5
Bacterial Culture Collections: Their Importance to Biotechnology and Microbiology

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Introduction

Micro-organisms are indispensable for the continued existence of plant and animal life. Louis Pasteur was the first to recognize the link between micro-organisms and the process now regarded as applied microbiology and biotechnology. He observed that micro-organisms had a specific role in human and animal disease and in the production and spoilage of food and drinks. Understanding and control of micro-organisms has developed over the years and the practice of biotechnology in which micro-organisms are employed as biological 'tools' is rapidly increasing and will no doubt continue to do so.

The use of biological fertilizers, growth stimulators and bioinsecticides promises to increase production capacity and ultimately to convert barren lands to fertile areas. In medicine, novel pharmaceutical products, blood

Abbreviations: ATCC, American Type Culture Collection; CAB, Commonwealth Agricultural Bureaux; CBS, Centraalbureau voor Schimmelcultures; CEC, Commission of the European Communities; CMI, Commonwealth Mycological Institute; CODATA, Committee on Data for Science and Technology; DSM, Deutsche Sammlung von Mikroorganismen; ECCO, European Culture Collection Organization; HES, hydroxyethyl starch; IAMS, International Association of Microbiological Societies; IBP, International Biological Programme; ICCC, International Culture Collection Conferences; ICRO, International Cell Research Organization; IDA, International Depository Authority; IHEM, Institute of Hygiene and Epidemiology; IUMS, International Union of Microbiological Societies; JFCC, Japanese Federation of Culture Collections; LMG, Laboratorium voor Microbiologie en Microbiële Genetica, Gent; LN_2, liquid nitrogen; MINE, Microbial Information Network for Europe; MIRCEN, Microbial Resource Centre; MSDN, Microbial Strain Data Network; MUCL, Mycothèque de l'Université Catholique de Louvain; NCTC, National Collection of Type Cultures; PVC, Polyvinyl chloride; PVP, Polyvinyl pyrolidone; UKFCC, United Kingdom Federation of Culture Collections; UNEP, United Nations Environmental Programme; UNESCO, United Nations Educational, Scientific and Cultural Organization; UPU, Universal Postal Union; USFCC, United States Federation of Culture Collections; WDC, World Data Centre; WFCC, World Federation of Culture Collections; WIPO, World Intellectual Property Organization.
NB: Acronyms of individual Culture Collections are listed in Appendix A, pages 168–191.

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proteins, hormones, interferon, cell growth stimulators, insulin etc. are already being produced and new therapeutic products are being actively sought (Blanch, Drew and Wang, 1985a).

It is possible to produce gases, power-alcohols, petroleum substitutes and other renewable energy sources using microbial agents. Biotechnologically based recycling processes often offer cheap and effective means of effluent disposal (Robinson and Howell, 1985b). In addition micro-organisms are required for strain diagnosis of pathogens in human, plant and animal health and in numerous taxonomic and ecological studies. All such applications in microbiology and biotechnology depend upon a supply of authentic and viable cultures of micro-organisms. Research and development of bacteriology and biotechnology require that the cultures of micro-organisms described or mentioned in publications and patent applications are available for independent study. It is thus necessary to keep and preserve representatives, types and micro-organisms of potential value for industry, medicine, agriculture and other scientific activities. This important function is the responsibility of the Culture Collections which have several roles in addition to deposition, documentation, maintenance, preservation and distribution of useful, representative and type strains (Clark and Loegering, 1967). Microbial collections are living libraries, a continuing reference source and an essential base for related research studies. Generally, collections hold a wide range of micro-organisms which are of past, present and future interest. Culture Collections, associated with biological and biotechnological industry, have also proved their worth by acting as depositories for the preservation and maintenance of important and diverse groups of prokaryote and eukaryote recombinant and non-recombinant strains. Among other standard functions, such Collections regulate the transfer and maintenance of strains as needed for patent rights and/or patent filing procedures.

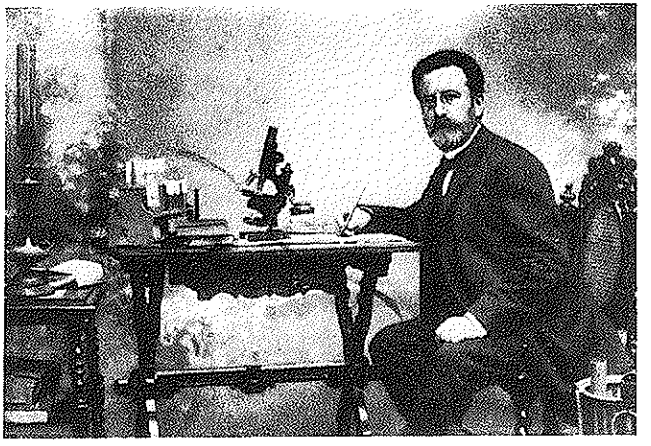
Professor Frantisek Kral (1846–1911) of Prague was the first to realize the importance of Culture Collections: he collected cultures which he made available for a fee to other workers (*Figures 1a,b*). This Collection was later transferred to the University of Vienna in 1915 by Professor Ernst Pribram. The next oldest Collection, Centraalbureau voor Schimmelcultures (CBS), was founded in 1906 and still exists at Baarn, The Netherlands.

Until the 1920s the main reason for the existence of Collections was their value for taxonomic and epidemiological studies. In the 1930s, studies of microbial physiology and biochemistry entailed the preservation of micro-organisms with special properties and productive qualities. Since then many other Collections have developed; the history of such Collections has been reviewed by Porter (1976).

Functions

NETWORK AND COLLABORATION

The importance of Culture Collections, including those associated with industry, is now widely recognized. The usefulness of Culture Collections in



KRÁL'S BAKTERIOLOGISCHES LABORATORIUM, PRAG,
I., KLEINER RING 11.

Kral's bakteriologisches Museum
WIEN, IX/2,
Zimmermanngasse Nr. 3.

DER GEGENWÄRTIGE BESTAND

DER KRÁL'SCHEN SAMMLUNG

VON

MIKROORGANISMEN

MÄRZ 1911

PRAG

Figure 1. (a) Frantisek Kral (1846–1911) founder of the world's first collection of microorganisms. (b) The first page of the catalogue of strains of the collection *Der Kral'schen Sammlung von Mikroorganismen*.

developing and developed countries, and their fundamental role in microbiology, has often been emphasized in the International Culture Collection Conferences (Martin, 1963, 1976; Iizuka and Hasegawa, 1970; Pestana de Castro *et al.*, 1976; Fernandes and Pereira, 1977; Anonymous, 1984; Kocur and Da Silva, 1984) and by the world network of Microbiological Resource Centres—MIRCEN (Da Silva, Burgers and Olembo, 1977).

In 1962, during the First Conference on Culture Collections (Martin, 1963), recommendations were made to the International Association of Microbiological Societies (IAMS) to form a section on Culture Collections. This section was set up in 1963 and on the recommendations of IAMS in 1970 it emerged as the World Federation of Culture Collections (WFCC). The WFCC is a Federation of the International Union of Microbiological Societies (IUMS) and a Commission of the International Union of Biological Sciences in the division of Botany and Zoology, linking it with other organizations concerned with problems of biological preservation such as herbaria, zoological gardens and museums. The aims of the WFCC include the collection of information on strains held by the Collections and more detailed information on the strain data. In addition, WFCC organizes training courses on the modern methods of preservation and on the operation and management of Culture Collections. For more details on WFCC *see Report of the WFCC* (Anonymous, 1975), *Statutes of WFCC* (Anonymous, 1972), and the *WFCC News Letters* (published by The Department of Microbiology, University of Queensland, St Lucia 4067, Australia).

Culture Collections aid biotechnological work by screening, selection, computer search, identification, patent processing and many other services. Several Culture Collection federations and associations such as the United Kingdom Federation of Culture Collections (UKFCC), United States Federation of Culture Collections (USFCC), Japanese Federation of Culture Collections (JFCC), etc. have emerged at national and regional bases. A new association, the European Culture Collection Curators' Organization (ECCCO), was established in 1982, to ensure more efficient service to biotechnology and industry by assisting collaboration between member Collections to avoid duplication of effort (ECCCO, 1984); this has now been renamed the European Culture Collection Organization (ECCO). ECCO member Culture Collections are also affiliated to the WFCC. Interaction and collaboration between Culture Collections through an international network has been described in detail in a review article by Hawksworth (1985).

ROLE AND APPLICATIONS

The role of Culture Collections in microbiology and biotechnology and the services provided by these have been reviewed by Kirsop (1983a,b) and Sly (1984).

According to the World Data Center of micro-organisms (WDC) about $0{\cdot}5 \times 10^6$ cultures are on records in 356 Culture Collections from 52 countries (Martin and Skerman, 1972; McGowan and Skerman, 1982; Skerman, 1984). The numbers of representative and type strains of micro-organisms held in Culture Collections of the world are shown in *Table 1*.

Table 1. Representative and type strains of micro-organisms collected in the world*

Micro-organisms	Number of species	Numbers of collections holding these
Algae	2000	49
Bacteria	3500	261
Fungi (filamentous)	12500	162
Protozoa	300	28
Yeasts	2000	125
Viruses		
Animal	1850	54
Bacterial	180	45
Insect	43	6
Plant	306	8

* McGowan and Skerman, 1982; Sly, 1984.

The majority of such cultures are of regional and international interest, or of scientific and industrial importance and represent an enormous financial investment. About 60% of WDC registered collections are concerned with applied microbiology in fields such as agriculture, industry, dairy, food and geomicrobiology. Such collections are a world resource, the significance of which may be recognized only in the light of future scientific discoveries. The main interests of some bacterial service Culture Collections, types of their holdings and services offered to microbiology and biotechnology are summarized in *Appendix A* (pages 168–191).

The scope of bacterial collection activities important to agriculture is wide ranging. Microbes are indispensable for recycling compounds and elements that are essential for proper conditioning of soil and the continued existence of plant and animal life. By the symbiotic union of legume plants and nitrogen-fixing bacteria of the genus *Rhizobium*, nodules are formed on the roots. Efficient strains of *Rhizobium* from standard reference Culture Collections are thus used to inoculate seeds of legumes before planting for effective nodulation. The collections of *Rhizobia* (Allen, Hamatova and Skinner, 1973; Skinner, Hamatova and McGowan, 1983) are also important in research into the genetics of bacteria and their interactions with the host plants in, for instance, the transfer of nitrogen-fixing capability to other organisms in attempts to induce symbiotic nitrogen-fixing associations with non-leguminous species (Lyons *et al.*, 1981). Such research depends on availability of a diversity of nitrogen-fixing micro-organisms in a viable and stable state (Malik, 1986). This need was emphasized during the National Work Conference on Microbial Collections of Major Importance to Agriculture in 1980 in the United States (Rogosa, 1981).

Micro-organisms are indispensable in many food production processes because foods are often made by controlled fermentation. Maintenance of a stable food supply and expansion of a food production capacity mainly depends on recycling of organic material through microbial decomposition (Blanch, Drew and Wang, 1985b; Knorr and Sinskey, 1985). The biochemical importance of micro-organisms in the degradation of cellulose, proteins,

organic compounds, minerals and in the recycling of nitrogen, carbon dioxide, oxygen, sulphur and other elements has already been established (Bull and Dalton, 1985; Robinson and Howell, 1985a).

Collection activity in industry has been stimulated for many years by the search for new antibiotic-producing organisms and for micro-organisms used in chemotherapeutic studies. In addition to the wide range of products from the drug industry, the chemical industry produces organic solvents, organic acids, enzymes and many other compounds derived from micro-organisms (Blanch, Drew and Wang, 1985c). The use of microbial enzymes in biosensors and their application in fermentation and environmental control, medicine and the food industry is at present of particular interest (Mullen and Vadgama, 1986). In the mining industry the bacteria *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans* rapidly oxidize and solubilize sulphide minerals and sulphur and are utilized in waste or leaching operations for the recovery of copper and uranium (Robinson and Howell, 1985a).

For the production of fuel and petroleum substitutes such as methane, biogas and hydrogen, several methanogenic bacteria and phototrophic bacteria are used (Schlegel and Barnea, 1977; Malik, 1979; Wise, 1984; Malik, Knobloch and Seifert, 1987). Similarly, several *Clostridium* species are available for the production of acetone-butanol and ethanol (Blanch, Drew and Wang, 1985c). Major service Culture Collections hold such bacteria, fungi and yeasts with specific potential applications in biotechnology. The source of availability of some bacteria with special applications in biotechnology is shown in *Appendix B* (pages 192–197).

General range of services

The detailed services offered by Culture Collections depend upon the size and interests of the Collection.

General services provided by major service Collections involve selection, accession, maintenance, preservation, documentation, cataloguing, supply, distribution, identification, safe keeping, screening, special testing, computer search and patent deposition of micro-organisms. In addition, training, consultancy, contract research and information services may also be provided. The range of services provided by individual collections may vary considerably; the services offered by some major bacterial Collections world wide are summarized in *Appendix A*.

Accession and deposition of cultures

In a service culture collection new depositions or accessions are essential. Each service collection has developed its own criteria for acceptance depending on interests, resources and assessment of probable future needs. Specialized curators usually are responsible for each specific group of micro-organisms. These curators conduct research in their appropriate fields, maintain contact with active workers in the field and are responsible for the development (enlargement) of their collections and new acquisitions. Some general categories of bacteria considered for acquisition are as follows:

1. Published strains of newly named taxa;
2. Type, neotype and selected reference strains, specific and unique biotypes;
3. Strains with special properties and applications (bioassay, quality control, resistance test, degradation, etc.);
4. Strains of special significance to agriculture, biotechnology, medicine, education etc.;
5. Strains mentioned in patent applications;
6. Genetically manipulated strains, plasmid carriers, special mutants etc.

According to the new *International Code of Bacteriological Nomenclature* (Revised Code) (Lapage *et al.*, 1975), it is a requirement for a valid publication of a new species that its type strain should be designated and be deposited in one or more of the permanently established Culture Collections. Similarly, for adequate documentation of each newly isolated strain, the editors of scientific journals recommend proper deposition of new isolates before publication. Research workers are encouraged to deposit their strains and to provide relevant published literature, on taxonomic and other important characteristics. Depositors of strains are usually given free access to their isolates and are offered an equal number of strains in exchange if the deposition is made at the request of a Culture Collection. Major Culture Collections require completion of an Accession Form for each deposit in which specific information such as the source of isolation, taxonomic status, history, published data, special properties, reason for deposit, growth medium, growth temperature, pH and optimum procedure of long-term maintenance of a strain are to be given. The Accession Form used at the Deutsche Sammlung von Mikroorganismen (DSM) is shown in *Figure 2*.

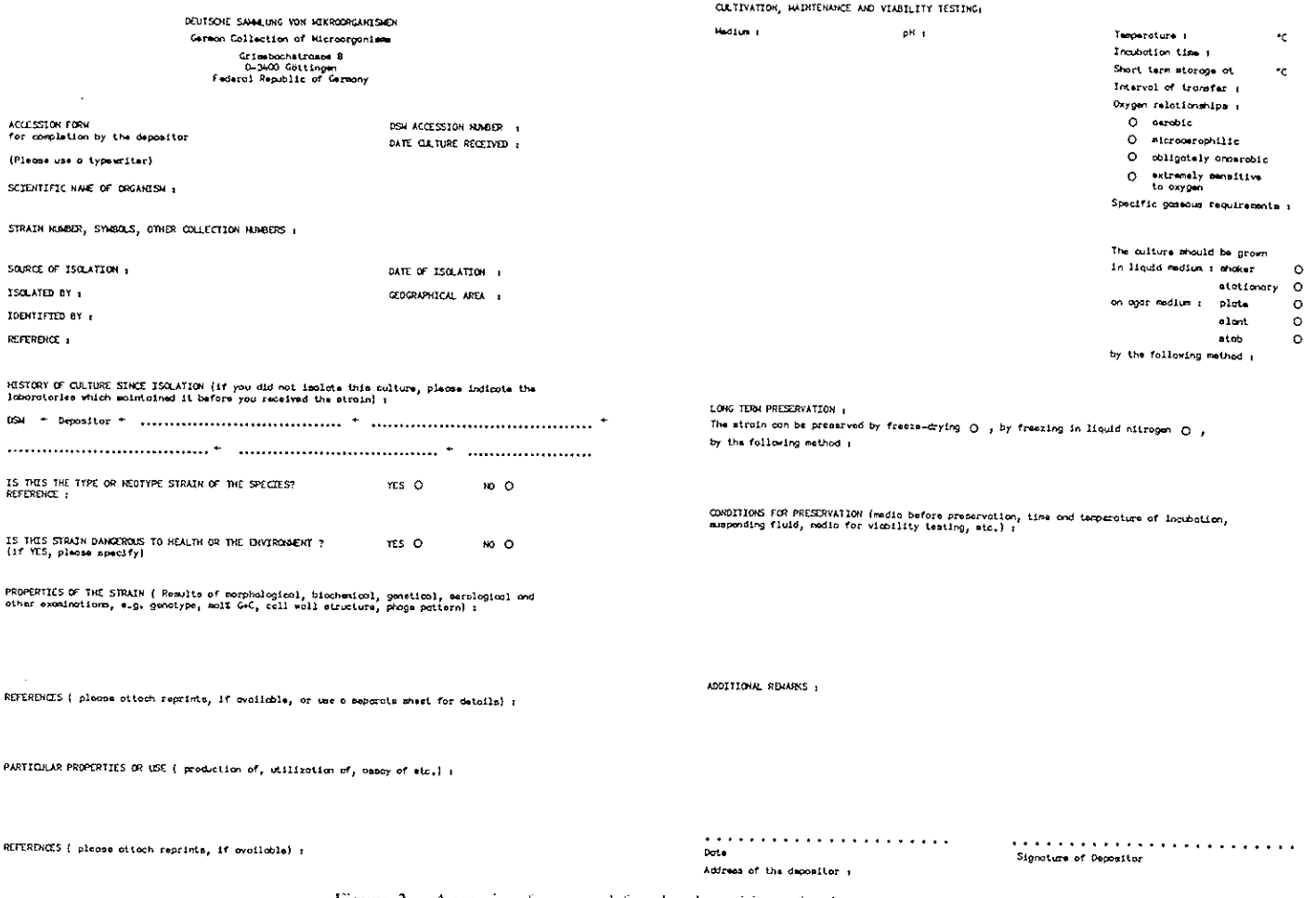
Supply of cultures

All major service culture collections supply authentic and viable strains for research, teaching or applied purposes. Normally, a fee is charged for each culture supplied but as a general rule, Culture Collections supply cultures to other Collections on an exchange basis.

Patent cultures are usually restricted and are supplied only to authorized persons under certain conditions (Crespi, 1985).

Cultures purchased from a Collection are guaranteed for viability, purity and, to a certain extent, according to the properties cited in publications or Culture Collection catalogues. However, the characteristics of certain mutated strains, phage hosts and strains with plasmids may change and so differ from the properties cited in the literature. For this reason, Culture Collections often stress that recipients of strains should report all discrepancies about a strain received from them. Collections bear responsibility only for such strains which have been directly received from the Collections and not for strains which bear the number of that Collection but which have been received from some other source.

Certain quarantine regulations must be observed while importing cultures from abroad: licences or import permits may be required for this purpose.



DEUTSCHE SAMMLUNG VON MIKROORGANISMEN
German Collection of Microorganisms
Grisebachstrasse 8
D-3400 Göttingen
Federal Republic of Germany

ACCESSION FORM
for completion by the depositor

(Please use a typewriter)

DSM ACCESSION NUMBER :
DATE CULTURE RECEIVED :

SCIENTIFIC NAME OF ORGANISM :

STRAIN NUMBER, SYMBOLS, OTHER COLLECTION NUMBERS :

SOURCE OF ISOLATION :
DATE OF ISOLATION :

ISOLATED BY :
GEOGRAPHICAL AREA :

IDENTIFIED BY :

REFERENCE :

HISTORY OF CULTURE SINCE ISOLATION (if you did not isolate this culture, please indicate the laboratories which maintained it before you received the strain) :

DSM + Depositor + ... + ... +

..... + ... + ...

IS THIS THE TYPE OR NEOTYPE STRAIN OF THE SPECIES? YES O NO O
REFERENCE :

IS THIS STRAIN DANGEROUS TO HEALTH OR THE ENVIRONMENT ? YES O NO O
(if YES, please specify)

PROPERTIES OF THE STRAIN (Results of morphological, biochemical, genetical, serological and other examinations, e.g. genotype, mol% G+C, cell wall structure, phage pattern) :

REFERENCES (please attach reprints, if available, or use a separate sheet for details) :

PARTICULAR PROPERTIES OR USE (production of, utilization of, assay of etc.) :

REFERENCES (please attach reprints, if available) :

CULTIVATION, MAINTENANCE AND VIABILITY TESTING:

Medium : pH :

Temperature : °C
Incubation time :
Short term storage at °C
Interval of transfer :
Oxygen relationships :
O aerobic
O microaerophilic
O obligately anaerobic
O extremely sensitive to oxygen
Specific gaseous requirements :

The culture should be grown
in liquid medium : shaker O
stationary O
on agar medium : plate O O
slant O O
stab O
by the following method :

LONG TERM PRESERVATION :
The strain can be preserved by freeze-drying O ; by freezing in liquid nitrogen O ,
by the following method :

CONDITIONS FOR PRESERVATION (media before preservation, time and temperature of incubation, suspending fluid, media for viability testing, etc.) :

ADDITIONAL REMARKS :

....
Date Signature of Depositor
Address of the depositor :

Figure 2. Accession form used for the deposition of cultures at the DSM.

Universal Postal Union (UPU) lays down common regulations about packing and shipping of cultures. The details on export and import restrictions of micro-organisms are often published in the News Letters of WFCC.

Research, consultancy, training

Apart from service functions, Collections carry out research on actual collection and related matters and are concerned with the taxonomy and identification of micro-organisms. Several major Culture Collections offer training facilities in their fields of specialization and provide advisory and consultation services on such matters as cultivation, enrichment, isolation, identification, preservation and strain selection. Among their special scientific services, major service Collections also conduct contract research for industry or research organizations.

Training courses on Culture Collection management, modern preservation techniques and in other related areas in applied microbiological research are organized and conducted by experienced staff of major Culture Collections at national and international level. Under the auspices of WFCC such Training Courses are mostly linked with International Conferences on Culture Collections.

STRAIN DATA AND INFORMATION

All major service Culture Collections obtain and document data concerning their strains. Records are maintained on strain characteristics, nutritional requirements and qualities of special interest. These are periodically published in the form of catalogues and catalogue-supplements (for details see *Appendix A*), which are thus a major source of information on strains (growth medium, temperature, pH, biochemical characters, literature references, application and history).

For systematic information exchange between collections (about holdings, type of work, type of strain data, etc.) an International Centre for Information on Culture Collections has been established at Lausanne, Switzerland. The information about small Culture Collections and about their interests and stocks can be obtained from the WDC (Skerman, 1984) which has published a *World Directory of Collections of Cultures of Micro-organisms* (McGowan and Skerman, 1982), and the *IBP World Catalogue of Rhizobium Collections* (Skinner, Hamatova and McGowan, 1983) and is now planning to expand its services to more specific information about strain data by publishing strain catalogues. The WDC at Brisbane is one of the co-ordinating Microbiological Resource Centres (MIRCEN), and is now, as part of the world network, establishing a regional network of collaborating institutes and laboratories in developing and developed countries (Da Silva, Burgers and Olembo, 1977; Annon, 1979). WDC has now been transferred to RIKEN, (Institute for Chemical and Physical Research) at Saitama, Japan and the third edition of the *World Directory* will be published from there in 1988.

Management of data and data exchange has grown in importance as a Culture Collection function. Culture Collections associated with biotechnology and genetic engineering organizations or industry maintain computer data-bases for their particular interest. Major Culture Collections also are increasingly using computers for maintaining and managing data and to facilitate computer search for strains with particular qualities. An International Microbial Strain Data Network (MSDN) is being established under the sponsorship of the Committee on Data for Science and Technology (CODATA) and WFCC, (Hill and Krichevsky, 1986). The Microbial Information Network for Europe (MINE) has recently been established. MINE is funded by the Commission of the European Communities (CEC) Biotechnology Action Programme. Its main objective is the production of an integrated catalogue of the holdings of culture collections in Europe by a computer network. It incorporates the European Network of Microbial Culture Collections Databanks which has been established initially with the collaborative efforts of the Commonwealth Agricultural Bureaux (CAB), UK; Commonwealth Mycological Institute (CMI), UK; Centraalbureau voor Schimmelcultures (CBS), The Netherlands; Deutsche Sammlung von Mikroorganismen (DSM), FRG; the Belgian Coordinated Collection of Microorganisms (LMG, MUCL, IHEM), Belgium and Gulbenkian Institute of Science and Oeiras, Portugal. Similarly, at the national level several advanced countries are considering establishment of their data bases, e.g. the Microbial Culture Information Service (MiCIS) in the United Kingdom. More detail on data management systems and the information system of Culture Collections has been described in detail by Krichevsky and Norton, 1976; Jong, 1984; Sly, 1984; Hawksworth, 1985.

Strain maintenance and preservation

In nature, micro-organisms occur in mixed populations of diverse or closely related species. The individual isolates in pure cultures are known as strains and strains with common modal characteristics are recognized as species. Different strains of a species may exhibit differing non-modal properties. Thus examination of pure cultures of several strains of a species is normally essential in selecting microbes for specific use.

After isolation from nature strains may become unstable and change on continued maintenance in artificial media and conditions. Strains containing plasmids and genetically engineered micro-organisms containing extrachromosomal DNA and manipulated genes may also change. Successful maintenance of such cells is a major problem because loss in viability and stability of such micro-organisms may cause serious disruption to industrial processes (Calam, 1964; Hesseltine and Haynes, 1973).

The high costs of strain selection, research, development and patent application or processing underline the need to maintain the authenticity and production efficiency of the strains which form the basis of biotechnological industries. Thus it is a major concern that all microbial strains be maintained, or preserved in such a way as to assure long-term viability, stability and

accessibility. The maintenance of microbial cultures and living cells requires specialized knowledge of each organism and familiarity with sophisticated and modern preservation techniques. It is essential that the applied methods of preservation cause minimal damage to cells and stabilize genotypic and phenotypic behaviour as well as viability. Major culture collections therefore frequently test and adopt new methods of preservation (Nei, 1968; Lapage *et al.*, 1970; Malik, 1976, 1984a,b,c, 1985, 1986; Heckly, 1978; Hatt, 1980; Gherna, 1981; Hippe, Hoffman and Malik, 1981; Rogosa, 1981; Dietz, 1982; Kirsop and Snell, 1984). A preservation method generally is judged by its ability to retain significant properties of the culture and to ensure viability for long periods. There is no universal method of long-term preservation because there is great variation in susceptibility to the various preservation methods within groups, genera and species (Redway and Lapage, 1974; Lapage *et al.*, 1970; Malik, 1976, 1986, 1987).

MODERN METHODS OF PRESERVATION

Generally the long-term viability and stability of the microbial cell is secured by reducing the metabolic activity to a minimum. This can be achieved by the removal of water from the cell (to a defined extent) in the presence of certain protective agents, by freezing at ultra-low temperatures (−196°C in liquid nitrogen), by freeze-drying (lyophilization) and by liquid drying (L-drying). These sophisticated methods to various degrees secure viability and strain stability. Such techniques are commonly used by the major service Culture Collections and industrial and scientific laboratories and need to be learned in order to handle and store microbes adequately and in a well organized manner (*Figures 3a, b*). The L-drying method (drying without freezing) was developed by Annear (1954, 1956, 1962) and later was modified by Banno and Sakane (1979). It is successfully used by many Culture Collections for the routine preservation of various bacteria and yeasts which are not otherwise easy to conserve.

Preservation by freeze-drying

For long-term preservation, freeze-drying is the commonest preferred method. Unlike L-drying, freeze-drying causes little shrinkage and results in a product which is readily dispersible, can easily be stored and is usually stable over a period of many years.

The final objective of freeze-drying is to reduce the metabolic activity of the cells to a minimum and to keep these cells dormant but viable. This is achieved by lowering the temperature by rapid freezing to reduce enzymic activity and then stopping metabolism by the removal of cell water (Greaves, 1964). The dehydration is achieved by applying a vacuum to remove water as water vapour by direct sublimation. However, there are micro-organisms which fail to survive lyophilization or which lose viability during subsequent long-term storage.

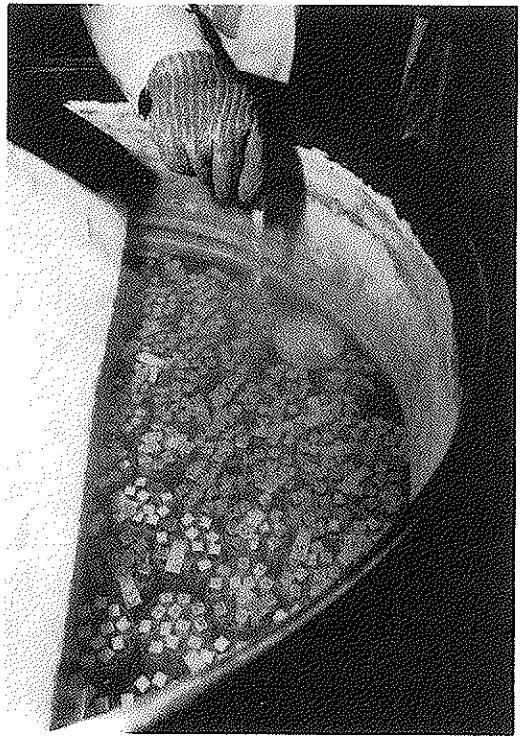


Figure 3. (a) Collection of lyophilized ampoules maintained at 9°C. (b) Cultures preserved in liquid nitrogen at the Deutsche Sammlung von Mikroorganismen (DSM).

The freeze-drying stresses affect sensitive bacteria in two main ways: (1) by creating loss of viability; (2) by loss of genetic properties. To obtain a true representation of original cultures it is very important that there should be a high survival rate after lyophilization, as only freeze-drying resistant mutants may survive if losses are high. Quality control testing is essential because some sensitive microbes show no loss in viability after lyophilization although their genetic stability may be affected and this causes loss of important qualities. This is especially likely for bacteria with properties depending on the presence of plasmids. Many industrially useful strains, especially those obtained as a result of selective screening, mutations or genetic engineering may suffer loss of useful qualities after lyophilization. At the American Type Culture Collection (ATCC), lyophilization of plasmid-containing *Escherichia coli* cultures resulted in loss neither of plasmid nor of viability (Nierman and Feldblyum, 1980). At the German Collection of Microorganisms (DSM) during a systematic control about 250 plasmid-bearing strains of *E. coli* (host of recombinant or isolated plasmid DNA) were checked for their plasmid content and plasmid markers and about 97% of strains were found to maintain their plasmids after lyophilization (Rohde and Claus, 1987). However, in some comparative lyophilization studies diazotrophic bacteria proved very sensitive during unprotected freeze-drying and in many cases complete loss of viability and stability (loss of chemolithoautotrophy, diazotrophy and plasmids) resulted (Malik, 1986). Using a simplified freeze-drying method (involving protectants) successful lyophilization was achieved with diazotrophic and phototrophic bacteria without loss of plasmids or other desirable characters (Malik, 1986, 1987). In some lyophilized cultures mutant colonies were isolated; these showed loss of useful properties without any apparent plasmid loss. Possibly only a plasmid injury or damage could have caused the loss of such characteristics (plasmid located), but at present no method is available to demonstrate such plasmid damage or injury.

Generally Gram-positive bacteria survive better than Gram-negative bacteria when freeze-dried under comparable conditions. Similarly, spore-forming bacteria can better survive freeze-drying. The age of a culture can also have a profound effect on the survival of micro-organisms to be freeze-dried. Our results show that usually cells in a culture at the early stationary phase are most resistant to the freeze-drying process. Optimal incubation periods for various bacteria to be freeze-dried are shown in *Table 2*. Similarly it is known that high cell concentration usually results in an increased percentage of cells surviving freeze-drying (*Table 3*). The reason for this phenomenon is not clearly understood.

The cells of the micro-organisms to be lyophilized should be suspended in medium containing agents which protect against freezing and drying injuries (Greaves, 1964; Lapage *et al.*, 1970; Redway and Lapage, 1974; Hatt, 1980; Malik, 1976, 1986). Protective agents are needed to prevent both ice crystal formation and total desiccation (which damages DNA and kills the cells) as well as to neutralize harmful effects (from electrolytes, for instance). Most protective compounds, in general, are hydrophilic in nature. These are

Table 2. Optimal time of preincubation for some bacteria to be freeze-dried

Organism	Preincubation (hours)
Acetobacter	48–72
Acinetobacter	24
Aeromonas	24
Agrobacterium	48
Alcaligenes	24
Arthrobacter	72
Azotobacter	72
Azomonas	72
Bacillus	96
Bacteroides	48
Bifidobacterium	24
Brevibacterium	72
Cellulomonas	24–48
Chromobacterium	24
Citrobacter	24
Clostridium	72
Corynebacterium	24
Enterobacter	24
Erwinia	36
Escherichia	24
Klebsiella	24
Lactobacillus	24–48
Leuconostoc	24
Micrococcus	16–18
Nocardia	96 or more
Pediococcus	24
Propionobacterium	24–72
Proteus	24
Pseudomonas	24
Serratia	24
Sporosarcina	24
Staphylococcus	18
Streptococcus	24–48
Vitreocilla	48
Zymomonas	24–48

Table 3. Cell concentration and survival of some bacteria after freeze-drying

Species	Before freeze-drying	After freeze-drying
Serratia marcescens	$3{\cdot}5 \times 10^{7}$	2×10^{5}
	2×10^{10}	1×10^{10}
Escherichia coli	2×10^{6}	3×10^{4}
	3×10^{10}	3×10^{10}
Pseudomonas putida	4×10^{6}	1×10^{4}
	5×10^{10}	4×10^{10}
Lactobacillus plantarum	1×10^{9}	1×10^{8}
	1×10^{11}	1×10^{11}

assumed to fit into water lattices by forming parallel structures (tetrahydric and hexagonal, etc.) and are able to bind water through hydrogen bonding. A few selected sugars, and honey (a natural combination of almost all favourable substances) have proved to be good protective agents during lyophilization (Malik, 1976, 1978).

To summarize, the protective agents in the suspending fluids should assist retention of residual water to about 1% (0·133 Pa), could neutralize toxic carbonyl radicals, electrolytes, etc. and should provide an easily water-soluble final porous solid. This 'porous cake' is intended to facilitate sublimation of ice, protect the dried culture from mechanical damage and prevent aerial dispersion during sublimation of ice and when the ampoule is opened to recover the micro-organisms. In addition, such factors as high glass transition point (for preventing ice crystal formation within the cell during super-cooling) of the substance and colloidal nature (for providing a protective film around the cell to help prevent mechanical damage during the process) and high buffering capacity also prove helpful.

A variety of protective agents, such as amino acids, carbohydrates (e.g. sugars or dextran) and hydrophilic compounds, proteins (such as haemoglobin, gelatin and serum albumin) and materials such as milk and honey, have all been used with some success.

At the DSM we have tested about 30 of such substances on more than 40 different microbial species to determine their ability to ensure:

1. Maximum viability;
2. Minimum losses during storage at low (4°C) and relatively higher (25°C) temperatures;
3. Maximum phenotypic and genetic stability.

Only four of these additives offered good protection for a variety of bacteria (*Table 4*). During more than 12 years' experience these additives have proved successful for the lyophilization of a broad spectrum of micro-organisms. Special precautions are needed for the lyophilization of micro-organisms sensitive to light, oxygen, temperature, osmotic pressure or other factors. As an example, freeze-drying has seldom been used to preserve blue-green

Table 4. Some protective agents for successful lyophilization *

Additives (protective agents)	Average survival (%)	Storage temperature		
		4°C	10°C	25°C
1. Skim Milk† 20% and Honey 10%	90–100	Good	Moderate	Poor
2. Skim Milk 20% and Glutamate 5%	60–90	Good	Good	Good
3. Skim Milk 20% and m-Inositol 5%	70–90	Good	Good	Moderate
4. Skim Milk 20% and Raffinose 5%	50–60	Good	Moderate	Moderate

* Malik, 1986, 1987.
† Bacto-Skim milk, Difco.

algae or phototrophic bacteria. Failure to preserve such cultures by ordinary methods is mainly due to their sensitivity to oxygen in the presence of light (Malik, 1983, 1984c). Photochemically active agents such as carotenoids, bacteriochlorophylls or bacteriophytins (porphyrins) offer good protection against photo-oxidation by absorbing light and trapping oxygen during the freeze-drying process. Using this method, several species of phototrophic bacteria were successfully preserved (Malik, 1978, 1984a). During recent experiments porphyrins were replaced by fine neutral active carbon powder used as a protective additive, together with 5% raffinose or 5% meso-inositol (Malik, 1987). Several species of *Rhodospirillaceae* (brown and oxygen-sensitive), and a few species of *Chlorobiaceae* and *Chromatiaceae* retained viability and stability after lyophilization and after 1–2 years of storage at +9°C.

There have been few reports of lyophilization of methanogenic bacteria so far. At the DSM a mixture composed of horse serum, glucose and ferrous sulphide has been used as suspending fluid for lyophilization of several species of methanogens (Hippe, 1984). The outline of a modern freeze-drying process and the major steps involved are shown in *Figure 4* (Malik, 1985, 1986).

Cryopreservation in liquid nitrogen

Micro-organisms to which freeze-drying cannot be applied are often preserved in liquid nitrogen (LN_2). With controlled freezing and thawing it is possible to freeze-store microbes and maintain genetic stability and viability (Bridges, 1966; Jarvis, Wynne and Telfer, 1967; Swoager, 1972; Nagel and Kunz, 1972; Daily and Higgens, 1973; Dietz, 1975; Simione and Daggett, 1977; Hatt, 1980; Kirsop and Snell, 1984; Malik, 1984b).

At ultra-low temperatures (−196°C of liquid nitrogen) metabolic activities are reduced to a minimum. A broad range of living cells (from small prokaryotic to large eukaryotic cells) can remain viable for long periods (Bridges, 1966; Nei, 1968; Hatt, 1980; Kirsop and Snell, 1984). Nearly all micro-organisms so far tested have survived in LN_2 and possibly can survive for prolonged periods with little loss in viable count. Liquid nitrogen is safer (non-burning, non-toxic) and cheaper than other inert gases. It provides a storage temperature of −196°C and storage containers and additional equipment for LN_2 storage are commercially available.

Cells undergo severe temperature changes during freezing, for example from growth or room temperature to below 0°C. During this step cells are particularly susceptible to injury and loss of viability, thus suffering 'cold shock' which can be avoided or limited by slower cooling and by the addition of cryoprotectants (Daily and Higgens, 1973; Calcott and Gargett, 1981).

High recovery of cells from the frozen state is important to minimize the possibility of selection of cells resistant to the technical manipulation employed during the process of cryopreservation. Successful deep-freezing of most bacterial suspensions requires certain procedures:

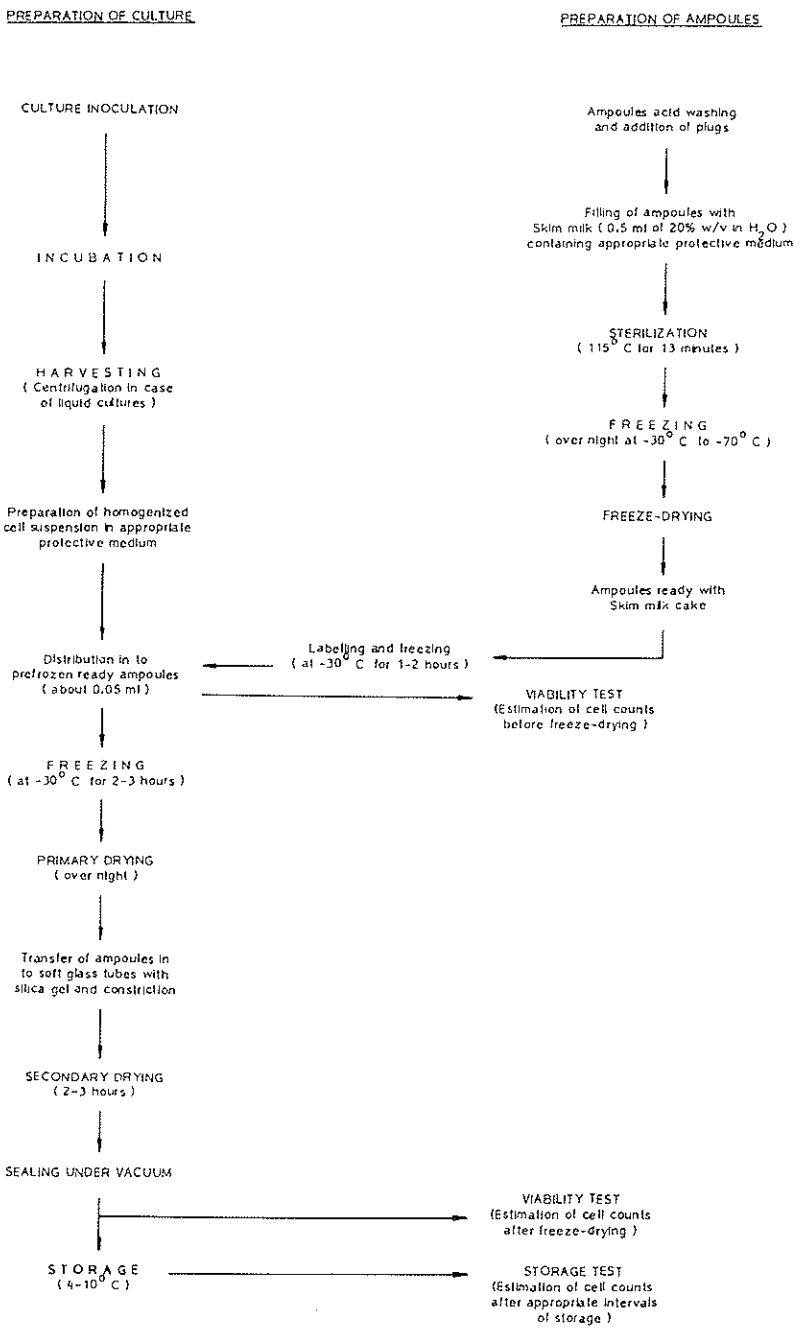


Figure 4. Flow diagram of a freeze-drying process (Malik, 1986).

1. Low cooling rate down to −30°C;
2. Fast cooling rate below −30°C;
3. Fast warming rate during thawing;
4. Minimum concentration of electrolytes in the freezing suspension;
5. Addition of cryoprotectants to the freezing suspension.

Compounds which protect living cells against freeze–thaw injury are termed cryoprotectants and belong to two main classes on the basis of their permeability to cells (*Table 5*). Penetrating compounds of low molecular weight, e.g. glycerol, dimethylsulphoxide (DMSO), methanol, etc. are used as cryoprotectants in preserving many types of bacteria. These penetrating compounds are generally applied in high concentration (0·5–1·5 M) whereas non-penetrating agents such as HES and PVP are used at much lower concentrations (0·01 M). Toxicity of these compounds at higher concentrations may cause problems. At low cooling rates it can be assumed that ice crystals are formed only outside, not inside, the cell because the interior of a cell has a stronger freeze-depression and withstands supercooling. During thawing (warming) of the sample from the deep-frozen state, all the processes during deep-freezing will reverse and could damage the cells. Generally, a low cooling rate of 1–10°C per minute and rapid thawing is recommended for obtaining good survival in the case of bacteria, yeasts and fungi. There are several exceptions for which a modified freezing procedure or suspension medium is essential for survival. For effective preservation of anaerobic bacteria, several special methods are available (Gilmour *et al.*, 1978; Hippe, 1984; Malik, 1984a,b).

Considerable progress has been made during recent years in low temperature preservation methods (Hatt, 1980; Kirsop and Snell, 1984; Malik, 1985). Cryopreservation in liquid nitrogen or in deep freezers requires specialized

Table 5. General cryoprotective agents for deep-freezing

I.	*Penetrating Compounds*
	1. Dimethylsulphoxide (DMSO)
	2. Glycerol
	3. Methanol
	4. Ethylene glycol
II.	*Non-penetrating Compounds*
	1. Hydroxyethyl starch (HES)
	2. Polyvinyl pyrolidone (PVP)
	3. Sugars, proteins, etc.
	4. Polyethylene glycol
III.	*Complex Undefined Agents*
	1. Extract of boiled microbial cells
	2. Malt extract
	3. Blood proteins, serum
	4. Milk, skim milk

Table 6. Miniaturized methods for freeze-preservation of micro-organisms

Methods	Storage temperature
Glass capillary tubes*	−196°C (Liquid nitrogen)
Plastic capillary tubes (PVC)†	−196°C (Liquid nitrogen)
Glass beads‡	−70°C
	−196°C (Liquid nitrogen)
Mini-screw cap glass ampoules§	−196°C (Liquid nitrogen)

* Pautrizel and Carloz, 1952; Jarvis, Wynne and Telfer, 1967; Hippe, 1984.
† Dietz, 1975; Gilmour *et al.*, 1978; Hippe, Hoffman and Malik, 1981.
‡ Nagel and Kunz, 1972; Feltham *et al.*, 1978.
§ Malik, 1984b.

expensive storage. Problems of space and cost can be overcome by the use of novel miniaturized methods. These techniques (*Table 6*) reduce the volume of the cell suspension to be preserved and of the unit holding this. For this purpose small glass beads, ampoules, glass capillary tubes and plastic polyvinylchloride (PVC) straw or capillary tubes are used to prepare several parallel samples from the same batch of the cells (Jarvis, Wynne and Telfer, 1967; Nagel and Kunz, 1972; Feltham *et al.*, 1978; Gilmour *et al.*, 1978; Hippe, Hoffmann and Malik, 1981; Hippe, 1984; Malik, 1984a,b).

Some of the major steps involved during a general process for liquid nitrogen preservation of bacteria are shown in the flow diagram of *Figure 5* (Malik, 1985).

Patent deposition

A patent is valid only if the micro-organisms cited in the patent application are deposited with a recognized International Patent Depository Authority (recognized by the World Intellectual Property Organization (WIPO) under the 'Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure', 1977).

Culture Collections (International Depository Authorities or IDAs) can advise scientists on procedures for patent deposition, provide help in the deposition of microbes in an International Depository and provide advice and consultancy services on similar matters. An International Form for the deposition of a patent micro-organism with a recognized International Patent Depository Authority is shown in *Figure 6*.

There are 14 IDAs that have been recognized by WIPO under the Budapest Treaty (*Table 7*). The bacterial culture collections which act as IDAs and the number of patent strains deposited at these are shown in *Table 8*. According to patent treaties, Culture Collections (International Depository Authorities) holding patent cultures must ensure viability, stability and availability of the patent deposits for at least 30 years (Saliwanchik, 1982).

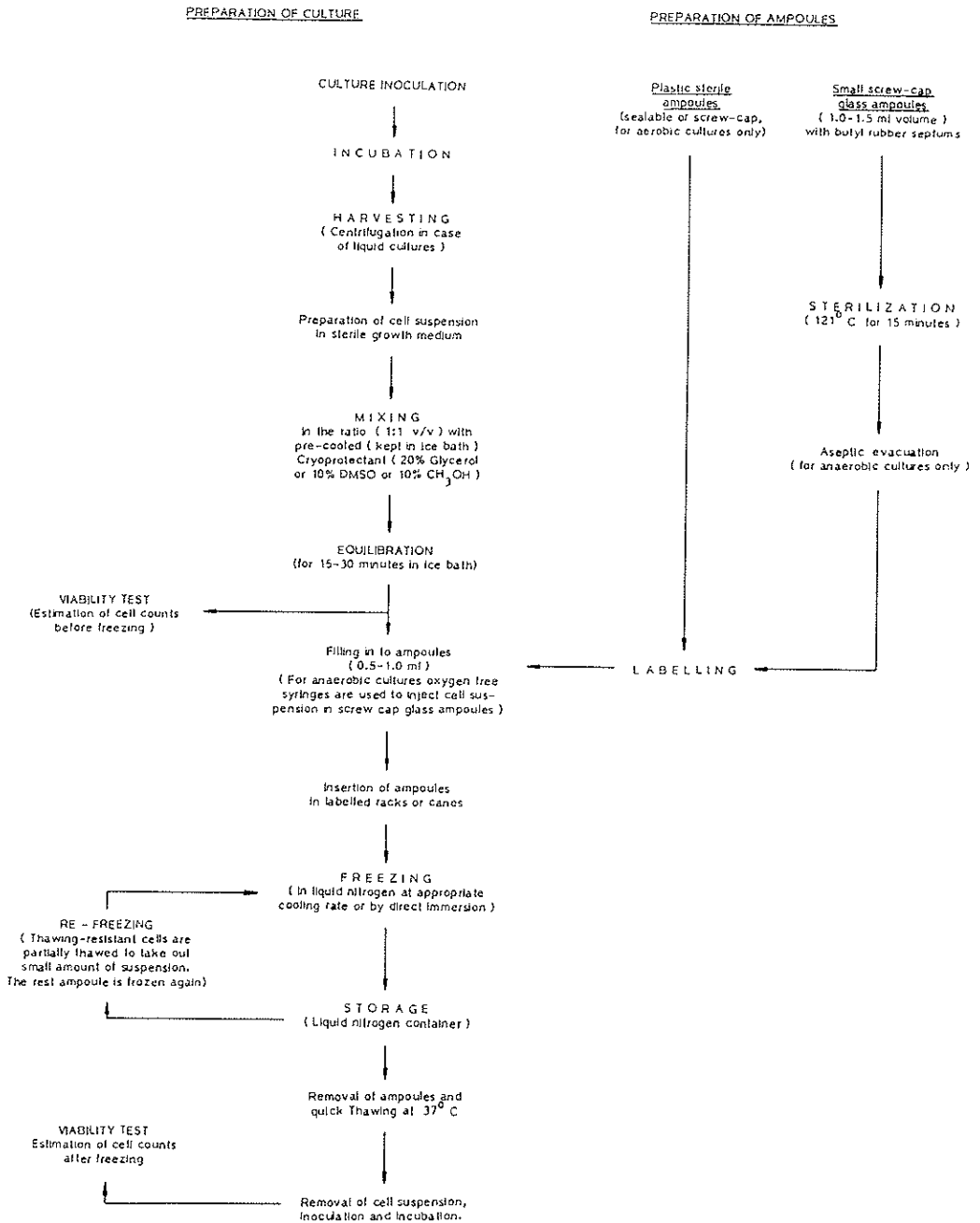


Figure 5. Flow diagram of a general procedure for cryopreservation in liquid nitrogen (Malik, 1985).

Table 7. Culture collections recognized under the Budapest Treaty as International Depository Authorities for Patent micro-organisms*

Country	Name and address of the International Depository Authority	Kind of micro-organisms that may be deposited	Deposition fees (30 years)	Provision of cultures (fee)
USA	Agricultural Research Culture Collection (NRRL) 1815 North University Street Peoria, Illinois 61604 USA	Agriculturally and industrially important bacteria, yeast, moulds and Actinomycetales with a few exceptions. Plasmids	US $500	US $20
USA	American Type Culture Collection (ATCC) 12301 Parklawn Drive Rockville, Maryland 20852 USA	Algae, animal viruses, bacteria cell lines, fungi, hybridomas, oncogenes, phages, plant tissue cultures, plant viruses, plasmids, protozoa, seeds, yeasts	US $870	US $40–64
Netherlands	Centraalbureau voor Schimmelcultures (CBS) Oosterstraat 1 Postbus 273 NL–3740 AG Baarn Netherlands	Fungi, including yeasts, actinomycetes, bacteria	Hfl. 2000	Hfl. 40–90
France	Collection National de Cultures de Microorganisms (CNCM) Institut Pasteur 28 rue du Dr Roux F–75724 Paris Cedex 15 France	Bacteria, filamentous fungi, yeasts, viruses with few exceptions	F.Fr. 3500	F.Fr. 600
UK	Culture Collection of Algae and Protozoa (CCAP) The Ferry House Far Sawrey Ambleside, Cumbria, LA22 0LP United Kingdom	Algae and protozoa with few exceptions	£275	£10

(cont'd)

Table 7. *Continued*

Country	Name and address of the International Depository Authority	Kind of micro-organisms that may be deposited	Deposition fees (30 years)	Provision of cultures (fee)
UK	Culture Collection of The Commonwealth Mycological Institute (CMICC) Ferry Lane Kew Richmond, Surrey TW9 3AF United Kingdom	Fungi and yeasts with few exceptions	£400	£35
FRG	Deutsche Sammlung von Mikroorganismen (DSM) Gesellschaft für Biotechnologische Forschung mbH Grisebachstrasse 8 3400 Göttingen, FRG	Bacteria, fungi, yeasts, bacteriophages with few exceptions. Plasmids	DM 950	DM 60
UK	European Collection of Animal Cell Cultures (ECACC) Vaccine Research and Production Laboratory, PHLS Porton Down Salisbury, Wiltshire SPU 0JG United Kingdom	Cell lines and viruses with few exceptions	£600–800	£50–80
Japan	Fermentation Research Institute (FRI) 1–3 Higashi 1-chome Yatabe-machi Tsukuba-gum, Ibaraki-ken 305 Japan	Bacteria, fungi and yeasts with few exceptions	Yen 170 000	Yen 6900
USA	In Vitro International Inc. (IVI) 611 (P) Hammonds Ferry Road Linthicum, Maryland 21090 USA	Algae, bacteria, bacteriophages, cell cultures, fungi, protozoa, animal and plant viruses	Variable depending on period	(variable): US $25–30

Hungary	Mezogazdasagi Es Ipari Mikroorganizmusok Magyar Nemzeti Gyüjtemenye (MIMNG) Dept. of Microbiology University of Horticulture Somloi ut 14–16 1118 Budapest Hungary	Bacteria and fungi with few limitations	FT. 15 000	Ft. 2000
UK	National Collection of Industrial Bacteria (NCIB) Torry Research Station PO Box 31 135 Abbey Road Aberdeen AB9 8DG United Kingdom	Bacteria and bacteriophages with few limitations. Plasmids	£225	£9-18
UK	National Collection of Type Cultures (NCTC) Central Public Health Laboratory 175 Colindale Avenue London NW9 5HT United Kingdom	Bacteria with few limitations	£250	£40
UK	National Collection of Yeast Cultures (NCYC) Food Research Institute Colney Lane Norwich, Norfolk NR4 7UA United Kingdom	Yeasts with few limitations	£240	£10

* There are 16 states (Austria, Bulgaria, Denmark, France, Federal Republic of Germany, Hungary, Japan, Liechtenstein, Philippines, Soviet Union, Spain, Sweden, Switzerland, United Kingdom, United States of America), members to the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure (*Industrial Property: January 1985, 24th year* No. 1 and *January 1987, 26th year* No. 1).

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSE OF PATENT PROCEDURE

STATEMENT IN THE CASE OF AN ORIGINAL DEPOSIT
pursuant to Rule 6.1

To
DEUTSCHE SAMMLUNG
VON MIKROORGANISMEN
Grisebachstr.8

D-3400 Göttingen

DSM accession number:
Date culture received:

THE UNDERSIGNED HEREBY DEPOSITS UNDER THE BUDAPEST TREATY THE MICROORGANISM IDENTIFIED HEREUNDER AND UNDERTAKES NOT TO WITHDRAW THE DEPOSIT FOR THE PERIOD SPECIFIED IN RULE 9.1[1]

Please use a typewriter

I. IDENTIFICATION OF THE MICROORGANISM

Identification reference[2]:

Taxonomic designation[3]:

The culture to be deposited is
☐ a pure culture
☐ a mixture of microorganisms
(Mark with a cross where applicable)

II. CONDITIONS FOR CULTIVATION ☐ [4]

Medium:

pH before sterilisation:
Sterilisation min at C
pH after sterilisation:
Oxygen realtionships:
☐ aerobic
☐ microaerophilic
☐ obligate anaerobic
Specific gaseous requirements:

Incubation temperature: C
Incubation time:
Short term storage at: C
Interval of transfer:

[1] This form may also be used if the undersigned converts into a deposit under the Budapest Treaty the deposit of a microorganism that he or his predecessor in title has already deposited, outside the Budapest Treaty, with the same depository institution either before (Rule 6.4(d)) or after the acquisition by that institution of the status of international depository authority.

[2] Number, symbols, etc., given to the microorganism by the depositor.

[3] It is stronly recommended that the taxonomic designation and/or scientific description (see under VII.) of the microorganism be indicated.

[4] Mark with a cross if additional information is given on an attached sheet.

Form DSM-BP/1 (first page) 0382

III. CONDITIONS FOR LONG TERM STORAGE ☐ [4]

IV. CONDITIONS FOR TESTING VIABILITY ☐ [4]

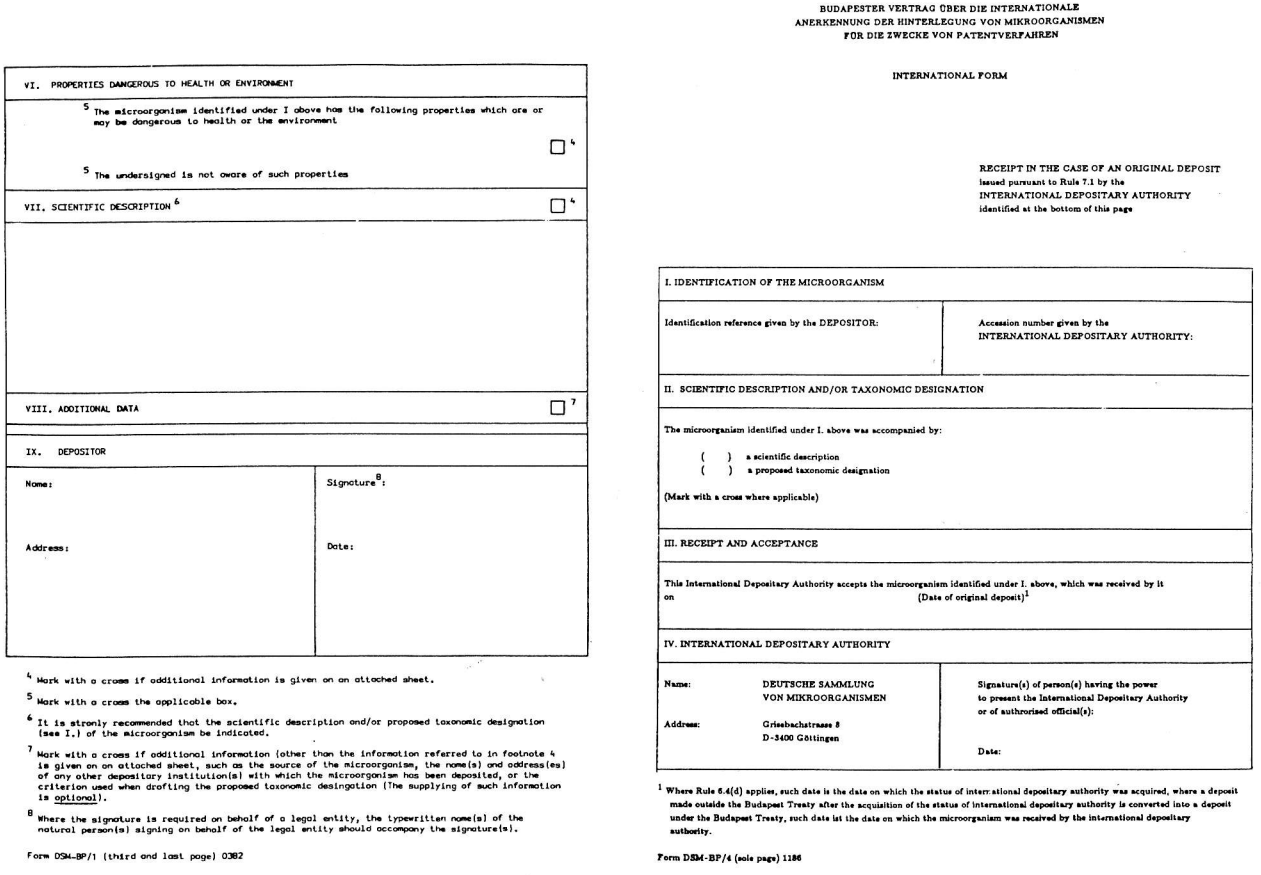
V. COMPONENTS OF MIXED CULTURES (WHEN APPLICABLE) ☐ [4]

Description of components:

Method(s) for checking presence of components:

[4] Mark with a cross if additional information is given on an attached sheet.

Form DSM-BP/1 (second page) 0382



VI. PROPERTIES DANGEROUS TO HEALTH OR ENVIRONMENT

[5] The microorganism identified under I above has the following properties which are or may be dangerous to health or the environment ☐ [4]

[5] The undersigned is not aware of such properties

VII. SCIENTIFIC DESCRIPTION [6] ☐ [4]

VIII. ADDITIONAL DATA ☐ [7]

IX. DEPOSITOR

Name: | Signature [8]:

Address: | Date:

[4] Mark with a cross if additional information is given on an attached sheet.

[5] Mark with a cross the applicable box.

[6] It is strongly recommended that the scientific description and/or proposed taxonomic designation (see I.) of the microorganism be indicated.

[7] Mark with a cross if additional information (other than the information referred to in footnote 4 is given on an attached sheet, such as the source of the microorganism, the name(s) and address(es) of any other depositary institution(s) with which the microorganism has been deposited, or the criterion used when drafting the proposed taxonomic designation (The supplying of such information is optional).

[8] Where the signature is required on behalf of a legal entity, the typewritten name(s) of the natural person(s) signing on behalf of the legal entity should accompany the signature(s).

Form DSM-BP/1 (third and last page) 0382

BUDAPESTER VERTRAG ÜBER DIE INTERNATIONALE ANERKENNUNG DER HINTERLEGUNG VON MIKROORGANISMEN FÜR DIE ZWECKE VON PATENTVERFAHREN

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM

Identification reference given by the DEPOSITOR: | Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:

II. SCIENTIFIC DESCRIPTION AND/OR TAXONOMIC DESIGNATION

The microorganism identified under I. above was accompanied by:

( ) a scientific description
( ) a proposed taxonomic designation

(Mark with a cross where applicable)

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I. above, which was received by it on (Date of original deposit)[1]

IV. INTERNATIONAL DEPOSITARY AUTHORITY

Name: DEUTSCHE SAMMLUNG VON MIKROORGANISMEN

Address: Grisebachstrasse 8
D-3400 Göttingen

Signature(s) of person(s) having the power to present the International Depositary Authority or of authorised official(s):

Date:

1 Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired, where a deposit made outside the Budapest Treaty after the acquisition of the status of international depositary authority is converted into a deposit under the Budapest Treaty, such date is the date on which the microorganism was received by the international depositary authority.

Form DSM-BP/4 (sole page) 1186

Figure 6. A typical International Form used (at the DSM) for the deposition of a microorganism for patent purposes under the Budapest Treaty with a recognized International Patent Depository Authority.

Table 8. Micro-organisms deposited in some bacterial culture collections recognized under the Budapest Treaty for the purposes of patent procedure*

International Depository Authority	Number of deposits in 1983 and 1984†	Number of deposits in 1985 and 1986‡
Agricultural Research Culture Collection (NRRL), United States of America	801	236
American Type Culture Collection (ATCC), United States of America	826	1507(460)§
Centraalbureau voor Schimmelcultures (CBS), Netherlands	—	58
Collection National de Cultures de Microorganisms(CNCM), France	49	56 (for 1985 only)
Deutsche Sammlung von Mikroorganismen (DSM) Federal Republic of Germany	314	308
Fermentation Research Institute (FRI), Japan	689	552
In Vitro International Inc. (IVI) United States of America	41	80
Mezogazdasagi es Ipari Mikroorganizmusok Magyar Nemzeti Gyüjtemenye (MIMNG) Hungary	—	41 (for 1986 only)
National Collection of Industrial Bacteria (NCIB) United Kingdom	76	165
National Collection of Type Cultures (NCTC) United Kingdom	10	2

* Culture collections are unable to provide detailed information on such strains deposited with them under the Budapest Treaty although this information is available to the public after the announcement or publication of the patent.
† Data from WIPO Publication IP/STAT/1984/A (Publication A)
‡ Data received directly from the collections
§ Deposited under the Budapest Treaty

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We are grateful to Dr M. Kocur and Mr R. Radloff for the illustrations (*Figures 1a, b* and *Figures 3a, b*, respectively). We are indebted to Mrs H. Meunier, Mrs B. Neu and Mrs W. Hanneman for their technical assistance during the preparation of this review.

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Appendix A. Some bacterial service Culture Collections, their holdings and services to microbiology and biotechnology*

Country	Acronym	Name and address	Main interests†	Types of micro-organisms held‡	Services offered§
Argentina	—	Biotechnology MIRCEN, Planta Piloto de Process Industrials Microbiologicos (PROIMI), Avenida Belgranoy, Pasaje Caseros, 4000 SM de Tucuman Argentina	A,B,T,M	B,Y,R	1,4,9,10
	CCMA	Collection Catedra Microbiologia Agricula Facultad Agronomis Univ. de Buenos Aires Avda San Martin 4453 Buenos Aires Argentina	A,I,T	B,F,Y	9
	CCM–A	Collection de Cultivos Microbianos Facultad de Farmacia y Bioquimica de la Universidad de Buenos Aires Piso 4, BA, Argentina	A,I,T	B,F,Y	1,4,9,10
	IMA	Depto. de Microbiologia Inst. Nacional de Technologia Agropecuaria C.C.25- (1712) Castelar, BA, Argentina	A,T	B,R	9
Australia	WR	Azotobacteriaceae Collection, Queensland Wheat Research Institute 13 Holberton Street, Toowoomba 4350, Queensland, Australia	A,T	B	1,4,9

	DRLDFR	CSIRO Starter Culture Collection CSIRO Division of Food Research, Graham Road Highett 3190, Victoria Australia	B,J,T	B	4,9,10
	CB	Rhizobium Strain Collection, CSIRO Division of Tropical Crops and Pastures, 306 Cormody Road St Lucia 4067, Queensland, Australia	A,T	B,R	4,9,10
	UQM	Department of Microbiology University of Queensland St Lucia 4067, Queensland, Australia	A,B,T	A,B,F,R,Y	1,4,6,9,10
Austria	TRSG	International Research Society for Geomicrobiology and Soil Hygiene Mikrobiologisches Institut Penzinger Straße 99 A-1140 Vienna Austria	A,B,I,T	B,F,Y	1,9
Belgium	MCITM	Bacterial Culture Collection Institute of Tropical Medicine Nationalstraat 155 B-2000 Antwerpen, Belgium	M,T	B	1,4,9
	LMFW	Collection Lab. voor Microbiologie (LMG) Fakulteit Wetenschappen-Rijksuniversiteit Gent Ledeganckstraat 35 B-89000 Gent, Belgium	A,T	B	2,3,4,6,7
	—	Rhizobium Collection Faculté des Sciences Agronomiques Chaire de Microbiologie Gembloux Belgium	A,T	B,R	9

(*contd*)

Appendix A (*contd*)

Country	Acronym	Name and address	Main interests†	Types of micro-organisms held‡	Services offered§
Brazil	IAL	Colecao de Culturas Adolf Lutz Instituto Adolfo Lutz Av. Dr. Arnaldo 355 CEP 7027, Sao Paulo, Brazil	A,I,M,T	B,F,Y	1,4,9
	CERS	Colecao de Estirpes de Rhizobium Ssp. Inst.Agronomico de Campinas Av. Barao de Itapura 1481 Campinas, Sao Paulo, Brazil	A,T	B,R	1,4,9,10
	OCF	Culture Collection of Pathogenic Bacteria Oswaldo Cruz Foundation Avenida Brasil 4365 Rio de Janeiro, Brazil	M,T	B	1,4,9
	—	Rhizobium MIRCEN IPAGRO, Postal 776, 90000 Porto Alegre, Rio Grande do Sul Brazil	A,T	B,R	1,4,8,9,10
Bulgaria	BTCC	Bulgarian Type Culture Collection Inst. for State Control of Drugs Ministry of Health Vladimir Zaimov No. 26 Sofia, Bulgaria	A,B,M	B,F,Y	1,4,9,10

	PIR	National Agro-Industrial Union, N. Paushkarov Institute of Soil Sciences Shosse Bankja Str. No. 5 Sofia Bulgaria	A,T	B	9
Canada	DMCUWO	Dept Microbiology UWO Collection, University of Western Ontario Health Sciences Centre London, Ontario N6A 5C1, Canada	T,M	B	9
	FFPL	Microbiology Culture Collection Forintek Canada Corporation East Forest Products Laboratory 800 Montreal Road Ottawa, Ontario Canada K1A 0W5	A,T	B,F,Y	9,10
	—	Rhizobium Collection Research Institute Canada Agriculture University sub. PO London, Ontario Canada	A,T	B,R	9
	LSCC	Salmonella Genetic Stock Centre Department of Biology University of Calgary Calgary A, Alberta T2N 1NA, Canada	M,T	B	9
Chile	DMUC	Department de Microbiologia Universidad de Concepcion Casilla 272, Concepcion Chile	A,T	B,R	9

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Appendix A (*contd*).

Country	Acronym	Name and address	Main interests†	Types of micro-organisms held‡	Services offered§
Chile (*contd*)	UC	Laboratory of Microbiology and Immunology Department of Cellular Biology Catholic University of Santiago Casilla 10409, Santiago, Chile	M,T	B,F,Y	1,9
China	CCCCM	China Committee for Culture Collections of Microorganisms (CCCCM) Chinese Acad. Sciences Institute Micro. Zhongguancon, Beijing, China	A,B,I,M,T	B,F,Y	1,4,9,10
Colombia	CIAT	CIAT Rhizobium Collection Centro de International de Agricultura Tropical AA 67–13 Cali Colombia	A,T	B,R	4,9,10
Czechoslovakia	CCM	Czechoslovak Collection of Microorganisms J.E. Purkynje University of Brno Tr. Obrancu Miru 10 66243 Brno, Czechoslovakia	A,B,M,T	BF	1,2,3,4,7,9,10
	IEM	Czechoslovak National Collection of Type Cultures Inst. of Hygiene & Epidemiology Srobarova 48 10042 Praha 10, Czechoslovakia	M,T	B,F,Y	1,2,4,9,10

	CCEB	Culture Collection of Entomogenous Bacteria Institute of Entomology, CSAV Flemingovo N.2 Praha 6, Czechoslovakia	M,T	B	1,4,9,10
	CRIPP	Rhizobium Culture Collection Research Inst. of Crop Production Drnovska 507 Prague 6, Ruzyni 101 06, Czechoslovakia	A,T	B,R	9,10
Denmark	BCIH	Bacillus Collection Institute of Hygiene University of Aarhus Universitetsparken Building 161 DK-8000 Aarhus, Jylland, Denmark	M,T	B	1,9,10
	SSIC	Collaborating Centre for Reference and Research on Klebsiella (WHO) Statens Serum Institute 80 Amager Boulevard DK-2300 Copenhagen, Denmark	M,T	B	1,4,9,10
	—	Rhizobium Collection Department of Bacteriology State Laboratory for Soil and Crop Research Lyngby Denmark	A,T	B,R	9
Ecuador	INH	Biblioteca Instituto Nacional de Higiene y Medicina Inst. Minist. Salud Publica Cassila No. 3961 Guayaquil, Guayas, Ecuador	M,T	B,F	9,10

(*contd*)

Appendix A (*contd*)

Country	Acronym	Name and address	Main interests†	Types of micro-organisms held‡	Services offered§
Egypt	—	Biotechnology MIRCEN Ain Shams University, Faculty of Agriculture Shobra-Khaima, Cairo Arab. Republic of Egypt	A,B,I,T	B,F,Y,R	1,4,8,9,10
Finland	CMUH	Culture Collection of the Dept. of Microbiology University of Helsinki Vikki SF-00710, Helsinki 71, Finland	A,B,I,T	B,F,A	9,10
	—	Rhizobium Collection Biochemical Research Institute Kalevankatu 56B, Helsinki 18, Finland	A,T	B,R	9
	VTT	VTT Collection of Industrial Microorganisms Biotechnological Laboratory Technical Research Centre of Finland Tietotie 2 SF-02150 Espoo Finland	B,I,T	B,F,Y	1,3,4,7,10
France	CNCM	Collection Nationale de Cultures de Microorganisms Institut Pasteur 25–28 Rue du Docteur Roux F-75724 Paris 15 France	M,T	B,F,Y	1,2,3,4,9,10

	CUETM	Collection Unit Ecotoxicologie Microbienne INSERM Jules Guesde, 369 Villeneuve de Ascq, Nord, France	A,B,I,M,T	B	9
	IEMVT	Institut D'Elevage et de Medecine Veterinaire des Pays Tropicaux 10 Rue Pierre Curie F-94704 Maison-Alfort Val de Marne, France	M,T	B	1,9,10
German Democratic Republic	IMET	IMET Kulturensammlungen Zentralinstitut für Mikrobiologie und Experimentelle Therapie Beutenbergstrasse 11 Jena 69 DDR	I,M,T	B	1,2,9,10
Germany, Federal Republic	HIPU	Bakteriensammlung HIM Zentrum für Hygiene und Med. Mikrobiologie Klinikum der Phillips-Universität Pilgrimstein 2 Marburg, Hessen, FRG	M,T	B	1,9
	CCDAM	Culture Collection of Dept. Agricultural Microbiology University of Giessen Senckenbergstraße 3 D-6300 Giessen, FRG	A,I,T	B,F,Y	9
	DSM	Deutsche Sammlung von Mikroorganismen Grisebachstrasse 8 D-3400 Göttingen FRG	A,B,I,T	B,F,Y,R	1,2,3,4,7,8,9,10

(*contd*)

Appendix A (*contd*).

Country	Acronym	Name and address	Main interests†	Types of micro-organisms held‡	Services offered§
Germany, Federal Republic (*contd*)	IFAM	Institut für Allgemeine Mikrobiologie University of Kiel Olshausenstrasse 40/60 D-2300 Kiel Schleswig-Holstein, FRG	T	B,F	9,10
Greece	NUA	Department of Microbiology National University of Athens PO Box 1540 Athens, Greece	M,T	B	1,9
Hungary	DACT	Dept. Agricult. Chem. Technology Technical University Budapest Gellert Ter 4, 1111 Budapest XI, Hungary	A,B,I,T	B,F,Y	9,10
	CNCMB	Hungarian National Collection of Medical Bacteria National Institute of Hygiene Gyali Ut 2–6 H-1097 Budapest Hungary	M,T	B	1,9,10
	HMGB (MIMNG)	Microbiological Gene Bank University of Horticulture Somloi 14–16 Budapest H-1118, Hungary	A,B,I,M,T	B,F,R,Y	1,2,4,8,9,10

India	ICRISAT	ICRISAT Rhizobium Collection International Crops Research Institute for the Semi-Arid Tropics Hyderabad Andhra Pradesh 50324, India	A,T	B,R	4,9,10
	IARI	Microbiology Division IARI Culture Collection Indian Agricultural Research Institute New Delhi 110012, India	A,T	B,R,Y	9
	NCIM	National Collection of Industrial Microorganisms Biochemistry Division National Chem. Lab., CSIR Poona, Maharastra, 411-08, India	A,B,I,T	A,B,F,Y	4,9,10
	—	Rhizobium Collection Department of Microbiology University of Agricultural Sciences, Bangalore 24 India	A,T	B,R	9
Indonesia	BTCCI	Bakitwan-I Culture Collection Research Inst. for Animal Disease Jalan Martadinata Bogo, Java, Indonesia	M,T	B	9
	DMUIJ	Department of Microbiology University of Indonesia 16 Pegangsaan Timur Jakarta, Jakarta Pusat, Indonesia	M,T	B	9

(contd)

Appendix A (*contd*).

Country	Acronym	Name and address	Main interests†	Types of micro-organisms held‡	Services offered§
Iran	PTCC	The Persian Type Culture Collection Iranian Research Organisation for Science and Technology (IROST) Biological Science and Fermentation Technology Department 118 Felestine Ave. Opp. Kalantorry 7, Teheran Iran	I,T	B,F	1,9,10
Ireland	DIM	Dublin Industrial Microbiology Department of Industrial Micro. University College, Dublin Ardmore Stillorgan Road Dublin 3, Ireland	I,T	B,F,Y	9,10
	—	Rhizobium Collection Biology Department Agricultural Institute Johnstown Castle, Wexford, Ireland	A,T	B,R	9
Israel	GCL	Government Central Laboratories Ministry of Health 86 Jaffa Street Jerusalem, POB 6115, Israel	M,T	B	1,9
Italy	RV	Collection of Leptospira Strains Istituto Superior di Sanita Viale Regina Elena 299 Roma-Nementano 00161, Italy	M,T	B	1,4,9,10

	CSMA	Istituto di Microbiologia Agraria e Tecnica Universita di Firenze Piazzale Delle Cascine 27, Firenze, Italy	A,I,T	A,B,F,Y	9
	IMUP	Istituto de Microbiologia Universita de Parma Ospedali Riunita - Via Gramsci 14 Parma Italy	M,T	B,F,Y	9
	IPV	Istituto di Patologia Vegetale Culture Collection Cathedra die Micologia, Via Celoria 2 20133 Milano Italy	A,T	B	1,9,10
Japan	IFO	Culture Collection of the Institute for Fermentation Osaka Juso-Homachi 2-17-85 Yodogawa-ku, Osaka, Japan 532	A,B,I,T	B,F,Y	4,9,10
	IAM	Institute for Applied Microbiology University of Tokyo Yayoi 1-1-1, Bunkyo-ku, Tokyo, Japan 113	A,B,I,T	B,F,Y	4,9,10
	JCM	Japan Collection of Microorganisms Riken, Wako-shi Saitama 351 Japan	A,B,T	B,F,Y	1,3,4,6,9,10
	FRI	Fermentation Research Institute 1–3 Higashi 1-Chome Yatabe-machi, Tsukuba-gum, Japan	A,B,I	BFY	2,4,9,10

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Appendix A (*contd*).

Country	Acronym	Name and address	Main interests†	Types of micro-organisms held‡	Services offered§
Jordan	FACCUOJ	Faculty of Agriculture Culture Collection Univ. of Jordan University of Jordan, Amman, Jordan	A,T	B	9
	JVI	Jordan Vaccine Institute Ministry of Health Al Ashrafiah, Amman, Jordan	M,T	B	1,9
Kenya	—	Rhizobium MIRCEN Department of Soil Sciences and Botany University of Nairobi PO Box 30197 Nairobi Kenya	A,T	B,R	1,4,6,8,9,10
Malaysia	RRIM	Crop Protection and Microbiology Division Rubber Research Inst. Malaysia 260 Jalan Ampang Kuala Lumpur, Selangor, Malaysia	A,T	B,F	9
	MARDI	Rhizobium Culture Collection Malaysian Agricultural and Research Development Institute Serdang, Selangor, Malaysia	A,T	B,R	9
	SKUK	Simpanan Kultur Universiti Kebangsaan National Univ. Malaysia Department Microbiology Bangi, Selangor, Malaysia	A,I,T	B,F,Y	9

	—	Rhizobium Collection Department of Soil Sciences University Pertanian Malaysia Serdand, Selangor, Malaysia	A,T	B,R	9
Mexico	CDBB	Coll. Del Depto. De Biotec. y Bioinginiera Centro de Investigacion Del IPN Av. Inst. Politechnico Nac. 2508 Esguina Ticoman, Mexico 14	A,I,T	A,B,F,Y	9
	ENCB	Lab. Microbiol. Agricola, ENCB Instituto Politecnico Nacional Carpio y Plan de Ayala Mexico City, Mexico.	A,M,T	B,F,Y	4,9
New Zealand	NHI	National Health Institute NZ Reference Culture Collection 52–62 Riddiford Street Wellington, New Zealand	M,T	B,F,Y	1,4,9,10
	NZRCC	New Zealand Reference Culture Coll. DSIR Plant Diseases Division Private Bag Auckland, New Zealand	A,T	B,R	9
	PDDCC	Plant Diseases Division Culture Coll. Plant Diseases Division, DSIR Mt. Albert Road, Private Bag Auckland, New Zealand	A,T	B,F	9,10

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Appendix A (*contd*).

Country	Acronym	Name and address	Main interests†	Types of micro-organisms held‡	Services offered§
Nigeria	IITA	The IITA Coll. of Cultures Rhizobium International Institute of Tropical Agriculture PMB 5320, Oyo Road, Ibadan, Oyo State, Nigeria	A,T	B,R	9,10
Pakistan	—	Culture Collections of Microorganisms Botanical and Zoological Divisions Pakistan Museum of Natural History (PMNH), PSF Al-Markaz F-7/2 Islamabad Pakistan	A,I,T	B,F	1,4,9
	—	Culture Collection of Microorganisms Department of Microbiology University of Karachi Karachi Pakistan	A,I,M,T	B,F	1,4,9
Papua New Guinea	NGR	New Guinea Rhizobium Department of Primary Industry PO Box 2417, Konedobu Port Moresby, Papua New Guinea	A,T	B,R	9
Philippines	BIOTECH	Biotech Microbial Culture Coll. National Inst. Biotechnology and Applied Microbiology UPLB College Laguna 3720 The Philippines	B,I,T	B,F,Y	9

	UPCC	Natural Science Research Centre Culture Collection University of The Philippines Quirino and Roces Aves. Quezon City, Rizal, The Philippines	A,B,I,T	B,F,Y	1,4,9,10
	PTCC	Philippine Type Culture Collection Bureau of Research and Laboratories Department of Health PO Box 911 Manila, The Philippines	M,T	B	9
	NIST	Pre-Pilot and Culture Collection Microbiol. Research Department Nat. Inst. Science and Technology Pedro Gil St Malate, Manila, The Philippines	A,B,I,T	A,B,F,Y	4,9,10
Poland	PCM	Central Centre of Microorganisms Collection Microbiologic Committee of Polish Acad. Sciences Czerska 12, Wroclaw, PL-53114 Poland	M,T	B	9,10
	LOCK	Centre of Industrial Micro-organisms Collection Inst. of Fermentation and Technology Ul. Gdanska 166 Lodz, Poland	A,I,T	B,F,Y	9,10
	IPF	Collection of Industrial Microorganisms Inst. of Fermentation Industry Rakowieka 36 Warzawa, Poland	I,T	B,F,Y	9,10

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Appendix A (*contd*).

Country	Acronym	Name and address	Main interests*	Types of micro-organisms held‡	Services offered§
Poland (*contd*)	CRS	Collection of Rhizobium Strains Department of Microbiology Osada Palocowa Pulawy, Lublin, Poland	A,T	B,R	9,10
Puerto Rico	—	University of Puerto Rico Mayaguez Campus Department of Agronomy and Soils Mayaguez Puerto Rico 00708	A,T	B,R	9
Romania	ICFF	Collection of Industrial Micro-organisms (ICCF) Institutui de Cercetari Chimico-Farmaceutice 112, Soseana Vitan Bucharest 3, Romania	A,B,I,T	B,F,Y	9
	CPPBSF	Collection of Plant Bacteria and Fungi Industrial Interests Institute of Biological Sciences Splaiul Independentei 296 Bucharest Romania	A,I,T	BF	9
	—	Rhizobium Collection Department of Microbiology Research Institute of Cereal and Technical Plants, Bulev. Ion-Ionescu dela Brad No. 8, Bucuresti-Baneasa Romania	A,B	B,R	9

Senegal	—	Rhizobium MIRCEN Centre National de Recherches Agronomiques, d'Institut Sénégalais de Recherches Agricoles, BP51 Bambey, Senegal	A,T,	B,R	1,4,9,10
Singapore	USDB	Mycological Collection Department of Botany National University of Singapore Kent Ridge, Singapore 1025	A,I,T	B,F,R,Y	9
Spain	CECT	Coll. Espanola de Cultivos Tipo Dep. de Microbiologia Facultad de Ciencias Biologicas Valencia Burjasot, Valencia, Spain	I,T	B,F,Y	1,3,9,10,4
	CCMCU	Culture Coll. of Microorganisms Dept. Microbiology (Escuela Tecnica Superior de Ingenieros Agronomos) Ciudad University Madrid 3, Spain	A	B,F,R,Y	1,3,4,9,10
	—	Unidad de Fijaction de Nitrogeno Centro de Edafologia y Biologia Aplicada CSIC, Apartado 257 Salamanca Spain	A,T	B,R	9
S. Africa	SAMCC	S. African Mycobacterial Culture Coll. Tuberculosis Res. Inst. of S. African Med. Res. Council Private Bag X365 Pretoria, 0009, S. Africa	A,M,T	B	1,9,10

(*contd*)

Appendix A (*contd*).

Country	Acronym	Name and address	Main interests†	Types of micro-organisms held‡	Services offered§
S. Africa (*contd*)	SARC	South African Rhizobium Collection Plant Protection Research Institute Private Bag No. 134 Pretoria 0001, South Africa	A,I,T	B,R	9
Sri Lanka	MRICC	MRI Culture Collection Medical Research Institute Baseline Road PO Box 527 Colombo 8, Sri Lanka	M,T	B	9
	PDTCC	Peradeniya Type Culture Collection Department Microbiology Faculty Med. University of Peradeniya Peradeniya, Sri Lanka	M,T	B	9
Sweden	CCUG	Culture Collection of University of Goteborg Department of Clinical Bacteriology Guldhetsgatan 10 S-41346 Goteborg Sweden	M,T	B	1,3,4,7,9
	—	Rhizobium Collection Department of Microbiology Agricultural College, Ultana S-75007 Uppsala 7, Sweden	A,T	B,R,F	9

Switzerland	CHUV	Centre de Collections de Types Microbiens Rue de Bugnon 44 CH-1011 Lausanne, Switzerland	M,T	B	1,4,9
	ETHZ	Department of Microbiology Swiss Federal Institute of Technology (ETHZ) Universitätsstrasse 2 CH-8006 Zurich, Switzerland	I,T	B,F,Y	9
Thailand	DMIV	Dept. of Microbiology and Immunology Faculty of Tropical Medicine, University of Medical Sciences 42016 Rajvithi Road Bangkok 4 Thailand	M,T	B	9
	TISTR	TISTR Culture Collection and Food and Waste Recycling MIRCEN Thailand Institute of Scientific and Technological Research 196 Phahonyothin Road Bangkok 9, Thailand	A,B,I,T	B,F,Y	1,4,5,6,8,9,10
The Netherlands	—	Culture Collection Laboratory of Microbiology Delft University of Technology Julianalaan 67 A Delft The Netherlands	I,T	B	9
	LMD	Rhizobium Collection Institute of Soil Fertility Costerweg 41, Haren, Groningen, The Netherlands	A,T	B,R	9

(*contd*)

Appendix A (*contd*).

Country	Acronym	Name and address	Main interests*	Types of micro-organisms held‡	Services offered§
The Netherlands (*contd*)	CBS	Centraalbureau voor Schimmelcultures Oosterstraat 1 Postbus 273 3740 AG Baarn The Netherlands	A,B,I,T	B,F,Y	1,2,3,4,5,7,9,10
Turkey	KÜKENS	Culture Collection Department of Microbiology Istanbul Med. Fac. of Istanbul Univ. Temel Bilimler Binasi Capa-Topkapi, Istanbul, Turkey	M,T	B,F,Y	1,4,9
	RSTCC	Refik Saydam Type Culture Coll. Central Institute of Hygiene Cebeci Caddesi, Ankara, Turkey	M	B,Y	4,9,10
United Kingdom	NCDO	National Coll. of Dairy Organisms Institute of Food Research Shinfield, Reading, Berks, RG2 9AT UK	A,T	B	1,4,9
	NCIMB	National Collection of Industrial and Marine Bacteria Ltd. Torry Research Station, PO Box 31 135 Abbey Road, Aberdeen, AB9 8DG, UK	B,I,T	B	1,2,4,7,8,9,10

	NCTC	National Coll. of Type Cultures PHLS Central Public Health Lab. Service 61 Colindale Avenue London, NW9 5HT, UK	M,T	B	1,2,4,8,9,10
	RCR	Rothampsted Rhizobium Collection Rothamsted Experimental Station Harpenden, Hertfordshire, AL5 2JQ, UK	A,T	B,R	4,9,10
United States of America	TAL	Rhizobium MIRCEN NIFTAL Rhizobium Germplasm Resource, NIFTAL Project PO Box 'O' Paia, Hawaii 96779, USA	A,T	B,R	1,4,8,9,10
	—	Rhizobium MIRCEN Nitrogen-fixation and Soybean Genetic Laboratory, USDA Building, 011-A BARC-West, Beltsville, Maryland 20705, USA	A,T	B,R	1,4,9,10
	ATCC	American Type Culture Collection 12301 Parklawn Drive Rockville, Maryland 20852, USA	A,B,I,M,T	A,B,F,R,Y	1,2,3,4,5,6,7,8,9,10
	NRRL	ARS Culture Collection Northern Regional Research Centre Agricultural Research Service 1815 Nth University St. Peoria, Illinois, USA	A,I,T	A,B,F,R,Y	1,2,4,9,10
	IVI	In Vitro International Inc. 611 (P) Hammonds Ferry Road Linthicum, Maryland 21090, USA	A,B,I	A,B,F,Y	2,4,9

(*contd*)

Appendix A (*contd*).

Country	Acronym	Name and address	Main interests†	Types of micro-organisms held‡	Services offered§
Union of Soviet Socialist Republics	AUCM	All-Union Coll. of Microorganisms Institute of Biochemistry and Physiology of Microorganisms USSR Academy of Sciences Pushino, Moscow Region 142292 USSR	A,B,T	B,F,Y	9
	LIA	Cryobank of Microorganisms USSR Res. Technological Institute Antibiotics and Enzymes for Med. Use Ogorodnikov Prospekt, 41 Leningrad USSR	B,I,M,T	B,F,Y	1,4,9,10
	—	Rhizobium Collection Institute of Microbiology Armenian SSR Academy of Sciences Tscharentsa 19 Erevan 25 Armenian SSR USSR	A,T	B,R	9
	—	Rhizobium Collection All-Union Scientific Research Inst. of Agricultural Microbiology Gerzenstreet 42 Leningrad USSR	A,T	B,R	9

Venezuela	CME	Centro de Microskopia Electronica Universidad de Los Andes Apartado 163 Merida Venezuela	T	B	9
	INT	Instituto Nacional de Tuberculosis El Algodonal, Antimand Caracas 102 Venezuela	M,T	B,F	4,9,10
Yugoslavia	ISS	Institute of Soil Sciences Centre for Agricultural Research and Development T. Drajzera 7 PO Box 469 11000 Beograd, Serbia Yugoslavia	A,T	B,R	9
Zimbabwe	MAR	Grasslands Rhizobium Collection Soil Productivity Research Station P.Box 757 Marandellas, Zimbabwe	A,T	B,R	9

* Table does not include all the service collections in a country. This information is based on the *World Directory of Collections of Cultures of Microorganisms* (McGowan and Skerman, 1982), *IBP World Catalogue of Rhizobium Collections* (Allen, Hamatova and Skinner, 1973), *MIRCEN News* (published by UNEP/UNESCO/ICRO Panel on Microbiology, and UNESCO, Paris) and from the European Culture Collections Organization.

† A = Agriculture; B = Biotechnology; I = Industry; M = Medicine; T = General, Teaching, Research, Training etc.

‡ A = Algae; B = Bacteria (in general); F = Fungi; Y = Yeasts; R = Rhizobium (in particular).

§ 1 = Identification; 2 = Patent deposits; 3 = Safe deposits; 4 = Preservation; 5 = Biodeterioration testing; 6 = Computer search for strains; 7 = Contract work by negotiation/consultancy; 8 = Others, screening, isolation, pollution testing; 9 = General collection services, distribution, supply etc.; 10 = Catalogue of strains published.

Appendix B. Bacterial strains with special applications in biotechnology and their source

Special applications	Name of species or genera	Source* (collection numbers etc.)
Production of enzymes		
Agarase (EC 3.2.1.81)	*Cytophaga flevensis*	DSM 1076, ATCC 27944
	Pseudomonas elongata	ATCC 10144
Amylases (various)	*Bacillus macerans*	DSM 24
	Bacillus polymyxa	DSM 356
	Bacillus subtilis	DSM 7, 1060, ATCC 15841
	Bacillus amyloliquefaciens	ATCC 23842, 23845
	Streptomyces	Several strains at ATCC
Amylase (thermostable)	*Bacillus coagulans*	DSM 2357
	Bacillus licheniformis	ATCC 27811
Cellulase (EC 3.2.1.4)	*Cellulomonas uda*	DSM 20108
	Clostridium thermocellum	DSM 1237, 2360
	Thermomonospora fusca	ATCC 27730
Hydrogenase (EC 1.18.99.1)	*Acetobacterium woodii*	DSM 1030, ATCC 29683
	Clostridium pasteurianum	DSM 525
	Chemolithotrophic hydrogen bacteria	Several strains at DSM
Invertase (β-fructofuranosidase) (EC 3.2.1.26)	*Clostridium pasteurianum*	DSM 525
Nitrogenase (EC 1.18.6.1)	*Azotobacter, Azospirillum, Xanthobacter, Rhodospirillaceae*	*Several strains at ATCC, DSM etc.*
Protease	*Acremonium chrysogenum*	DSM 880
	Bacteroides amylophilus	DSM 1361
	Bacillus amyloliquefaciens	ATCC 23842–23845
Protease (thermostable)	*Streptomyces rectus*	ATCC 21067
Restriction endonuclease	Several species	Several strains at DSM
Ribonuclease	*Alcaligenes eutrophus*	DSM 541
	Bacillus amyloliquefaciens	ATCC 23842–23845

Miscellaneous	Several species	Several strains at ATCC, DSM etc.
Production of metabolites		
Alkaloids, quinoline	*Kitasatoa griseophaeus*	ATCC 29759
Amino acids	Several genera and species (see strains for specific amino acids)	Several strains at ATCC, DSM etc.
Antibiotics (Streptomycin, Penicillin, Tetracycline etc.)	*Streptomyces* and several other bacterial species (see strains for specific antibiotics	Several strains at ATCC, DSM etc.
Antifungal agents	*Pseudomonas lindbergii* *Streptomyces* species	ATCC 31099 Several strains at ATCC
Antitumour agents	*Clostridium perfringens* *Flavobacterium* sp. *Streptomyces pyogenes*	ATCC 29348 ATCC 21044 ATCC 21059, 21060, 21546
Insecticides (insect toxins etc.)	*Bacillus cereus* *Bacillus thuringensis*	DSM 508, ATCC 21281 Several strains at ATCC, DSM
Insulin	*Escherichia coli*	ATCC 31448, 31449
Steroids	*Micrococcus*, *Pseudomonas*, *Streptomyces*, etc.	Several strains at ATCC, DSM etc.
Organic acids		
Citric acid	*Acinetobacter lwoffii* *Arthrobacter paraffineus* *Arthrobacter* sp. *Bacillus licheniformis* Several other species	ATCC 21683 ATCC 15590, 15591 DSM 312 ATCC 21610
Lactic acid	*Bacillus coagulans* *Bacillus* sp. Several *Lactobacillus* species	DSM 2311, ATCC 10545 DSM 442, 444, 445 and several other strains ATCC 11443, 9649, 8041
Tartaric acid	*Acinetobacter tartarogenes* *Agrobacterium viscosum* *Nocardia tartaricans*	ATCC 31105 ATCC 31113 ATCC 31190, 31191

(*contd*)

Appendix B (*contd*).

Special applications	Name of species or genera	Source* (collection numbers etc.)
Polysaccharides	*Lysobacter gummosus*	ATCC 29489
	Xanthomonas campestris	ATCC 13951, DSM 1706
	Leuconostoc mesenteroides	DSM 20187, 20240, 20241
	Azotobacter vinelandii	DSM 85, several other strains at ATCC, DSM
Poly-β-hydroxybutyrate	*Alcaligenes latus*	DSM 1122–1124
	Azotobacter sp.	DSM 1721–1723
	Azotobacteriaceae	Several strains at DSM
Single-cell protein	*Alcaligenes eutrophus*	DSM 428, 531, 541
	Cellulomonas uda	DSM 20108
	Cellulomonas cartae	DSM 20106, ATCC 21681
	Arthrobacter petroleophagus	ATCC 21494
	Mycobacterium petroleophilum	ATCC 21497
Fatty acids	*Bacillus alvei*	DSM 29
Cellulose	*Acetobacter aceti* subsp. *xylinum*	DSM 2004, 2325
	Sarcina ventriculi	DSM 286, 300
	Acetobacter pasteurianus	ATCC 10821, 23769
	Acetobacter aceti	ATCC 23747
Vitamins		
B_{12}	*Arthrobacter* sp.	ATCC 31263
	Streptomyces sp.	ATCC 11071, 11072
	Ancylobacter sp.	DSM 1106, several other strains at DSM
C	*Escherichia coli*	ATCC 9492
K	*Serratia marcescens*	DSM 30121
Biotin	*Corynebacterium primorioxydans*	ATCC 31015, 31016
	Pseudomonas mutabilis	ATCC 31014

Production of fuel and petroleum substitutes		
Acetone and butanol	*Clostridium acetobutyricum*	DSM 792, 1731, 1732, 1739 ATCC 824, 4259, 10132
	Clostridium sp. *Clostridium saccharoperbutylacetonicum*	DSM 2152 ATCC 27022
Ethanol	*Clostridium acetobutylicum* *Clostridium thermocellum* *Zymomonas mobilis* subsp. *mobilis* *Zymomonas mobilis*	DSM 1739, ATCC 10132 DSM 1213, 2360, ATCC 31924 DSM 424, ATCC 10988 ATCC 31821–31823
Hydrogen	*Anabaena* sp. *Rhodobacter capsulatus*	ATCC 33047 DSM 152, 155 ATCC 17013, 17014
	Rhodospirillaceae, Chromatiaceae and Chlorobiaceae	Several strains at DSM
Methane	*Methanobacterium, Methanococcus, Methanogenium, Methanosarcina, Methanolobus* and other methanogenic bacteria	Several strains at DSM
Biogas	Depending on the raw material, several methanogenic bacteria	Several strains at ATCC, DSM
Biological control of:		
Fly larvae	*Bacillus cereus*	ATCC 21282
Mosquitos	*Bacillus cereus* *Bacillus sphaericus*	ATCC 21282 ATCC 33203
Dipteran larvae	*Bacillus thuringiensis* subsp. *israelensis*	ATCC 35646
Frost damage	*Erwinia ananas*	ATCC 31225
Control of environmental pollution		
Hydrocarbon degradation	*Acinetobacter calcoaceticus* *Arthrobacter paraffineus* *Rhodococcus* sp.	DSM 590, 1139 ATCC 15590, 15591 ATCC 29671, 29673 DSM 43290

(*contd*)

Appendix B (*contd*).

Special applications	Name of species or genera	Source* (collection numbers etc.)
Petroleum degradation (oil spills etc.)	*Achromobacter* sp.	ATCC 21910
	Arthrobacter sp.	ATCC 21908
	Acinetobacter calcoaceticus	ATCC 31012
	Rhodococcus sp.	ATCC 21504, 21507
Asphalt degradation	*Nocardia coeliaca*	DSM 43190
Desulphurization of fuels	*Acinetobacter* sp.	Cited in patent applications
	Pseudomonas sp.	
Cellulose degradation	*Acetovibrio cellulolyticus*	ATCC 33288, DSM 1870
	Polyangium cellulosum subsp. *ferrugineum*	ATCC 25531
	Clostridium thermocellum	DSM 1237, 1313, 2360
		ATC 31924
	Polyangium cellulosum subsp. *fascum*	ATCC 25569
	Cellulomonas	Several strains at DSM
Poly-β-hydroxybutyrate	*Pseudomonas delafieldii*	DSM 64
	Pseudomonas facilis	DSM 550, 620, 649
Pulp and paper mill waste water	*Pseudomonas aeruginosa*	ATCC 31482
Phosphorus removal from waste water	*Acinetobacter calcoaceticus*	DSM 1532
Nitrate removal from waste water	Unidentified bacterial mixed culture	ATCC 31192
Cyanide and nitrile removal from waste water	*Bacillus subtilis*	ATCC 21697
	Corynebacterium sp.	ATCC 21698
	Rhodococcus rubropertinctus	ATCC 21930
	Bacillus subtilis	ATCC 21697
Hydrogen sulphide removal	*Chromatiaceae* and *Chlorobiaceae*	Several strains at DSM
Iron pyrites removal from coal	*Thiobacillus ferrooxidans*	ATCC 19859
Sulphur degradation (reduction)	Several genera and species of Thermoproteales	Several strains at ATCC, DSM, NCIB
Sulphur degradation (oxidation)	*Thiobacillus thiooxidans*	DSM 504, 612
	Sulfolobus brierleyi	DSM 1651
	other *Thiobacillus* and *Sulfolobus* spp.	Several strains at ATCC, DSM, NCIB

Sulphate removal (reduction)	*Desulfobacter, Desulfomonas, Desulfotomaculum, Desulfovibrio, Desulfosarcina* and other related genera	Several strains at ATCC, DSM, NCIB
Miscellaneous		
Leaching of ores (recovery of iron, copper, uranium and other metals)	*Thiobacillus ferrooxidans* *Thiobacillus thiooxidans* *Leptospirillum ferrooxidans* *Sulfolobus* species	Several strains at ATCC, DSM, NCIB
Cleaning of metallic surfaces	*Thiobacillus ferrooxidans* *Thiobacillus thiooxidans*	Several strains at ATCC, DSM, NCIB
Degradation of aromatic compounds	*Pseudomonas putida* *Acinetobacter calcoaceticus* *Arthrobacter* sp. *Rhodococcus erythropolis*	DSM 291, 50202, 50208 DSM 590 DSM 20389 DSM 43066
Production of vaccines		
Cholera Colibacillosis	*Vibrio cholerae* *Escherichia coli*	ATCC 9459 ATCC 21972 ATCC 31616–31619
Haemophilus influenzae	*Haemophilus influenzae*	ATCC 31517
Miscellaneous	Several genera and species	Several strains at ATCC, NCTC
Assay of amino acids, antibiotics, vitamins and other compounds	Several genera and species	Several strains at ATCC, DSM, NCIB, NCTC
Resistance, sensitivity and other testing	Several genera and species	Several strains at ATCC, DSM, NCIB, NCTC

* ATCC = American Type Culture Collection; DSM = Deutsche Sammlung von Mikroorganismen; NCIB = National Collection of Industrial Bacteria; NCTC = National Collection of Type Cultures. For more details see the catalogues of such collections which also enlist supporting literature on the application of such strains and use in patent applications (Claus, Lack and Neu, 1983; Gherna, Nierman and Pienta, 1985).