	BVb
	Rothamsted Inoculum Collection, Soil Microbiology Department, Rothamsted Experimental Station	Harpenden, Hertfordshire, England	Dr. P. S. Nutman	G	B
	British National Collection of Yeast Cultures, Brewing Industry Research Foundation	Nutfield, Surrey, England	Dr. A. H. Cook	I	Y
	Glaxo Laboratories Ltd.	Greenford, Middlesex, England	Dr. P. Muggleton	I	BFYP

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Table 1.-- Collections Containing Industrially Useful Microorganisms--continued

Nation	Collection and Parent Organization	Address	Dept. Head or Curator	Sponsors[o]	Contents[*]
United Kingdom (cont.)	Culture Collection of Algae and Protozoa, Cambridge University	Cambridge, England	Dr. E. A. George	U	AlP
	Agricultural Division, Imperial Chemical Industries Ltd.	Jealotts Hill Research Station, Bracknell, Berkshire, England	Dr. M. J. Geoghegan	I	BFY
	Akers Culture Collection, Pharmaceuticals Division, Imperial Chemical Industries Ltd.	P.O. Box 25, Alderley Park, Macclesfield, Cheshire, England	Dr. D. Broadbent	I	BFY
	The Wellcome Bacterial Collection, Wellcome Foundation Ltd.	Langley Court, Beckenham, Kent, England	Dr. M. Sterne	P	B
	National Collection of Industrial Bacteria, Ministry of Technology	P.O. Box 31, 135 Abbey Road, Aberdeen, Scotland	Dr. J. M. Shewan	G	BVb
	National Collection of Marine Bacteria, Ministry of Technology	P. O. Box 31, 135 Abbey Road, Aberdeen, Scotland	Dr. J. Lovern	G	BY
United States of America	American Type Culture Collection (ATCC)	12301 Parklawn Drive, Rockville, Maryland 20852	Dr. W. A. Clark	GIP	BFYAc AlPVabC

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Table 1.--Collections Containing Industrially Useful Microorganisms--continued

Nation	Collection and Parent Organization	Address	Dept. Head or Curator	Sponsors[o]	Contents[x]
United States of America (cont.)	ARS Culture Collection, Northern Regional Research Laboratory, Agricultural Research Service, U.S. Dept. of Agriculture	1815 N. University St., Peoria, Illinois 61604	Dr. T. G. Pridham	G	BFYAc
	Culture Collection of the U.S. Army Materiel Command, U.S. Army Natick Laboratories	Natick, Massachusetts	Dr. E. G. Simmons	G	BFY
	The Culture Collection of Algae, Department of Botany, Indiana University	Bloomington, Indiana	Dr. R. C. Starr	U	Al
	IMRU Collection, Institute of Microbiology, Rutgers, The State University	New Brunswick, New Jersey 08903	Dr. R. E. Gordon	U	BAc
	Actinoplanaceae (Actinomycetales), Botanical Department, University of North Carolina	Coker Hall, Chapel Hill, North Carolina	Dr. J. N. Couch	GUIP	AcVb
	Department of Bacteriology, College of Agricultural and Life Sciences, University of Wisconsin	Madison, Wisconsin 53706	Dr. K. B. Raper	U	F

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Table 1.--Collections Containing Industrially Useful Microorganisms--continued

Nation	Collection and Parent Organization	Address	Dept. Head or Curator	Sponsors[o]	Contents[x]
United States of America (cont.)	Department of Plant Pathology and Botany, Agricultural Experiment Station	P.O. Box H, Río Piedras, Puerto Rico 00928	Dr. J. E. Pérez	U	B
	Abbott Laboratories	14th Sheridan Road, North Chicago, Illinois 60064	Dr. J. C. Holper	I	BVa
	Affiliated Laboratories Stock Culture Collection, Affiliated Laboratories Corporation	Lincoln Road, White Hall, Illinois	Dr. E. Baldwin	I	BVa
	Bristol Laboratories	P.O.Box 657, Syracuse, New York 13201	Dr. K. E. Crook, Jr.	I	BF
	Fermentation Products, Eli Lilly and Company	307 East McCarty St., Indianapolis, Indiana	Dr. G. E. Mallett	I	BFAc
	Grain Processing Corp.	1600 Oregon Street, Muscatine, Iowa 52761	Mr. C. Smith	I	BFYA1
	IMC Culture Collection, Growth of Sciences Center, International Minerals and Chemical Corporation	P.O. Box 192, Libertyville, Illinois 60048	Dr. M. H. Rogoff	I	BFVb
	Lederle Microbiology Research Collection, Lederle Laboratories Division, American Cyanamid Company	No. Middletown Road, Pearl River, New York	Dr. E. J. Backus and Dr. H. Tresner	I	BFYA1Ac

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Table 1.--Collections Containing Industrially Useful Microorganisms--continued

Nation	Collection and Parent Organization	Address	Dept. Head or Curator	Sponsors[o]	Contents[*]
United States of America (cont.)	Merck Sharp and Dohme Research Laboratories Culture Collection, Merck and Company, Inc.	Rahway, New Jersey	Dr. T. H. Stoudt	I	BFYA1Ac
	Chas. Pfizer and Company	Groton, Connecticut	Dr. J. Routien	I	BFAC
	Rohm and Haas Stock Culture Collection, Rohm and Haas Company	P.O. Box 219, Bristol, Pennsylvania	Dr. H. H. Kuehn	I	BFY
	Schering Corporation	86 Orange Street, Bloomfield, New Jersey	Dr. M. J. Weinstein	I	BFVa
	Bioanalytical Culture Collection, Smith Kline and French Laboratories	1500 Spring Garden Street, Philadelphia, Pennsylvania 19101	R. J. Ferlauto	I	BFYVa
	Culture Collection, Squibb Institute for Medical Research, E. R. Squibb and Sons, Inc.	Georges Road, New Brunswick, New Jersey	Mrs. F. Arnow	I	BFYAc
	Wyeth Laboratories Collection, Wyeth Laboratories	P.O. Box 8299, Philadelphia, Pennsylvania 19101	Dr. G. H. Warren	I	BFY
	The Upjohn Stock Culture Collection, The Upjohn Company	301 Henrietta Street, Kalamazoo, Michigan 49001	Dr. G. B. Whitfield, Jr.	I	BFYA1AcP

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Table [illegible] Industrially Useful Microorganisms--continued

Nation	Collection and Parent Organization	Address	Dept. Head or Curator	Sponsors[o]	Contents[*]
United States of America (cont.)	Sanraz Services International Div.	240 Washington Street, Mt. Vernon, New York 10551	B. K. Blenkinship	P	BFY
U.S.S.R.	All-Union Collection of Type Cultures, Dept. of Type Cultures, Inst. of Microbiology, U.S.S.R. Acad. Sci.	Pretchistenskaya Str., 7a, Moscow, B-133	Prof. V. Kudriavtsev	G	BFYAc
	Institute of New Antibiotics	Moscow	Dr. G. F. Gauze	G	Ac
Venezuela	Centro de Microscopía Electrónica, Facultad Central, Universidad de Los Andes	Apartado 163, Calle Vargas, Merida	Dr. J. A. Serrano	UP	BF

[o] G = Government, U = University, I = Industry, P = Private.
[*] B = Bacteria, F = Fungi, Y = Yeasts, Ac = Actinomycetes, Al = Algae, Va = Animal Viruses, Vb = Bacteriophages, Vp = Plant Viruses, P = Protozoa, C = Cell lines.

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. For example, at the Northern Regional Research Laboratory the ARS Culture Collection is part 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 give guidance as to what microorganisms 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 microorganisms, 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 microorganisms 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 microorganisms. 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 partially developed 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. Ideally, this work should be in the area of chemotaxonomy. 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 advise, cultures, and information. This point is an important one that we have stressed before (Hesseltine et al., 1968).

8. College-trained personnel in collections 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 decision making.

In turn, the members of a culture collection should be informed of developments in associated fermentation research areas and in problems in a fermentation plant. Currently, all reports and papers from our Fermentation Laboratory are circulated to all the other senior scientists. Also, 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 (general, medical, reference, or agricultural) should be sponsored and where they should be located are under study by the Section on Culture Collections of the International Association of Microbiological Societies. Our concern, which overlaps theirs, is the narrower. Where should collections of industrial microorganisms 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." Because of the scarcity of scientists trained in culture collection science in developing countries, it seems unrealistic to support the idea of national

collections of any sort. It seems to us that the source of cultures of microorganisms should be limited to a relative 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 microorganisms are often located too far from the emerging nations to furnish the support assistance that is needed in handling the cultures. Also, the cost of the cultures is prohibitive because hard currency is difficult to come by in many of the developing areas. These are our conclusions based upon practical experience and on frank discussions with a number of knowledgeable persons from various countries.

We think it would be well 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 U.S., USSR, U.K., Netherlands, Japan, Canada, and South Africa would serve many areas. The excellent Dutch collection would probably fill the needs of Central Europe. The USSR All-Union Collection could adequately meet the requirements of Poland, Romania, and Bulgaria. Well- staffed and financed collections need to be established in (1) South America (perhaps in Brazil or Argentina), (2) India, (3) Central Africa, and (4) the Middle East and North Africa. We do not mean to imply

that none exist in these regions but rather to suggest that they need to be enlarged in size, better equipped, and more adequately staffed. We believe that if these proposed 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 Department 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 microorganisms 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 product destined for human consumption?" In this instance, the developmental work should be done in some central research laboratory. To ensure good reliable inoculum, this 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 go to completion in spite of contaminants. Thus, 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 who makes, packages, and distributes the inoculum.

Procedures for Isolation and Selection of Microorganisms from Nature

Innumerable techniques for isolation of microorganisms 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 family or fungus 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 to give a uniform style. Eventually, the whole will be published as a source book of information.

Techniques for isolating microorganisms, which can grow free of other living things, can be classified into several general categories. Microorganisms are here interpreted in a broad sense 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 microorganisms into a selective medium followed by incubation of the culture under conditions of temperature, aeration, etc., that further contribute to favoring the growth of desired forms. A small

amount from the initial culture is transferred to a second that is set up in the same manner as the first, and this procedure is continued until the flora desired predominates and can be isolated in pure culture. For example, if you wanted a strain of Clostridium that would produce acetone on corn meal, you would make a sterile corn mash and inoculate it 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 and certain of these giving the appearance of vigorous anaerobic growth and having a solvent odor would be used to inoculate new flasks which, in turn, would be incubated until a vigorous solvent-producing culture emerged. Finally, the selected Clostridium would be plated out on a corn meal medium under anaerobic conditions and many strains would be isolated as pure cultures. Each would then be tested in the proposed industrial fermentation using corn meal as a basic ingredient. Assays of the solvent yields would be determined and the best strain selected for evaluation in the scaleup of the fermentation.

Sometimes, the isolation of pure cultures is not even necessary. For example, in the fermentation of cucumbers for the manufacture of pickles, conditions are established in the fermentation tanks such that part of the natural microbial flora on the cucumbers is favored. Certain bacteria grow at 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 pickle making 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 a 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, in the U.S. for the last 12 years a culture of Pediococcus has been used to inoculate commercial sausage. According to information supplied by the manufacturer, a search was made of cultures from 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 in spite of competing bacteria.

However, not always can these various approaches 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 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 microorganism 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 microorganism. 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 sterile techniques, some or all of a colony can be picked off the substrate and a pure culture established. 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, moistened in sterile agar, and the tip carefully brought into contact with the fruiting head. The adhering spores may then be transferred to suitable nutrient agar and a pure

cultures 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 for the isolation of 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 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 encounteres 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, a second antifungi antibiotic, actidione, can be incorporated into nutrient media to inhibit both yeasts and fungi without affecting the growth of 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 Microorganisms Used for Production of Fermentation Products

Since this topic is reviewed in great detail in various texts on fermentation (Peppler, 1967, Prescott & Dunn, 1959; and Smith, 1969), 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 quite 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 U.S. Other countries with a shortage of oil, but with enormous amounts of molasses, still make ethanol by fermentation.

Table 2.--Classification of Microorganisms Used for Commercial Production of Various Materials

Product	Genus	Type of microorganism*
Foods		
Ang-kak	Monascus	M
Bacterial starters (fermented dairy products and sausage)	Streptococcus	B
	Leuconostoc	B
	Lactobacillus	B
	Propionibacterium	B
	Pediococcus	B
Bantu beer	Saccharomyces	Y
	Lactobacillus	B
Blue cheese flavor	Penicillium	M
Bread (bakers' yeast)	Saccharomyces	Y
Cheese and fermented dairy products	Streptococcus	B
	Lactobacillus	B
	Penicillium	M
	Propionibacterium	B
Chinese yeast	Chlamydomucor	M
	Rhizopus	M
	Hansenula	Y
	Saccharomyces	Y
Fermented fish	Halophilic bacteria	B
	Aspergillus	M
Hamanatto	Aspergillus	M
Koji	Aspergillus	M
	Rhizopus	M
Nagou	Lactobacillus	B
Miso	Aspergillus	M
	Saccharomyces	Y
Nata	Acetobacter	B
Natto	Bacillus	B

--continued

Table 2.--Classification of Microorganisms Used for the Commercial Production of Various Materials--continued

Product	Genus	Type of microorganism[x]
Ontjom	Neurospora	M
Pickles and sauerkraut	Lactobacillus	B
	Streptococcus	B
Shoyu	Torulopsis	Y
	Saccharomyces	Y
	Aspergillus	M
Sufu	Actinomucor	M
	Mucor	M
Tempeh	Rhizopus	M
Yeast	Candida	Y
	Saccharomyces	Y
Beverages		
Beer	Saccharomyces	Y
Distilled spirits	Saccharomyces	Y
Sake	Saccharomyces	Y
	Aspergillus	M
Wine	Saccharomyces	Y
Protein	Torula	Y
	Saccharomyces	Y
	Chlorella	Algae
Amino Acids		
Aspartic acid	Pseudomonas	B
Glutamic acid (monosodium glutamate)	Bacillus	B
	Micrococcus	B
	Brevibacterium	B
Isoleucine	Pseudomonas	B

--continued

Table 2.--Classification of Microorganisms Used for the Commercial Production of Various Materials--continued

Product	Genus	Type of microorganism*
Lysine	Micrococcus	B
Phenylalanine	Micrococcus	B
Threonine	Bacillus	B
Valine	Micrococcus	B
Industrial Solvents		
Acetone	Clostridium	B
Butanol	Clostridium	B
2,3-Butanediol	Bacillus	B
	Aerobacter	B
Dihydroxy acetone	Acetobacter	B
Ethanol	Saccharomyces	Y
	Clostridium	B
Glycerol	Torulopsis	Y
	Saccharomyces	Y
Antibiotics	Penicillium	M
	Bacillus	B
	Streptomyces	A
	Cephalosporium	M
	Fusidium	M
	Aspergillus	M
	Micromonospora	A
	Nocardia	A
Organic Acids		
Acetic	Acetobacter	B
Citric	Aspergillus	M
Erythorbic	Penicillium	M
Fumaric	Rhizopus	M
Gluconic	Aspergillus	M

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Table 2.--Classification of Microorganisms Used for the Commercial Production of Various Materials--continued

Product	Genus	Type of microorganism[x]
Organic Acids--Cont'd.		
Itaconic	Aspergillus	M
Itatartaric	Aspergillus	M
2-Ketogluconic	Pseudomonas	B
5-Ketogluconic	Acetobacter	B
α-Ketoglutaric	Pseudomonas	B
Kojic	Aspergillus	M
Lactic	Lactobacillus	B
Vitamins		
Ascorbic acid (vitamin C) (in part)	Acetobacter	B
β-Cerotene	Blakeslea	M
B_{12}	Bacillus	B
	Propionibacterium	B
	Streptomyces	A
Riboflavin	Ashbya	Y
	Eremothecium	Y
Enzymes		
α-Amylase	Aspergillus	M
	Rhizopus	M
	Bacillus	B
	Endomycopsis	Y
Amyloglucosidase	Aspergillus	M
	Rhizopus	M
	Endomycopsis	Y
Asparaginase	Escherichia	B
	Erwinia	B

--continued

Table 2.--Classification of Microorganisms Used for the Commercial Production of Various Materials--continued

Product	Genus	Type of microorganism*
Enzymes--Cont'd.		
Catalase	Aspergillus	M
	Penicillium	M
Cellulases	Aspergillus	M
	Trichoderma	M
	Myrothecium	M
Dextranase	Penicillium	M
Glucose isomerase	Streptomyces	A
Glucose oxidase	Aspergillus	M
	Penicillium	M
Glucosidases	Aspergillus	M
Hemicellulase	(Unrevealed)	?
Invertase	Saccharomyces	Y
Lactases	Saccharomyces	Y
Laundry enzymes (alkaline proteases)	Bacillus	B
Lipases	Candida	Y
	Aspergillus	M
	Mucor	M
Milk-clotting enzymes	Mucor	M
	Endothia	M
Pectinase	Aspergillus	M
Penicillase	Bacillus	B
Penicillin amidase	Many microorganisms	
Pentosanases		

--continued

Table 2.--Classification of Microorganisms Used for the Commercial Production of Various Materials--continued

Product	Genus	Type of microorganism*
Protease	Streptomyces	A
	Aspergillus	M
	Mucor	M
	Conidiobolus	M
Streptokinase	Streptococcus	A
Miscellaneous Products		
Alkaloids	Claviceps	M
Bioinsecticides	Bacillus	B
Dextran	Leuconostoc	B
Gibberellin	Gibberella (Fusarium)	M
Inosinic acid	Bacillus	B
	Yeast	Y
Nucleotides	Brevibacterium	B
	Torula	Y
Rhizobium culture	Rhizobium	B
Silage	Lactobacillus	B
Sorbose	Acetobacter	B
Steroids transformations	Rhizopus	M
	Streptomyces	A
	Corynebacterium	B
	Aspergillus	M
	Curvularia	M

* A = Actinomycetes
M = Mold
B = Bacteria
Y = Yeast

Problems in Maintaining Stable Industrial Cultures

From years of experience in handling cultures of all kinds, we believe certain steps should be taken as soon as a microorganism is isolated in pure culture to ensure that 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 of other microorganisms; (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 microorganism 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 dilute 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 microorganisms, 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 microorganisms fail to survive this process and a different method of preservation must be used. 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 the lyophil preparation or from one put up by another method such as freezing in liquid nitrogen, records should be kept of the proper medium for growth and sporulation, and any other peculiar requirements such as temperature of incubation, length of incubation, etc. For example, the mold Blakeslea trispora will sporulate rapidly (3-4 days at 25° C.) but often in a matter of 10 days the spores will germinate in place and lyophilization will be a complete failure. The process works beautifully when the spores are processed when they have just reached maturity.

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

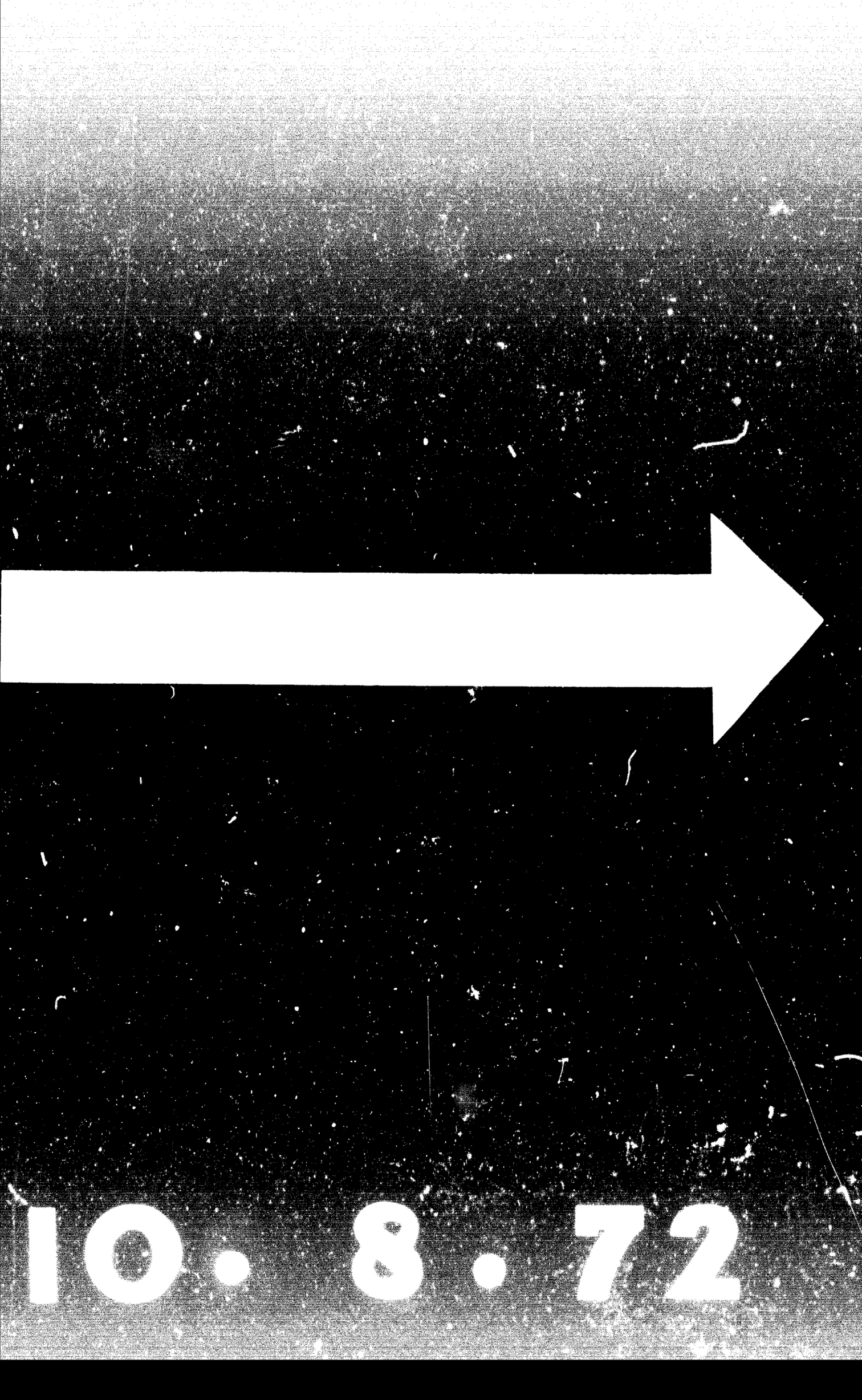
6. At the time of lyophilization, the culture should be examined in the appropriate way to determine its identity. Sometimes this examination may lead to species and variety identification but other times only to the approximate species and genus. The records should certainly show its approximate identity 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 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 now onto cards 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 is 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 on industrial strains, which 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,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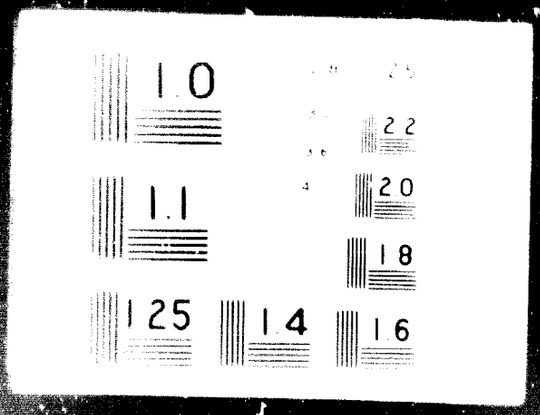

then the nonuniform culture is lyophilized; but also, the various forms are isolated separately, and each type is lyophilized individually. Sometimes a heterogeneous culture cannot be separated into its components. The philosophy of lyophilizing the sectoring culture is to try to preserve all the component 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 colony 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 consist 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 techniques of preservation require too much space to describe them here.

10. If a culture is received that has degenerated, certain steps need to be carried out to obtain a better culture before it is put away in the collection. Perhaps the degeneracy is in sporulating ability; often a series of dilution plates will give some colonies that grow more vigorously or sporulate more heavily in a natural manner. In some, 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, some cultures, even though they grow and fruit on a synthetic medium, 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 that we list a number of principles which we consider important for the cultivation of microorganisms 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 microorganism, as it occurs in nature, almost always is 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 shortened. Worse yet, the population imbalance mentioned in No. 1 might alter or destroy the usefulness of the culture. Appropriate media for use with a variety of microorganisms are given in a paper entitled "Maintenance of Cultures of Industrially Important Microorganisms" (Haynes et al., 1955).

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. In any case, they seem not to harm the microorganisms.

The pH of the medium is also important. Generally, bacteria are grown in neutral media, molds in unadjusted media that have a pH between 6 and 7, and yeasts are grown 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 microorganisms. We have encountered many cultures reputedly pure but which carried a second microorganism 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, bacterial cells that are dormant are removed with it and then when placed on a medium free of the inhibitor grow again. A

bacterial culture producing a thin growth may be obscured by the more luxuriant growth of the 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 is opened, moist air enters and upon cooling condenses upon the labels and cotton plugs. Some penicillia can grow on the moist cotton and the labels at 4 to 5° C. in a matter of 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.

2. Infestation by mites. Certain species of mites feed on fungus spores. These mites are extremely small and can barely 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 which hatch in a few days and the young will migrate into new cultures. Thus, in a relatively short time, hundreds of cultures are contaminated and a whole collection may be lost. If the

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 precautions should be taken with respect to cultures received from outside the culture collection. The further precaution of poisoning all the cotton plugs of stock cultures should be observed.

3. 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 of them. At one time, yeasts and mold species were believed never to be infected with phage, but this belief is now known not to be true.

4. Natural selection and mutation. Changes in the genetic population in a culture will occur in all microorganisms. It is our personal belief 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 carried 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 of the 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.

5. Untrained staff. Probably as serious as any cause of culture failure is the handling of cultures by untrained persons. Often media is improperly sterilized by people who do not comprehend that some complex media require more heat than others and that the larger the amount of media, the more sterilization is needed. Sometimes inadequately trained people just do not know how to transfer cultures so as 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 microorganisms 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 Microorganisms

The physical conditions that affect the growth and longevity of microorganisms 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 <u>in transit</u>.

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 climes without loss of life or change in character. (See Fennell, 1960, for a review of methods.)

Some microorganisms 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 typical after 5 years of preservation (Hwang, 1966). Ultra-low-temperature frozen cultures are sealed in glass vials or ampules so 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 the method is less convenient than lyophilization, it is becoming more and 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 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°-10° C.) but sometimes is at room or some other 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 sealing the cultures with rubber stoppers, corks, or by impregnating the cotton plugs with paraffin. The rate of growth is slowed by refrigerating the cultures, thus minimizing changes in pH, and oxidation/reduction potential, and reducing the danger that one or more cells mutated by stray radiations (cosmic rays) will gain predominance in the population.

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 microorganisms 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, we, at the Northern Regional Research Laboratory, have 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 September 1, 1969.

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 which 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 U.S. 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, in consequence of a ruling of the Patent Office, some depositors have used initially, or 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.

The ARS Culture Collection understands that the ruling referred to above is being appealed, but a final decision has not been reached. It is suggested that you seek advice from your attorney as to which type of statement you should use. Either one of these statements will be written

depending upon your wishes. 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. It is not necessary, of course, to provide a precise identification, but the microbiologist concerned should at least state to what genus the microorganism belongs. Also, if special media are required for its maintenance, the curators need to know this. Ordinarily, one or two agar slant cultures and/or one lyophilized preparation 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 preferably 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 5°-6° C. Bona fide letter requests for the culture would be shipped from this stock.

2. One lyophilised preparation, clearly labeled with the depositor's original strain designation. On receipt, the microorganism is cultivated on appropriate agar media and thirty 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 microorganism growing on an appropriate medium. Sufficient material is prepared by our depositors to make thirty 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 microorganism 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 [illegible] patent issues wherein the particular microorganism is involved.

[illegible]

Living cultures of microorganisms 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 collections, 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 microorganisms, primarily for their own research. In the course of time the large collections have learned how to solve the problems associated with packaging and shipping living cultures so that they arrive at their destinations whole, alive, and uncontaminated.

Except for microorganisms used in the pharmaceutical industry to produce various antibiotics, very few of the microbes used in the food, feed, and fermentation industries and included in the [illegible] 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 etiological agents and

[illegible]." These are discussed in a brochure published in 1966 by the American Type Culture Collection. In it four Departments of the U.S. [illegible]ment--Agriculture; Health, Education, and Welfare; Commerce; and [illegible]y--are listed as concerned with regulating potentially dangerous [illegible]anisms. Two Divisions of the U.S. Department of Agriculture are [illegible]. They are charged with responsibility to see that "no organisms [illegible]ay introduce or disseminate any contagious or infectious diseases [illegible]mals, including poultry)...shall be imported into the United States [illegible]ported from one State...to another State without a permit issued [illegible]ecretary and in accordance with the terms thereof." The Plant [illegible] Division requires a permit for the movement of any plant pest [illegible] through the United States or any of its territories and possessions. [illegible]al cultures are included in the definition of the term "plant pest." [illegible] U.S. Public Health Service of the Department of Health, Education, [illegible]fare through its Division of Foreign Quarantine enforces regulations [illegible]nt the introduction and spread of communicable disease from [illegible]ntries into the United States..., or from one state to another." [illegible] under a law which states, "A person shall not import into any [illegible]der the control of the United States, nor distribute after [illegible] any etiologic agent [illegible] of human disease...unless accompanied [illegible]it issued by the Surgeon General."

[illegible] primary concerns of the Department of Commerce, as it relates to [illegible]tion of living cultures, are safeguarding our national security [illegible]rying our foreign policies. It issues a general license to U.S. [illegible]s, which authorizes the export of living cultures to all friendly [illegible]tions. Special "validated" licenses are required for the export of [illegible] cultures to a few nations.

Shipments of living cultures are free of customs duty. The only reasons that the Treasury Department, through its Bureau of Customs, is involved are, first, to determine if the cultures are admissible to the United States and, second, to refer them to other governmental agencies for examination, permits, and release.

Some U.S. regulations specify how pathogenic microorganisms shall be packaged. The intent of these instructions is the same as those governing permits and licenses; i.e., to ensure that etiological agents do not escape and endanger the 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 U.S. 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 (1968). Another is the Catalogue of the Culture Collection of the Commonwealth Mycological Institute (1968) and the other is the List of Cultures of the Centraalbureau voor Schimmelcultures (1968).

Inasmuch as no mention is made in catalogs of other collections (Argentina, Czechoslovakia, England, India, Indonesia, Japan, Germany, Netherlands, Scotland, USSR) 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 themselves only with safe packaging and labeling of cultures destined for most nations.

The problems to be overcome in packaging are the selection of a sturdy mailing tube or carton that will remain intact in spite of rough handling and possible exposure to moisture, and will protect the enclosed cultures from breakage. Additionally, the container must be so made that, if, in spite of all precautions, breakage does occur, the released microorganisms 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.

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