"100 YEARS OF CULTURE COLLECTIONS"

PROCEEDINGS OF THE KRAL SYMPOSIUM TO CELEBRATE THE CENTENARY OF THE ESTABLISHMENT OF THE FIRST RECORDED SERVICE CULTURE COLLECTION

WORLD FEDERATION FOR CULTURE COLLECTIONS

100 YEARS OF CULTURE COLLECTIONS

Proceedings of the Král Symposium to celebrate the centenary of the establishment of the first recorded Service culture collection

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September 19, 1990
International House, Osaka

Organized by WFCC
Chaired by Mrs. Barbara Kirsop, President of WFCC
Convenors: Dr. L.I. SLY and Dr. T. IIJIMA

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FOREWORD

The World Federation for Culture Collection (WFCC) is an International organization committed to fostering the activities of culture collections of microorganisms and cell lines, and fostering communication between collections, their users and the scientific and industrial community in general. The WFCC is an Interdisciplinary Commission of the International Union of Biological Sciences and the International Union of Microbiological Societies (IUMS).

It was indeed a fortunate occurrence that the centenary of the establishment of the first recorded service culture collection coincided with the IUMS Congress of Bacteriology and Mycology in Osaka, Japan. The WFCC organized and held the Král Symposium "100 Years of Culture Collections" on Wednesday, September 19th, 1990 dedicated to Dr. Frantisek Král who established this collection one hundred years ago in Prague.

It was clear from the Symposium and from this book which records this event that Dr. Král was a man of vision. He foresaw the value of collections of microorganisms as resources for microbiology, and as sources of cultures for effective diagnosis, research, education and industry. Many of his innovative accomplishments remain the goals of culture collections one hundred years later. Culture collections continue to face the challenges of providing services of high quality. It is clear that if these challenges are faced with the vision of Král that the solutions will result in relevant, efficient and comprehensive culture collections able to meet the expanding demands of science and industry.

Perusal of the list of participants demonstrates the wide interest in this symposium and recognition of its fundamental significance to microbiology. The symposium was attended by many eminent microbiologists many from the major service culture collections of today.

We thank the IUMS conference organizer for including the symposium in the conference program. We also thank the Japan Federation for Culture Collections (JFCC) for financial support for this Symposium and the United Nations Educational, Scientific and Cultural Organization (UNESCO), and the Institute for Fermentation, Osaka (IFO) for their financial support which allowed the proceedings of the symposium to be published in this book.

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INTRODUCTION

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It seems probable that the first known service collection of micro-organisms was established a hundred years ago. A century later it is interesting not only to look back and see what has changed, but also to look forward and try to judge what developments will have taken place by 2090. The aim of the WFCC's Centenary Symposium, dedicated to the Czechoslovakian microbiologist, Dr. Frantisek Král, has been to mark this centenary by a consideration of past achievements and future possibilities.

In 1890, when the first service culture collection was established by Dr. Král, pure culture techniques were in their infancy: it was only in 1878 that Lister had developed the dilution technique that enabled the first pure bacterial cultures to be made. The debate about whether there could be fermentation without life was still continuing. Bacterial names had not the taxonomic significance that we recognize today. Under these circumstances, it is remarkable that a pure culture collection should have been established, and we can only speculate that the impetus for this revolutionary development was the earlier illegal importation of the lager process and lager yeasts into Czechoslovakia from Bavaria.

Dr. Král clearly had an unusual understanding of the future needs of the microbiological community of the time. By offering a culture service and producing catalogues of strains that were maintained in his collection, he prepared the ground for future developments world wide.

By the 1930's a number of important collections had been established and were providing a scientific service. Most had been

developed as support activities to existing microbiological laboratories or institutes. With time, many new collection were established, or existing ones expanded specifically to provide a service function. Their role was to collect, preserve and distribute authenticated material and to serve as taxonomic reference centers.

Responding to the recent developments in biotechnology, and using new technological tools, a whole range of support services have been added to these primary functions. As well as providing the traditional basic services, collections now offer professional consultancy and services for patents and regulatory requirements, are primary sources of expertise in taxonomy, identification and preservation, and provide specialized support (isolation, screening, industrial testing) in a growing number of areas. Computers have added to the range of activities available from collections and the essential data known previously only to the curatorial staff is now made readily available to the user community through databases and networks.

Now in 1990, there is a growing recognition of the need to record the biodiversity of the world's resources and to conserve the biological gene pool both as the basic resource for fundamental studies in ecology, molecular biology and genetics, and as the raw material for the development of novel products of importance to mankind. In these endeavours the significance of the worlds' "microbiological wealth" is increasingly understood and the conservation and taxonomic skills residing in the culture collections of the world must provide fundamental support for future studies.

Fortunately, culture collections have responded energetically to environmental and industrial needs and are well placed to support the new initiatives now being discussed. Increasingly, funding agencies understand the need for infrastructural support; increasingly it is recognized that taxonomic studies are an essential forerunner to developments in pure and applied microbiology; increasingly it is acknowledged that the preservation of biological material

in a stable, unchanged condition is essential for microbiological advancement.

Since 1972 the WFCC has played an important role in encouraging exchange of ideas and collaborative activities among the collections of the world. A number of committees have been set up to carry out specific tasks in different aspects of culture collection work. Books have been written, videos made, training courses and individual instruction provided, representations to patent and postal organizations have ensured the establishment of practicable regulations, databases and networks have been set up and sponsored, endangered collections have been 'saved' - for who can say what will be important in the future? -, guide lines for collections have been established and meetings held.

It is clear to us today that there is a major role for collections of pure cultures to play in the scientific effort of the future. It is unlikely that Dr. Král could have envisaged the microbiological developments that have taken place in the last century. But it is certain that had he been aware of the present day possibilities he would have responded vigorously.

The WFCC hopes the 'Král Symposium' at the IUMS Congress in Osaka, will serve as a landmark for the future, encapsulating the state-of-the-art in culture collections in September 1990. The meeting was addressed by a number of scientists highly experienced in work of culture collections and well qualified to assess past and present activities; the publication of the proceedings will serve as a permanent record of this occasion.

History of the KRAL Collection

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Masaryk University, Brno, Czechoslovakia

The idea of establishing culture collections arose in the years 1880 - 1890. It was at the time when solid cultivation media such as potato, gelatin and agar were first devised . Microbiologists were successful in the first isolation of pure cultures and this naturally led to the need for preservation of the isolated cultures for future work.

At that time several private collections arose, particularly within major microbiological institutes in Paris, Berlin, London and in Japan, too. Unfortunately, there are few documents about their existence.

Only a few microbiologists today know that one of the first collections was established in Prague in about 1890. It was founded by Frantisek Král (Fig 1). The collection was known under the name Král'sche Sammlumg von Mikroorganismen.

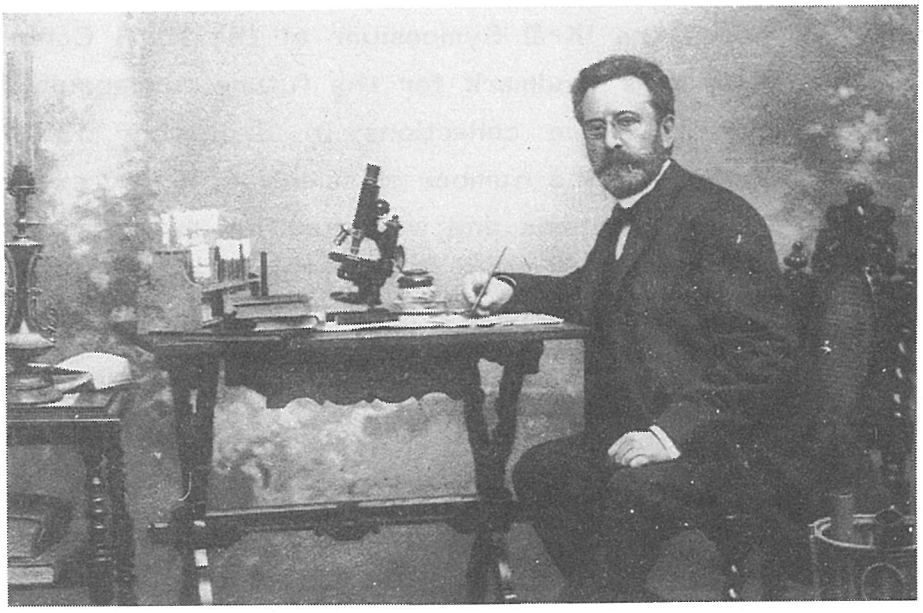


Fig. 1 Frantisek Král (1846-1911) founder of the first collection

Who was Frantisek Král? According to a report in the book entitled "Die K.K. Deutsche Technische Hochschule, Prag. 1806-1906", we know that F. Král was born in Prague on December 1, 1846. Having finished his apprenticeship he was employed with the firm Venceslaw (later Frantisek) Batka, manufacturing physical and chemical equipment. He worked with that firm for about 30 years. From 1887 he was employed at the Institute of Hygiene, German University, Prague, headed by Professor Isidor Soyka and from 1889 to 1892 at the Clinic of Dermatology, headed by Professor P.J. Pick.

At these institutes he acquired his experience with isolation, cultivation and maintenance of microorganisms. The experience, together with that of his previous employment (particularly the experience with manufacturing laboratory glass) was employed in establishing his private bacteriological laboratory and culture collection.

Frantisek Král must have been extraordinarily hard working and able since in 1894, despite being without formal university education, he was appointed Associate Professor of bacteriology at the German Technological University in Prague lecturing in bacteriology and mycology.

Král's collection was located on the first floor of a private house near the Old Town Square in Prague (Fig 2). Even at that time there were apparently objections from the authorities of the town to the fact that the collection maintaining pathogenic microorganisms was located in a private house (4).

How did Král come to the idea of establishing a collection of microorganisms? He was no doubt influenced by Prof. Soyka, head of the Institute of Hygiene, German University in Prague with whom he worked as a technical assistant in 1887 - 1889. Professor Soyka was the first to publish the method of preparation of the "museum display" cultures of microorganisms on solid media in 1887. A year

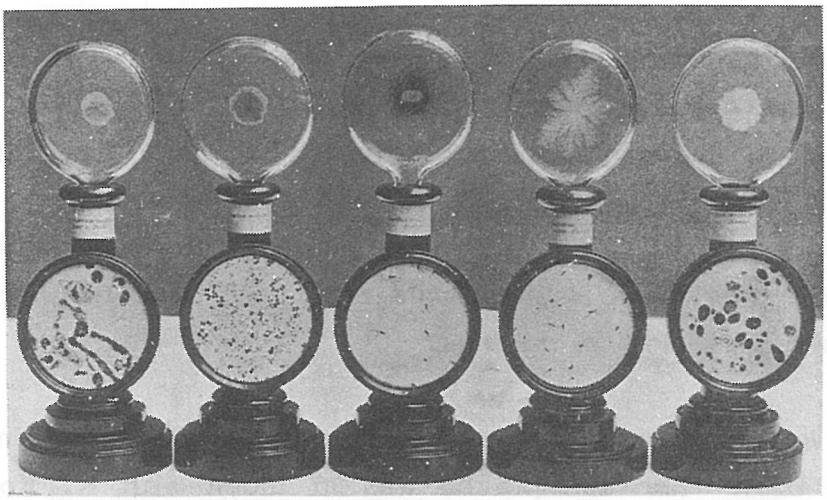
later, in 1888, Soyka and Král published a paper entitled "Suggestions and instructions for founding bacterial Museums" (1;8).

Fig. 2. Building where the Kral's culture collection was housed.

It was only later that Král found out that some of the cultures in those sealed preparations survived for a longer time than expected. This fact evidently crystallized the idea in his mind of establishing a long term collection of preserved microorganisms. The Král collection thus may be the first to collect and preserve strains of microorganisms for the general microbiological community.

What did Král and Soyka mean by "bacteriological museum"? They had in mind sets of permanent preparations of cultures of microorganisms for teaching purposes. Such "museum display" cultures were actually cultures on slant agar, gelatin or potato slices sealed in specially designed glass tubes or plates (Fig. 3).

Král's experience of almost 30 years with manufacturing glass laboratory equipment enabled him to achieve technical perfection in preparing these tubes.



Blastoderma salmonicolor Sarcina mobilis Bacillus cyanogenes Bacillus coli Saccharomyces anomalus

Fig. 3 A museum display of bacterial cultures which F. Král supplied for teaching purposes

About 1890 Král decided to open his own firm for manufacturing physical and chemical laboratory equipment. However, his application for a license was rejected by the municipal authorities and so instead Král established a private bacteriological laboratory, known as "Kral Bakteriologisches Laboratorium, Prag". This laboratory, which included the collection of microorganisms, was in existence for 21 years from 1890 until 1911.

Král's catalogue of cultures, which appeared in January 1900, is the first catalogue of cultures of microorganisms to have been published and thus of great importance to the history of microbiology. The catalogue included 40 pages and contained 7 figures with photographs of samples of the "museum display" cultures followed by photographs of samples of microscope slide preparations. The catalogue included about 800 strains of different microorganisms divided both alphabetically and according to other criteria, for instance, microorganisms pathogenic to man and animals, anaerobic bacteria, microorganisms for fermentation, dairy microorganisms, fungi and algae. From the introduction to the catalogue it is evident that it was not the first catalogue published by Král.

Earlier editions of the catalogue have, however, not yet been found.

How did Král acquire cultures for this collection? Král obtained cultures for his collection directly from the authors who isolated them and published their descriptions. Only a few strains of bacteria, yeasts and fungi were isolates from his own work. In the introduction to his catalogue Král appeals to microbiologists to deposit their cultures in the Král's collection, thus helping to preserve valuable biological material.

Král was in correspondence with many microbiologists in Europe and the USA. For example he was in touch with R. Koch, who deposited his strains of Mycobacterium tuberculosis in the Král collection, and Erwin Smith from Canada who deposited the strain of Agrobacterium tumefaciens (Bacterium tumefaciens) that he isolated from crown gall in 1907.

How did Král supply the cultures to the customers? Distribution of pathogenic microorganisms was restricted to the universities and to special bacteriological laboratories. The non-pathogenic microorganisms were supplied on a slant or stab of agar. Fungi and yeasts were mostly supplied on potato or beet slices in tubes. The tubes were sealed with wax to prevent drying during transport. Most cultures he supplied also survived overseas transport for several weeks. Král sold his cultures and teaching aids and it can be assumed that his collection brought in a reasonable profit.

Král published four further editions of his catalogue in the years 1902, 1904, 1906 and 1911. Unfortunately, only the catalogue published in 1911 has been preserved (Fig 4). The catalogue included 62 pages and several tables (2). It offered a wide range of microorganisms, such as microorganisms pathogenic to man and animals; plant pathogenic bacteria and fungi; acetic acid and dairy bacteria; thermophilic bacteria; luminous bacteria; strains of yeasts, fungi and algae.

The collection was later enriched by new groups of microorganisms, such as bacteria pathogenic for insects, wood rotting fungi


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

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

DER GEGENWÄRTIGE BESTAND

DER KRÁL'SCHEN SAMMLUNG

VON

MIKROORGANISMEN

MÄRZ 1911

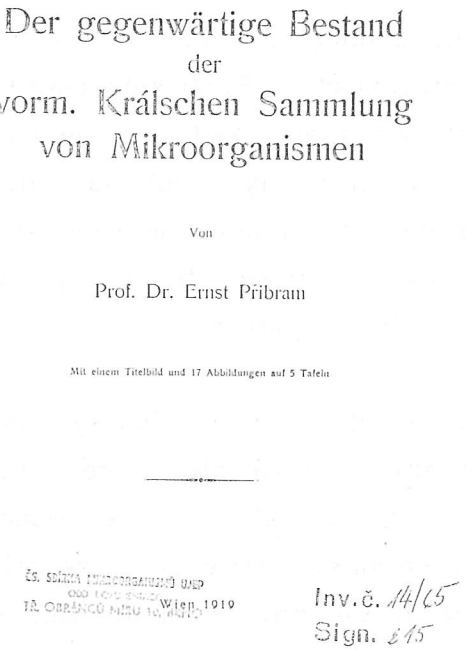
PRAG
SELBSTVERLAG. — DRUCK DER BÖHM. GRAPH. ACTIENGESELLSCHAFT „UNIE" IN PRAG

Fig. 4 Front page of the catalogue in 1911

and even cultures isolated from Charcot's South Pole expedition in 1905. In his last catalogue Král advertised an innovative set of "museum display cultures". Such sets could contain from 12 to 100 samples. Some of the specialized sets he supplied were: important human pathogens; a set of mycobacteria called "Tuberculosis museum"; important animal pathogenic microorganisms; plant pathogenic bacteria; sets of microorganisms for dairy, wine and brewery use, and soil and water microorganisms.

These new types of museum display cultures containing both colonies on agar and micrographs were distributed by Král from 1906. Other teaching aid that Král advertised in his catalogue were sets of slide preparations. Some of these still exist in the Museum of Natural History in Vienna. Král donated many such slides and museum display cultures to this museum in the years 1892 - 1896. He also manufactured projection slides of microscopic preparations

for the demonstration of different shapes, grouping and flagellar arrangement of bacterial cells. Král manufactured microscopic preparations from the cultures of his collection. However, he also obtained some unique microscopic preparations from various experts, for instance preparations of trypanosomes and spirochaetes from Professor Novy of Ann Arbor University, Michigan. The last edition of the catalogue of cultures which appeared in March, 1911 coincided with the end of Král's collection in Prague, as he died on July 22, 1911. The Král collection thus became one of the first endangered culture collections in the world. After Král's death in 1911 the collection was acquired by Professor Ernst Pribram and transferred to the State Serum Institute in Vienna in 1914. It seems that for at least 4 years nobody looked after it and consequently Prof. Pribram had to revive and re-examine all the cultures; unfortunately many of them had lost their viability. The work of reviving the collection was greatly complicated by the war events. Pribram re-examined the collection with the help of several other microbiologists and made considerable improvements including the addition of many strains.



Der gegenwärtige Bestand
der
vorm. Králschen Sammlung
von Mikroorganismen

Von

Prof. Dr. Ernst Přibram

Mit einem Titelbild und 17 Abbildungen auf 5 Tafeln

Wien 1919

Inv.č. 14/65
Sign. E 15

Fig. 5 Front page of Pribram's catalogue in 1911.

Figure 5 shows the front page of Pribram's catalogue. From its name and contents it is evident that he continued the work begun by Král. To keep continuity, Pribram also continued the original name of the collection - Král's Bakteriologisches Museum, Wien (5). He divided the cultures in his catalogue into five groups: water micro- organisms; saprophytic and parasitic microorganisms of man and animals; cultures important for agriculture; intestinal microorganisms and dairy microorganisms (5).

Pribram's catalogue already approaches modern catalogues in its arrangement. Whereas Král did not give any references in his catalogue to where the strains were published, Pribram, whenever it was possible, referred to the original literature. Pribram strongly stressed binomial nomenclature of organisms listed in his catalogue.

What has remained from this important collection? Several letters, a few copies of catalogues of cultures (1900, 1911, 1919); about 50 museum display cultures of bacteria and yeasts; a box with 72 slides with microscopic preparations of bacteria and yeasts. These materials are deposited in the Museum of Natural History in Vienna.

Some of Král's original subcultures were deposited by several American microbiologist (Breed, Hucker and others) with the American Type Culture Collection. We can still find some of them in the ATCC catalogue of cultures, for instance several Micrococcus roseus strains. The remaining cultures were most probably lost after the death of E. Pribram. in 1938.

We do not know the exact date when the Král collection definitely finished its activity. It could have been around 1927 when Pribram joined the Faculty of Loyola University Stritch School of Medicine in Chicago. He brought part of the Král Collection with him to USA. The second part which remained in Vienna was destroyed near the end of Warld War II.

Such was the history of the world-renowned Král collection. The next oldest collection was founded in 1906 at the CBS by the International Association of Botanists, we know, and of course it is still in existence in Baarn, The Netherlands. A full account of the history of culture cellections is given by Porter (6).

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The Impact of Chemotaxonomy on Culture Collections

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Chemotaxonomy was first applied to the study of relationships between plants based on such characteristics as flower pigments and essential oils. Recently, new approaches have been attempted to study microbial classification and identification based on information-bearing high molecular substances, semantides, such as DNA, RNA, and protein, and the interrelation and phylogeny of microorganisms have been described at the molecular level. Further, other cellular substances such as cell wall constituents, cell membrane constituents, respiratory systems, and enzymes are now included in the study of interrelation and identification of microorganisms.

In a broad sense, modern chemotaxonomic studies are focused on the cell constituents which deeply concern the maintenance of life. The term chemosystematics can more appropriately be used than chemotaxonomy, because chemosystematic studies include not only classification and identification but also phylogeny of microorganisms.

Within the context of the first publication of a comprehensive catalogue of microorganisms in Japan and the project of the reidentification of microbial cultures maintained in Japanese culture collections, the stability of cultures and impact of chemosystematics on the management of culture collections would be suggested.

In 1953, Kominami, who was the director of Nagao Institute in Tokyo, made a survey of microbial cultures maintained in Japanese collections. This project was sponsored by the Higher Education and Science Bureau, the Ministry of Education,

Japan. He collected data from 144 culture collections. These culture collections belonged to laboratories of universities, agricultural experimental stations of the Ministry of Agriculture and Forestry, foundations, and other organizations. As a result, "A General Catalogue of the Cultures of Microorganisms maintained in the Japanese Collections" was published in 1953, and contained data on 22,300 strains from 144 culture collections.

Further, after the publication of this catalogue, four collaborative projects were set up during the last 35 years for the reidentification of the cultures actually maintained in the culture collections (1st. 1954-1956, Coordinator, Dr. K. Sakaguchi; 2nd. 1958-1959, Coordinator, Dr. K. Sakaguchi; 3rd. 1961-1962, Coordinator, Dr. T. Asai; 4th. 1986-1988, Coordinator, Dr. K. Komagata). Reidentified cultures were deposited with culture collections affiliated with the Japan Federation for Culture Collections.

A large number of Japanese microbiologists participated in this projects, and showed their specialities in microbial taxonomy. Dr. Hasegawa, the previous director of the Institute for Fermentation, Osaka (IFO), took part in the reidentification of Rhodotorula strains, and collected the strains not only from Japanese culture collections but also from foreign culture collections.

Among the strains he collected, some originated from the same strain. However, these strains showed different cell shapes as if they were different strains. For example, one showed round cell shapes and other showed elongated cell shapes.

Cell size and shape of some species were described by the Dutch school as different from the original descriptions. For example, Torula koishikawaensis was described as being round

cells by Dr. Okunuki, the original author, in 1931, but this species was described as belonging to Rhodotorula longissima and to have elongated cells by Dr. Lodder in 1934 (1,2).

After that, a strain of Torula koishikawaensis played an important role in finding the sexual stage, teleomorph, of Rhodotorula glutinis, and this finding led to the establishment of the new genus Rhodosporidium, the first basidiomycetous yeast genus with a sexual life cycle, by Banno in 1967 (3).

Further, Dr. Hasegawa found that during subculturing in culture collections oval cells tend to produce elongated cells. The reason is not clear why such a change took place. However, it suggests that personnel in culture collections should handle cultures and records carefully and exactly.

Compared with morphological characteristics, biochemical characteristics seem to be rather stable. Nevertheless, during our studies, we often encountered differences of biochemical characteristics from the original descriptions, not only in yeasts but also in bacteria.

Chemosystematic characteristics are believed to be stable and reliable, and the data obtained are reproducible. Advantages of chemosystematics come from the following background:

1) The progress of molecular biology and related fields has made it possible to study the interrelation and phylogeny of microorganisms based on the information from semantides and cell constituents.

2) The development of instrumental analysis has made it possible to determine a small amount of cell constituents and a large number of specimens in a short time.

3) The development of computers has made it possible to analyze a large amount of data mathematically.

4) The development of preservation techniques of microbial cultures has made it possible to preserve fastidious

microorganisms and distribute them for the study to researchers all over the world.

5) The enrichment of culture collections has made it possible to obtain a large number of authentic cultures for taxonomic comparison.

At present, the following chemosystematic criteria are used: DNA base composition, DNA-DNA hybridization, cell wall composition, cellular fatty acid composition, phospholipid composition, quinone systems, cytochrome patterns, and electrophoretic comparison of enzymes and soluble proteins. Some of these characteristics are required to describe new bacterial taxa. Further, the phylogeny of microorganism has been studied based on 5S ribosomal RNA sequences and 16S and 18S ribosomal RNA partial sequences. The details are shown in Table 1. Recently, the 16S rRNA partial sequence has been used for the identification of bacteria that are not easily identified by routine techniques.

TABLE 1. Chemosystematic Criteria in Microbial Systematics

DNA Base composition
DNA-DNA hybridization
DNA-rRNA hybridization
Cell wall composition
1. Principal amino acid and type of peptide glycane in bacteria cell wall
2. Glycolyl test in coryneform and nocardia form bacteria
3. PMR spectra of cell wall mannans in yeasts
4. Cellular carbohydrate composition in yeasts
Cellular fatty acid composition
1. Major fatty acid composition
2. Hydroxy fatty acid composition
3. Mycolic acid composition
Phospholipid composition
Quinone system
Cytochrome pattern
Electrophoretic comparison of enzymes
Electrophoretic comparison of soluble proteins
Immunological studies
Pyrolysis
5S ribosomal RNA sequences
16S ribosomal RNA partial sequences
18S ribosomal RNA partial sequences

Gas chromatography, high performance liquid chromatography (HPLC), isotope facilities, and electrophoresis are used for the determination of chemosystematic characteristics. However, new techniques are being developed day by day. More correct, more reliable, and more simple techniques are required for better determinations; new and improved methods have been introduced in the study of modern chemosystematics. Major facilities for determination of chemosystematic characteristics are shown in Table 2. Computers are useful for data treatment in common.

TABLE 2. Major Facilities for Determination of Chemosystematic Characteristics

DNA base composition	Spectrophotometer equipped with a thermal controller. (Tm) HPLC
DNA-DNA Hybridization	Isotopic facilities Fluorometer
Cell wall composition	Amino acid analyzer (amino acids in bacterial cell wall) HPLC (sugars in yeast cell hydrolysate) NMR (cell wall mannans in yeast cells)
Cell fatty acid composition	Gas chromatography GC-Mass
Quinone systems	HPLC
Cytochrome patterns	Spectrophotometer
Electrophoretic comparison of enzymes	Electrophoresis Densitometer
5S rRNA sequence	Isotope facilities
16S rRNA partial sequences 18S rRNA partial sequences	Electrophoresis

For example, determination of DNA base composition is changing from the Tm method to the HPLC method. Melting temperature (Tm) of DNA has been used for the determination of DNA base composition for a long time. However, this is an indirect method and requires a rather expensive spectrophotometer equipped with a thermal controller. On the other hand, nucleotides or nucleosides derived from DNA are easily analyzed by HPLC (5). The HPLC method has the following advantages

compared with the Tm method: Direct determination; a small amount of DNA, (2 μg), for determination; a short time for analysis, 8 min for 1 run; and low standard error, approximately 1.0 %.

The photobiotin method for DNA-DNA hybridization is becoming popular because isotopes are not used for this method. Principal amino acids in the cell wall of coryneform bacteria and actinomycetes are analyzed by the use of the amino acid analyzer. GC-Mass spectrometry is used for the determination of the chemical structure of hydroxy fatty acids. Electrophoresis and isotope technique are used for sequencing of ribosomal RNAs. Computers are used with these analytical methods.

Chemosystematic data of microorganisms appear to be "finger prints" and are reliable for the characterization of individual microbial cultures. Thus the data are useful for correct maintenance of microbial cultures and smooth management of culture collections. In fact, recent catalogues of culture collections contain not only the history and taxonomic standing of cultures, but also chemosystematic characteristics as shown in Table 3.

TABLE 3. Strain Information in a Catalogue of Strains.

Corynebacterium glutamicum (Kinoshita, Nakayama and Akita) Abe, Takayama and Kinoshita
1318^T←ATCC 13032 ←Kyowa Hakko Kogyo Co., Ltd. 534.
=ATCC 13032 =CCM 2428 =DSM 20300 =IAM 12435 =IMET 10482 =KCTC 1445 =LMG 3730 =NCIB 10025.
Micrococcus glutamicus[1].
Type strain (596).
Source: Sewage.
Morphology (430,431,433,434); Biochemistry/Physiology (255,421); Cell wall, *meso*-DAP (253), acetyl type (276); Fatty acid (426,643); Quinone, MK-9(H_2) (263,391); G+C (mol%), 56.8 (Chemical) (423), 53.4 (Tm) (125); DNA-DNA(RNA) homology (259); 5S rRNA sequence (2499); Mycolic acids (392,425), Fatty acid synthase (1328); Taxonomy (250,256,422,424).
Produces L-glutamic acid (429).
Medium 22; 30C.

Cited from JCM Catalogue of Strains 4th ed. (1989)

In the old times of microbiology, microbial taxonomic studies needed rather simple optical microscopes and other equipment. The modern microbial taxonomic studies need much more upgraded facilities and much more expensive machines and equipment for its achievement.

The taxonomic study of microorganisms is one of the important concerns in culture collections. Therefore culture collections need to be equipped with gas chromatographs, high performance liquid chromatographs, isotope facilities, etc. Needless to say, computers are useful for the maintenance of chemosystematic data.

In order to strengthen the activity of culture collections, cooperation among them is needed to share the chemosystematic determinations, depending on the particular interests of individual culture collections. Consideration and realization of these aspects will contribute to the advancement of culture collections.

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The impact of computers on culture collections

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Campinas-SP, Brazil

INTRODUCTION

During the last decade, culture collections have been greatly affected by the rapid advance in biotechnology and microelectronics. The first increased the demand for cultures, specialized services and information, while the second affected scientific developments, data management and communication.

Most branches of science, and indeed many other aspects of human endeavor, have been radically transformed by computers. The electronic revolution, involving computers, microprocessors, and accompanying sophisticated software and increased storage capacity, has probably had the greatest overall affect on science, as a rapid tour of the laboratories of any scientific institution will show. Instrumentation technology has undergone dramatic developments and research activities are becoming more and more computer intensive. Microbiological research activities, such as taxonomy, physiology and genetics generate a great amount of information. The same applies to culture collection activities, such as collection administration, strain data storage and retrieval, catalogue preparation or identification.

Communication and dissemination of information, are other fields of activity which have undergone great changes recently, with telecommunication networks making computerized information and electronic mail available worldwide. Through database searching, it is now possible to combine literature searches with strain data in addition to patents information and business opportunities.

Different developments in computer science and technology are greatly affecting culture collections. Global developments in

hardware and software, computerization of laboratory equipment and of molecular biology, are just a few of the examples of the changes culture collections are undergoing. It is important to stress that the activities related to data standardization, database organization and management and electronic communication are also changing the role and importance of culture collections to the scientific, technological and industrial communities of the world.

GLOBAL DEVELOPMENTS

The rate of development of computer hardware is phenomenal and shows no sign of slowing down. More powerful processors with 386 and 486 chips, and drives with capacities in the order of "gigabytes" have changed personal computing dramatically in recent years. With greater capabilities, the 486 and eventually the 586 machines will evolve into super workstations. It will be possible to work on a PC using virtually any programming language and extremely powerful software is being developed. Local Area Network configurations with different micro-to-mainframe connections are also available.

Current hardware and software developments have made personal computers accessible, even to individuals. This is creating conditions for striking developments in the field of database management systems which were regarded as the exclusive province of large organizations with very large computers. This is now changing and new initiatives are being developed which will lead to a greater dissemination of information and integration of the scientific community worldwide.

Laboratory instruments such as gas-liquid chromatographs, high performance liquid chromatographs, mass spectrometers, spectrophotometers, scintillation counters and microdilution analyzers now include integrated computers or offer them as options (1). Research operation is becoming even more computer intensive and data logging and management of the resulting streams of microbial strain

data are creating the conditions for quantum leaps in the fields of microbial taxonomy and industrial screening programs (2).

The explosive growth in molecular biology, and especially DNA sequencing, has stimulated intensive computer use. Biological journals periodically devote entire issues on computer research recognizing the critical nature of computer based DNA analysis. Gene maps prepared for several species of microorganisms are now available, and data on gene sequences appear in the literature at such an accelerating rate that soon substantial libraries of genetic information will be available and subject to numerical taxonomic analysis.

Developments in computerization of microbiological data, associated with new technologies in immunology, oligonucleotide synthesis, robotic and microinstrumentation, has led to new screening programs in the major drug companies. Specialist screening companies have sprung up, and are playing an important role in the search and discovery of new pharmacologically active compounds (3). Computerization is fundamental to handle the great amount of data generated by the screening programs. According to Nisbet (4), it is now common to complete 1,000 assays in 30 minutes using automation and robotic systems. In the screening for novel therapeutics the number of samples tested is a major factor in the success rate. In order to be competitive, a screening program must achieve rates of 100,000 tests per year for as many as 20 different assay systems. Therefore it is clear that the rate limiting step is the number of strains available for testing.

DATABASES AND NETWORKS

Commercial and not-for-profit on-line databases are quickly gaining stature as effective information sources for biologists, engineers and other technical decision makers in the biotechnology industries and research institutions (5). Already widely used commercially, electronic communication is rapidly being adopted by the scientific community involved with culture collections (6). The

number of database users is increasing and there is a potential market to be explored (7). Specifically in the field of culture collections and strain data the function and differences of the major computerized databases for locating microorganisms have been reviewed by Kirsop (8). International, regional and national efforts in establishing databases and communication networks, and disseminating strain data are significantly affecting the development of culture collections worldwide.

The WFCC World Data Center for Collections of Microorganisms (WDC), created in 1972 and sponsored by UNEP and UNESCO, was relocated from Australia to the Institute of Physical and Chemical Research (RIKEN, Rikagaku Kenkyusho), in Japan in 1986. The WDC is involved with the development of an international directory of the description of culture collections and the production of a species oriented directory (9). The following WDC databases are available on-line: Culture Collection/Information (CCINFO), Species held in listed collections (STRAIN), World Catalogue of Collections of Algae (ALGAE), and Plant tissue culture bibliographic data (IRIS). The WDC activities of data collection, analysis and dissemination play an important role in the improvement of data quality of collections throughout the world.

The Microbial Strain Data Network (MSDN) is an internationally sponsored information network that provides mechanisms for locating microorganisms and cultured cells with specific properties, through an electronic communication system. Details of the origin, design and operation of the MSDN were discussed by Hill and Krichevsky (10). The MSDN electronic facilities are used by various scientific organizations in biosciences and biotechnology to fulfill their communication needs. Catalogue level information of important established service culture collections is accessible on-line through the MSDN network, and facilities for on-line ordering of cultures are provided (11). The MSDN is also a facilitating mechanism that builds links between databases. Recently links to the WDC at RIKEN in Japan, to the Data-star databases in Switzerland and to

the Tropical Data Base in Brazil were established. MSDN plays a fundamental role in stimulating the development of regional networks such as the Interamerican Microbial Information Service (IMIS) covering the Americas. Similar networks are being planned for Central and Eastern Europe and Island Nations.

Europe has realized the importance of information in the bioindustries' competitiveness (12), and several activities are being sponsored by the commission of the European Communities (CEC) to set up computerized microbial strain databases together with the European culture collections. The Information Center for European Culture Collections (ICECC) in Germany aims to become a central contact point for European scientists and an information center on culture collection related matters. The British database MiCIS (Microbial Culture Information Service) has been transferred to the ICECC as a first step in establishing a central European database.

The Microbical Information Network Europe (MINE) is a network of European culture collections cooperating in facilitating access and exchange of microbial strain data. A general format for computer storage and retrieval of data from different collections has been developed and the full data set contains much biotechnological and industrially important information.

In Japan, the Office for Life Science Promotion, recognizing the growing need for nationally unified information on laboratory organisms for life sciences, sponsored the development of a National Information System for Laboratory Organisms (NISLO). The program has been developed in collaboration with study groups specialized in laboratory animals, microorganisms, plants, algae, and animal and plant tissues and cell cultures. The processed information is available via computerized databases (9).

An Asian Information Network on Biomaterials is being developed and it is expected that the cooperation of Asian countries in this

field will promote further developments of the culture collections in the region (13).

In the United States of America, the American Type Culture Collection (ATCC) has created a highly specialized Department of Bioinformatics. The scope of its current projects includes design and development of scientific databases for internal use, customer support and for regional, national, and international networks. ATCC is playing an important role in the development of international standards for coding of microbiological data. The Bioinformatics Department is involved in numerous projects relating to biological data management, such as the Hybridoma Data Bank, the Database on Human and Mouse DNA Probes and Human Chromosome Libraries, the Directory of Biotechnology Information Resources, the Human Genome Project and the Microbial Databases as Taxonomic and Ecological Resources (14).

Also in the USA, BIOSIS has a database and bulletin board system for biologists, the Taxonomic Reference File (TRF). The databases focus on bacteriology, bacterial nomenclature, molecular biology and culture collections and is updated quarterly from BIOSIS previews (15).

The Microbial Germplasm Database is an on-line computerized database and network of American scientists and curators from culture collections containing microbial germplasm utilized primarily in plant-related research.

Developing countries have major difficulties, such as inadequate public infrastructure and lack of trained personnel. Information on regional activities is frequently scarce and the demand for specialized information services is normally lower. All this added together inhibits the development of databases and net works. Another major difficulty is the availability and cost of equipment. With the diminishing cost of microcomputers associated with the rapidly developing progress in computer technology, developing countries

are now able to establish databases and start organizing their scientific activities.

An example is the Tropical Data Base (BDT), an information center for culture collections in Brazil, which has its data on-line using a personal computer as host to the system. All software has been developed by the BDT and includes electronic mail facilities. The Brazilian experience shows how much can be achieved by data organization and dissemination associated with international cooperation (16). Developing countries setting up new regional initiatives should be encouraged to use existing communication and database networks to organize and disseminate regional information.

Inconsistencies in database organization and data coding greatly complicate, and may even prevent, electronic exchange of information between scientists. The committee on Data for Science and Technology, CODATA, is concerned with the quality and accessibility of data, as well as the methods by which data are acquired, managed, analyzed, and disseminated, and has established a Commission on the Terminology and Nomenclature of Biology. This commission aims to provide resources for the development of standards for terminology and nomenclature in the fields of biological sciences and bioinformatics. CODATA sponsored the publication of a coding system manual entitled "Coding Microbiological Data for computers" by Rogosa, Krichevsky, and Colwell (17). The manual, known as the "RKC Code", establishes a systematized language for describing the characteristics of microorganisms, such as bacteria, protozoa, algae, filamentous fungi and yeasts, and is being expanded to cover attributes of viruses and genetic strains. The code has been adopted by several international organizations, including the Directory of the Microbial Strain Data Network (MSDN). Software has been developed to manage and analyze phenotypic data on strains, permitting efficient storage and retrieval of this information. This software, called MICRO-IS, also incorporates the RKC codes and performs probabilistic identifications on the basis of test results (18).

FUTURE DEVELOPMENTS

It is important to stress that all taxonomic activity depends on an international network of communication and information. Although research can be, and often is, carried out individually, all taxonomy is dependent on a series of internationally agreed conventions concerning names, publications, and taxonomic structure. There is currently a renewed interest in taxonomic information. Systematic and evolutionary biology are no longer facing the problem of lack of information technology. The destruction of biological diversity is leading to the establishment of global programmes for species inventories and assessment of the role of microorganisms in the environment (19). The loss of biological diversity is a global crisis and will require international solutions. There will be new opportunities for real cooperation between industrialized and developing countries. Culture collections, as indispensable archives, will benefit from the species inventories programmes and electronic communication will play a fundamental role in facilitating cooperative projects. Developments in hardware and software, associated with decreasing prices, will allow the development of specific databases in the fields of biological diversity and strain data. The new possibilities of efficient gateways and efficient data retrieval, will make this wealth of data available to the user community.

CONCLUSIONS

We should find ahead of us an exciting era in the fields of taxonomy, computerization of culture collections, strain databases, and electronic communication. The ever growing information on microorganisms together with microcomputer developments, are leading to the development of comprehensive databases. Facilitated data transfer via standardization and speed of data storage and retrieval will lead to new adventures in biological exploitation.

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The Impact of Biotechnology on Culture Collections

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Biotechnology can be defined as "including any technique that uses living organisms (or parts of organisms) to make or modify products, to improve plants or animals, or to develop microorganisms for specific use." Recombinant DNA, cell fusion, and novel bioprocessing methods are all examples which can be cited (1). The "old biotechnology", i.e., the use of microorganisms for brewing and baking or selective breeding in agriculture and animal husbandry should be recognized as an important component of biotechnology and should not be discounted by the glitter of the so-called "new biotechnology."

In fact, biotechnology, both old and new, has become an integral part of research in industry, especially the pharmaceutical industry, with a strong move from technology development to clinical applications. The advances being made in biotechnology are swift and dazzling. For example, the first use of gene therapy was reported in late 1990 in the United States, whereby a four-year old child was treated at the National Institute of Health with engineered cells to correct an inborn error of metabolism. In a more practical vein, agriculture and hazardous waste management are rapidly adopting biotechnology-based methods for production and bioremediation, respectively. The source of the material and the repository are culture collection.

The effect of biotechnology on industry in the United States alone has been estimated by the U.S. Environmental Protection Agency to be ca. $20 billion currently and growing to ca. $100 billion by the year 2035 (2). Reviews of biotechnology applications by the Environmental Protection Agency for use of biotechnology

have increased exponentially in number. The types of applications for which patents have been applied are extensive, from agricultural chemistry to mineral recovery. The number of patents are more than 1,000 from 1980 to 1984. Similarly, the kinds of microorganisms involved in these patents include Gram positive and Gram negative bacteria, as well as fungi and yeasts, and the list expands almost daily.

The role of culture collections in the development of biotechnology is obvious in that accession and storage of cultures, including patent cultures, historically, are the responsibility of culture collections. However, new demands are being made on culture collections, as a result of the boom in biotechnology.

Modification of plants employing bacteria require that plasmids and other nucleic acid vectors, as well as the host bacteria, must be included in the inventories of culture collections. This task for the culture collection is, of course, not new, since bacteriophages have been among the stocks of culture collections for decades. More recently, mammalian tissues have become a part of the toolbox of biotechnology and cell lines, both modified and unmodified, are included in the holdings of many collections, worldwide. What is relatively new is that embryos of animals can now be stored frozen for genetic experiments. An example of the extension of biotechnology to animal systems is transgenic fish, produced by cloning and transfer of genes encoding the growth hormone, yielding fish which are larger in size and demonstrate a faster rate of growth (3).

Concomitant with the explosive growth in biotechnology, there is increasing recognition of the need to protect biodiversity throughout the world. Culture collections must respond to this new challenge by preparing to become the repositories of germ plasm, including nucleic acid sequences (the data bank of genetic diversity), and gene banks, thereby ensuring maintenance of genomes of endangered species. It can be anticipated that increasing attention

will be paid to culture collections as a repository of genetic information. For example, genetic sequences cloned within microorganisms could serve in the future as "living encyclopedias."

With the new methods for studying heredity that have been developed, using the technological advances of molecular biology, gene probes, nucleic acid sequences, and information on secondary and tertiary molecular structure of nucleic acids (coiling of molecules also imparts information, as well as the base sequences, i.e., the "building blocks" themselves) will comprise yet another set of entries for the culture collection informational array.

Interestingly, the future may find that large amounts of material need not be stored physically. The PCR method, for example, allows femtogram (fg) quantities of material to be amplified sufficiently to be detected. It may be necessary only to store tissues, small samples of soil, etc., as frozen "blueprints" or micro-ecosystems." The nucleic acids can be amplified and the ecosystem reproduced in future years.

Extreme environments are being explored extensively, such as the hydrothermal vents, and organisms residing therein when collected and studied in the laboratory will demand unusual conditions of incubation, such as both high pressure and high temperature for growth. These microorganisms and tissues of animals collected from these sites will comprised an expensive and challenging expansion of culture collection technology.

At the demand to protect biodiversity increases, along with a simultaneous rush to exploit the germplasm diversity of this planet, greater technical expertise and creativity will be required of culture collections. The Great Barrier Reef off the coast of Australia and coral reefs of many areas of the world offer enormous diversity and opportunity for exploitation for new pharmaceuticals and fine chemicals.

A new field of research affecting culture collections and the way they function is the zoological garden reproduction of endangered species by artificial insemination, surrogate parentage, and/or embryo implantation. Genetic material for this facet of preserving biodiversity, i.e., the germ plasm, will surely involve culture collections in the future. Other roles for culture collections in systematics, taxonomy, and as centers of excellence in systematics suggest a very exciting future for the curators of culture collections.

Systematics has moved aggressively to incorporate molecular methods and to use computerized data storage and analysis. These methods must be available at culture collections for modern curatorial work and research.

Molecular graphics, which has revolutionized protein chemistry and spawned biomolecular engineering is being adapted for systematics. In fact, a rebirth of systematics as a discipline, as well as population biology and evolution is underway. The "New biology of the 1940's " has transmagnified the "New Biology of the 1990's."

Bioremediation is an area where culture collections are very important, because they are the source of genetic material in microorganisms capable of degrading a variety of compounds. Bioremediation is rapidly becoming accepted and is viewed with great interest, especially in Eastern Europe, as a means to address environmental problems. The approaches taken to date are, essentially, nutrient addition to enrich for indigenous microorganisms capable of biodegradation of toxic compounds.

However, "customizing" microorganisms by genetic engineering, with genes degraditive pathways of interest is a very attractive alternative. Land treatment or use of bioreactors can be more effective in degradation of compounds in the environment if engineered microorganisms can be employed. Injection of

microorganisms to degrade chemicals contaminating ground water, composting of soil in landfills, have been used in environmental clean-up, but there is much yet to be done.

One example which can be offered is where PCB's and heavy metals are present in the same toxic dump site. Engineered microorganisms with genes coding for resistance to heavy metals can be engineered to incorporate genes coding for degradation of PCB. The result is a heavy metal-resistant, PCB-degrader, very useful for bioremediation of the heavy-metal PCB laden dump site.

Culture collections are the source of the genetic materials for such engineering feats. To fulfill this promise, engineered organisms will have to be released to the environment. Before this can be done, data on systematics and natural history of bacteria in soil, water, and air must be collected. Some of these microorganisms have been deposited in culture collections, but a great deal of work must be done before bioremediation can reach its full potential.

One of many capabilities of the "New Biology" is to identify organisms in ecosystems by extracting nucleic acids, purifying and sequencing them, and comparing computer-stored sequences with the freshly isolated sequences to identify the organism present in the environment, as has been done with Antarctic rock specimens (4).

Quite clearly, genetic engineering requires knowledge of both the genes and whole organism. The culture collection is the central player in all of this exciting activity. The creative collection curator must face squarely the dilemma of what materials to retain and what to discard. In this regard, it is important to keep in mind, the example of the polymerase chain reaction enzyme (PCR) from Thermus aquaticus, an important enzyme from a hot water geyser bacterium found Yellowstone Park. Had this quite ordinary organism, distinguished only by its ability to grow at elevated temperatures been discarded, an important and almost revolutionary

tool of molecular biology would have been lost. The culture collection of the future, it can be predicted, will play a much expanded role in science and society and will prove to be the source of new developments for biotechnology.

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APPENDIX 1

From the Poster Session of IUMS OSAKA '90
"Král's Bacteriologisches Laboratorium - Král Collection-
The First Recorded Service Culture Collection by
D. Fritze and M. Kocur

During IUMS Osaka '90, Dr. D. Fritze (Deutsche Sammlung von Mikroorganismen und Zellkulturen) and Dr. M. Kocur (Czechoslovak Collection of Microorganisms) presented historical material concerning the Král Collection, the first service culture collection in the world, at the poster session and the WFCC Král Meeting. This material is very interesting and valuable to microbiologists and staff in culture collections all over the world.
In the following pages, some of the material from their poster session is reviewed and presented.

THE PERSON OF FRANTISEK KRAL

Honorardozent **Franz Kral,**

Laboratoriums-Inhaber, geb. 1. Dezember 1846 zu Prag, absolvierte die Unterrealschule zu St. Jakob, trat dann 1860 in die Handlung der Firma Wenzel (später Franz) Batka mit physikalischen und chemischen Apparaten in Prag ein, in welcher er 30 Jahre hindurch, davon 24 Jahre als selbständiger Leiter und protokollierter Prokurist tätig war. Er arbeitete seit 1887 vier Semester hindurch am hygienischen Universitätsinstitute zu Prag unter der Leitung von Prof. J. Soyka, hierauf von 1889 bis 1892 acht Semester hindurch an der Dermatologischen Universitätsklinik zu Prag unter der Leitung des Prof. F. J. Pick; errichtete 1890 ein eigenes Privat-Laboratorium für bakteriologische Untersuchungen zu Prag, welches sich durch seine Arbeiten einen hervorragenden Wirkungskreis und weithin reichenden Ruf erwarb. Mit k. k. Minist.-Erlasse v. 15. November 1899 erhielt er den Lehrauftrag als Honorardozent für Bakterioskopie und bakteriologische Technik an der k. k. deutschen technischen Hochschule zu Prag, auf den Beginn mit dem Sommersemester 1900 lautend, in dessen Ausübung er bis jetzt tätig ist. Dozent *Kral* ist langjähriger Mitarbeiter am »Zentralblatte für Bakteriologie und Parasitenkunde«, am »Archiv für Dermatologie und Syphilis«, und am »Jahresbericht über die Fortschritte in der Lehre von den pathogenen Mikro-Organismen«.

Fig. 1. " Die Deutsche Technologische Hochschule Prag"

Page 389 of the centennial Book "Die Deutsche Technische Hochschule Prag 1806-1906" (Fig. 1) reads as follows :

Honorary Lecturer Franz Král, owner of a laboratory, born on the 1st of December 1846 in Prague, finished at the St.

Jacobs School (Unterrealschule), entered in 1860 the business of physical and chemical instruments of the firm Wenzel (later Franz) Batka in Prague, in which he worked for 30 years, 24 years thereof as a senior manager and authorized signatory. Since 1887, he worked for four semesters at the Institute of Hygiene of the University of Prague under the supervision of Professor J. Soyka, then from 1889 until 1892 for eight semesters at the Dermatological Clinic of the University of Prague under the supervision of Professor F.J. Pick; he founded in 1890 his own private laboratory for bacteriological investigations, which gained through its work a prominent sphere of activity and a far reaching good reputation. Through a ministry decree of November 15, 1899 he obtained a lecturership as an honorary Dozent for Bacterioscopy and Bacteriological Techniques at the German Technical University in Prague, starting with the summer semester 1900, in which position he is still active today. Dozent Král is a cooperator of many years' standing with the "Archiv für Dermatologie und Syphilis", and with the "Jahresbericht über die Fortschritte in der Lehre von den pathogenen Mikroorganismen".

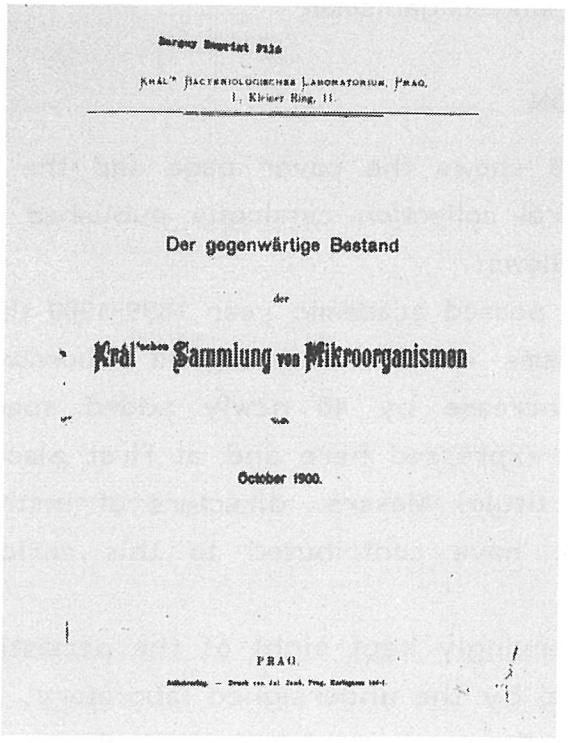
THE COLLECTION

Fig. 2 and 3 shows the cover page and the first two text pages of the Král collection catalogue published in 1900. The text reads as follows:

> During the passed academic year 1899-1900 the collection of microorganisms of the undersigned laboratory again can show an increase by 40 newly added species. Sincere thanks are expressed here and at first place to the P.T. (praemisso titulo) Messrs. directors of institutes and authors, who have contributed to this enrichment of the collection.
>
> It is unswervingly kept sight of the persistingly aimed at ultimate goal by the undersigned laboratory, to give to the researcher of any bacteriological field easy and painless

access to all microorganisms of special interest to him in the form of living and pure cultures. The sum of effort, expenditure of time and costs, known to all initiated in this field, which have to be devoted to the maintenance of (at present) approximately 800 species of microorganisms in the viable and pure state, does not suffice to approach this goal.

For this it needs further and broad general support from the side of the P.T. Messrs. directors of institutes and authors. To them, again the humble request is directed, to kindly supplement the collection by the donation of microorganisms which have been isolated and described by them or by their scholars, as well as of other microorganisms in their possession - as long as these are not listed in the present catalogue. Through this not only the purpose of public utility for bacteriology will be promoted and some microorganisms will be protected from extinction and accidental loss, but also the depositor will be spared the time consumption and cost of propagation, because the author or the institute, any time when wished, would receive cultures



Der gegenwärtige Bestand

der

Král'schen Sammlung von Mikroorganismen

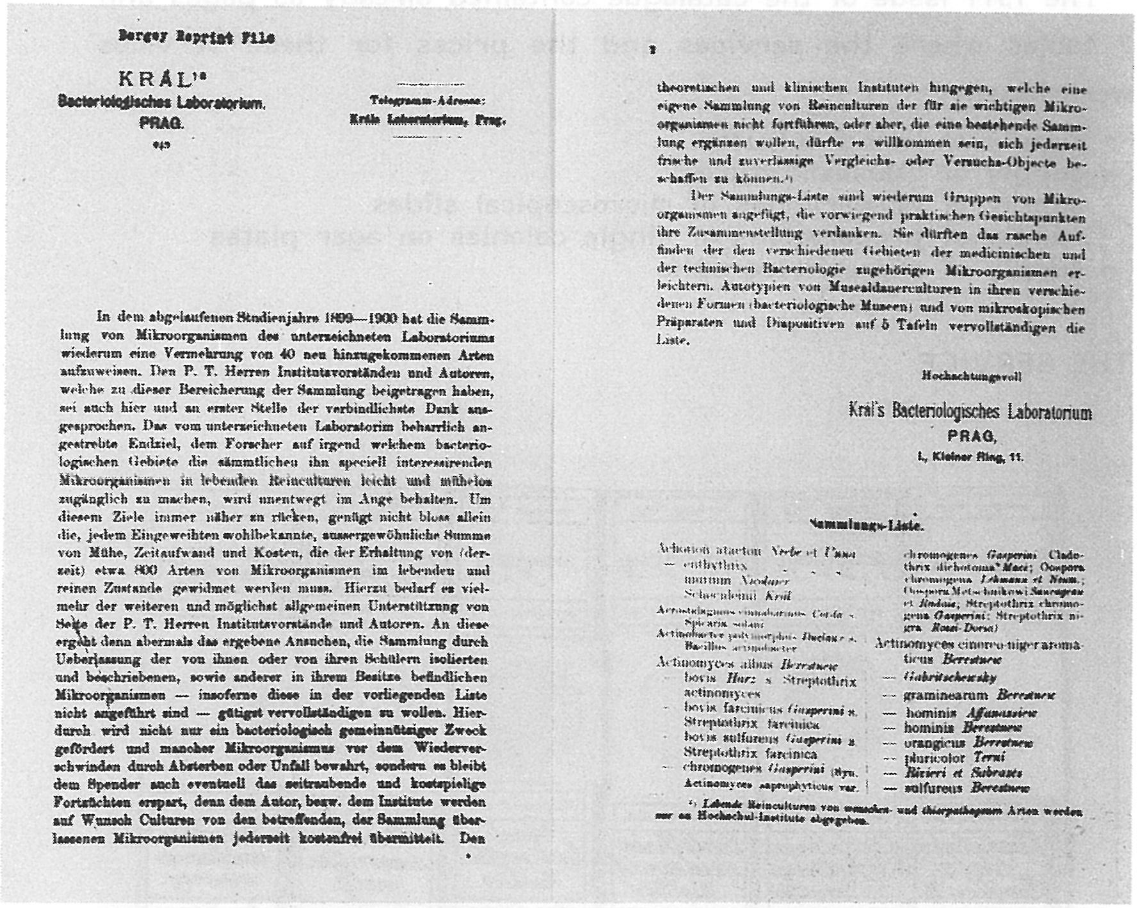
October 1900.

PRAG.

Fig. 2. The cover page of the Král collection catalogue in 1990

of the organism given to the collection without cost. However, for the theoretical and clinical institutes which do not carry on their own collection of pure cultures of microorganisms of importance for them, or which want to complete their existing collection, it must be welcome to procure at any time fresh and reliable comparison and test objects. * Again, groups of organisms have been added to the collection list, which have been arranged mainly according to practical aspects. These groups will ease the quick finding of the microorganisms affiliated to the different fields of medical and technical bacteriology.....

* Living pure cultures of species pathogenic to man or animals will be supplied only to university institutes.



Berger Reprint File

KRÁL's
Bacteriologisches Laboratorium.
PRAG.

Telegramm-Adresse:
Král's Laboratorium, Prag.

In dem abgelaufenen Studienjahre 1899—1900 hat die Sammlung von Mikroorganismen des unterzeichneten Laboratoriums wiederum eine Vermehrung von 40 neu hinzugekommenen Arten aufzuweisen. Den P. T. Herren Institutsvorständen und Autoren, welche zu dieser Bereicherung der Sammlung beigetragen haben, sei auch hier und an erster Stelle der verbindlichste Dank ausgesprochen. Das vom unterzeichneten Laboratorim beharrlich angestrebte Endziel, dem Forscher auf irgend welchem bacteriologischen Gebiete die sämmtlichen ihn speciell interessirenden Mikroorganismen in lebenden Reinculturen leicht und mühelos zugänglich zu machen, wird unentwegt im Auge behalten. Um diesem Ziele immer näher zu rücken, genügt nicht bloss allein die, jedem Eingeweihten wohlbekannte, aussergewöhnliche Summe von Mühe, Zeitaufwand und Kosten, die der Erhaltung von (derzeit) etwa 800 Arten von Mikroorganismen im lebenden und reinen Zustande gewidmet werden muss. Hierzu bedarf es vielmehr der weiteren und möglichst allgemeinen Unterstützung von Seite der P. T. Herren Institutsvorstände und Autoren. An diese ergeht denn abermals das ergebene Ansuchen, die Sammlung durch Ueberlassung der von ihnen oder von ihren Schülern isolierten und beschriebenen, sowie anderer in ihrem Besitze befindlichen Mikroorganismen — insoferne diese in der vorliegenden Liste nicht angeführt sind — gütigst vervollständigen zu wollen. Hierdurch wird nicht nur ein bacteriologisch gemeinnütziger Zweck gefördert und mancher Mikroorganismus vor dem Wiederverschwinden durch Absterben oder Unfall bewahrt, sondern es bleibt dem Spender auch eventuell das zeitraubende und kostspielige Fortzüchten erspart, denn dem Autor, bezw. dem Institute werden auf Wunsch Culturen von den betreffenden, der Sammlung überlassenen Mikroorganismen jederzeit kostenfrei übermittelt. Den

2

theoretischen und klinischen Instituten hingegen, welche eine eigene Sammlung von Reinculturen der für sie wichtigen Mikroorganismen nicht fortführen, oder aber, die eine bestehende Sammlung ergänzen wollen, dürfte es willkommen sein, sich jederzeit frische und zuverlässige Vergleichs- oder Versuchs-Objecte beschaffen zu können.*)

Der Sammlungs-Liste sind wiederum Gruppen von Mikroorganismen angefügt, die vorwiegend praktischen Gesichtspunkten ihre Zusammenstellung verdanken. Sie dürften das rasche Auffinden der den verschiedenen Gebieten der medicinischen und der technischen Bacteriologie zugehörigen Mikroorganismen erleichtern. Autotypien von Musealdauerculturen in ihren verschiedenen Formen (bacteriologische Museen) und von mikroskopischen Präparaten und Diapositiven auf 5 Tafeln vervollständigen die Liste.

Hochachtungsvoll

Král's Bacteriologisches Laboratorium
PRAG,
I., Kleiner Ring, 11.

Sammlungs-Liste.

Actinomyces albus *Berestnew*
— bovis *Harz* (= Streptothrix actinomyces)
— bovis farcinicus *Gasperini* (= Streptothrix farcinica)
— bovis sulfureus *Gasperini* (= Streptothrix farcinica)
— chromogenes *Gasperini* (Syn. Actinomyces saprophyticus var. chromogenes *Gasperini*; Cladothrix dichotoma* *Macé*; Oospora chromogena *Lehmann et Neum.*; Oospora Metschnikowi *Sauvageau et Radais*; Streptothrix chromogena *Gasperini*; Streptothrix nigra *Rossi-Doria*)

Actinomyces cinereo-niger aromaticus *Berestnew*
— *Gabritschewsky*
— graminearum *Berestnew*
— hominis *Affanassiew*
— hominis *Berestnew*
— orangicus *Berestnew*
— pluricolor *Terni*
— *Rivieri et Sabrazès*
— sulfureus *Berestnew*

*) Lebende Reinculturen von menschen- und thierpathogenen Arten werden nur an Hochschul-Institute abgegeben.

Fig. 3. The first two text pages of the catalogue

It will no longer be possible to find out precisely the year in which F. Král began building up his collection. In the last edition of his catalogue in 1911 he writes the following: "Das gefertigte Laboratorium führt seit seinem Bestand, also seit mehr als zwei Decennien, genaue Aufzeichnungen über jeden der Sammlung überlassenen Mikroorganismus". - "Since its foundation two decades ago, the laboratory keeps precise records about each microorganism given to the collection". From this sentence it can be judged that the collection originated from 1890 or earlier. It might even date back to 1884, because in that year Prof. Soyka, head of the Institute for Hygiene of German University with whom Král had been working (in the years 1887 and 1888) asked for a grant of 1234 Guldens for the equipment of the collection and of the museum for the purpose of lectures in hygiene.

The 1911 issue of the catalogue contained already 63 pages and 7 tables where the services and the prices for these services were listed.

- supply of living cultures
- supply of "Musealkulturen"
- supply of preparations of microscopical slides
- supply of preparations of single colonies on agar plates
- supply of microphotographs

THE SERVICE

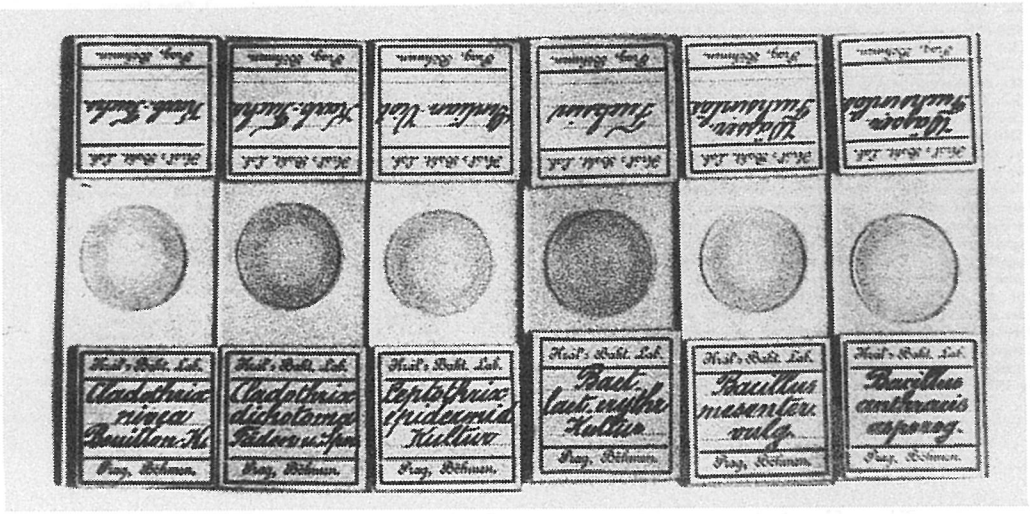


Fig. 4. Microscopic preparations

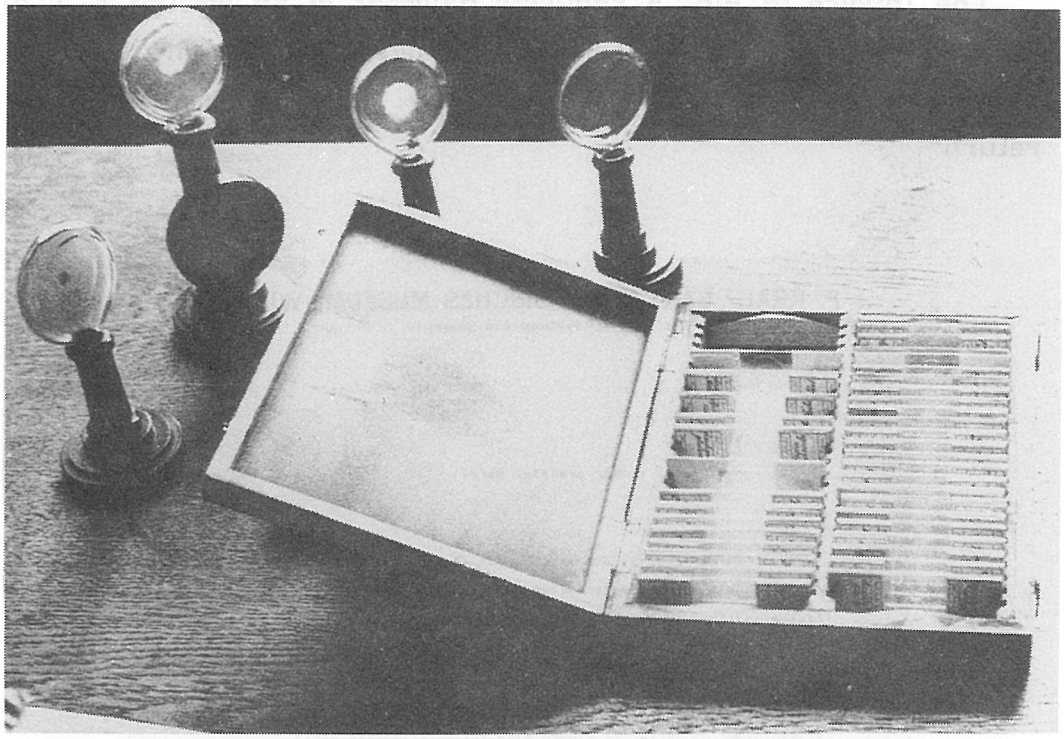
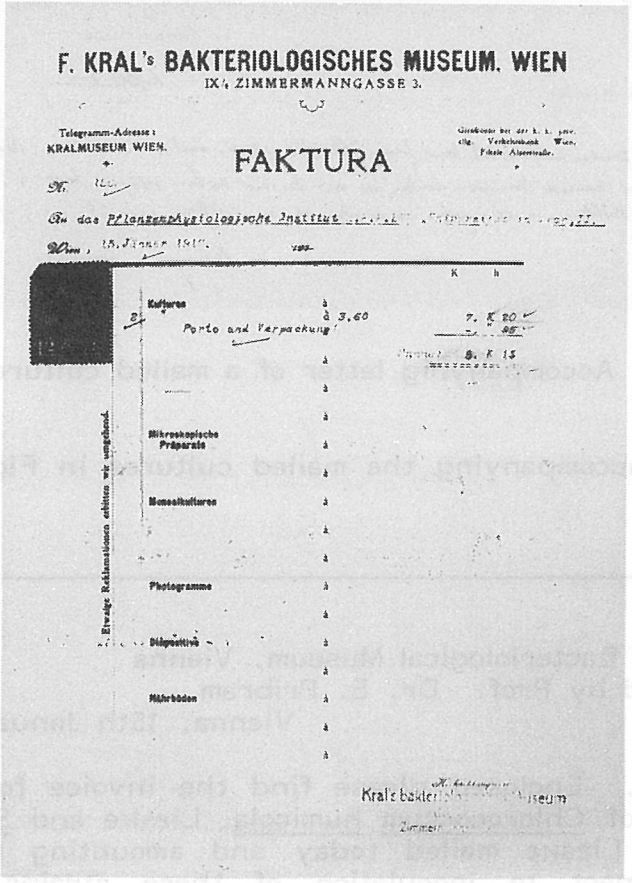


Fig. 5. "Musealkulturen"



F. KRAL's BAKTERIOLOGISCHES MUSEUM. WIEN
IX/4, ZIMMERMANNGASSE 3.

Telegramm-Adresse: KRALMUSEUM WIEN.

FAKTURA

An das Pflanzenphysiologische Institut ... in Prag II.

Wien, 15. Jänner 1911.

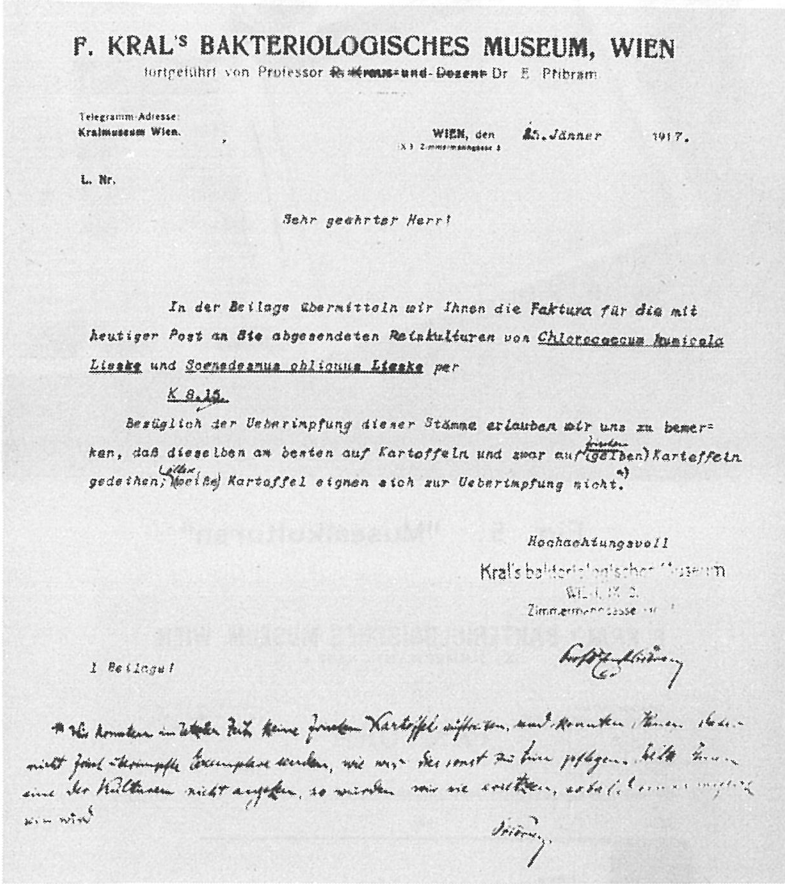
2	Kulturen	à 3,60	7. K 20
	Porto und Verpackung		85
			8. K 15
	Mikroskopische Präparate	à	
	Musealkulturen	à	
	Photogramme	à	
	Diapositive	à	
	Nährböden	à	

Etwaige Reklamationen erbitten wir umgehend.

Kral's bakter... Museum

Fig. 6. Invoice to the Institute of Plant Physiology of the University of Prague

The Invoice in Fig. 6 requests payment of an amount K 7.20 for two cultures and K 0.95 for postage and packing, with a comment "please let us know of any possible complaints by return".



F. KRAL's BAKTERIOLOGISCHES MUSEUM, WIEN
fortgeführt von Professor ~~R. Kraus und Dozent~~ Dr. E. Pribram

Telegramm-Adresse:
Kralmuseum Wien.

WIEN, den 15. Jänner 1917.

L. Nr.

Sehr geehrter Herr!

In der Beilage übermitteln wir Ihnen die Faktura für die mit heutiger Post an Sie abgesendeten Reinkulturen von Chlorococcum humicola Lieske und Scenedesmus obliquus Lieske per

K 8.15.

Bezüglich der Ueberimpfung dieser Stämme erlauben wir uns zu bemerken, daß dieselben am besten auf Kartoffeln und zwar auf (gelben) Kartoffeln gedeihen; (weiße) Kartoffel eignen sich zur Ueberimpfung nicht.*)

Hochachtungsvoll

Kral's bakteriologisches Museum
WIEN, IX. 2.
Zimmermanngasse

1 Beilage!

*) [illegible]

Fig. 7. Accompanynig letter of a mailed culture

The letter accompanying the mailed cultures in Fig. 7, reads as follows:

F. Král's Bacteriological Museum, Vienna
Continued by Prof. Dr. E. Pribram
Vienna, 15th January, 1917

Dear Sir, Enclosed please find the invoice for the pure cultures of Chlorococcum humicola Lieske and Scenedesmus obliquus Lieske mailed today and amounting to K 8.15. With respect to inoculation of these strains we would

mention that they grow best on potatoes, namely on fresh (yellow) potatoes; older (white) potatoes are not suitable for inoculation.

Respectfully
Králs bacteriological Museum,
Vienna, IX 2.

1 enclosure

* Recently, we have not been able to obtain fresh potatoes, and were therefore not able to send you freshly inoculated samples, as we usually do. If one of the cultures should not start to grow, we will replace it by a new one as soon as it is possible for us to do so.

Prof. F. Král died in 1911 and his collection was transferred in 1915 to Prof. Ernst Pribram in Vienna. He reorganized and enlarged the collection in the spirit of its founder and in 1919 he published a new updated catalogue. The publication had to be postponed because of the war 1914-18. (See Fig. 5 on page 10)

The preface of this catalogue ended with the words: " Eine Begründung der Existenzberechtigung der Sammlung ist wohl heute nicht mehr nötig; ein Blick in diese scheinbar trockene Zusammenstellung von Namen und Literaturzitaten genügt, um die Fülle und Reinhaltigkeit der Formen, ..."

" I am sure that any justification of the need for a collection to exist is no longer necessary. A look into this seemingly dry list of names and literature references provides evidence of the abundance of shapes, the tremendous diversity of fermentative properties, the enormous importance of microorganisms for health and the lives of human beings and animals, the versatility of bacteria, yeasts and filamentous fungi in many branches of industry, and is enough to justify the existence of this botanical garden of single celled beings."

APPENDIX 2

List of participants at the Kral Symposium

Norihide Amano	Suntory Ltd., Osaka, Japan
Esther Asaki	American Type Culture Collection, Rockville, U.S.A.
Isao Banno	Institute for Fermentation, Osaka, Japan
Yoshimi Benno	Japan Collection of Microorganisms, RIKEN, Saitama, Japan
Bobbie Brandon	American Type Culture Collection, Rockville, U.S.A.
Vanderli P. Canhos	Tropical Data Base, Campinas-SP, Brazil
Rita Colwell	Maryland Biotechnology Institute, University of Maryland, College Park, U.S.A.
Alma Dietz	Microtox, Kalamazoo, U.S.A.
W. Frederiksen	Department of Diagnostic Bacteriology, Statens Seruminstitut, Denmark
Dagmar Fritze	Deutsche Sammlung von Mikroorganismen, Braunschweig, F.R.G.
Tibor Deak	National Collection of Microorganisms, Budapest, Hungary
R. Gherna	American Type Culture Collection, Rockville, U.S.A.
Michael Goodfellow	Department of Microbiology, Newcastle University, Newcastle upon Tyne, U.K.
Makiko Hamamoto	Japan Collection of Microorganisms, RIKEN, Saitama, Japan
Toru Hasegawa	Institute for Fermentation, Osaka, Japan
Barry Holmes	National Collection of Type Cultures, Colindale, London, U.K.
Koichi Homma	Hitachi Software Engineering Co., Ltd., Yokohama, Japan
Teiji Iijima	Institute for Fermentation, Osaka, Japan
Ko Imai	Institute for Fermentation, Osaka, Japan
Tadayoshi Ito	Institute for Fermentation, Osaka, Japan
Peter Jackman	National Collection of Yeast Cultures, National Collection of Food Bacteria, Institute of Food Research, U.K.
Ken-ichi Joho	Snow Brand Milk Products Co., Ltd., Saitama, Japan
S. C. Jong	American Type Culture Collection, Rockville, U.S.A.
Takichi Kaneko	Tokyo University of Agriculture, Tokyo, Japan
Hiroko Kawasaki	Institute of Applied Microbiology, University of Tokyo, Tokyo, Japan
Barbara Kirsop	Microbial Strain Data Network, Cambridge University, Cambridge, U.K.
Noriaki Kishimoto	Mimasaka Women's Junior College, Okayama, Japan
Kou-ichi Kita	National Institute Agrobiological Resources, Tsukuba, Japan
M. Kocur	Czechoslovak Collection of Microorganisms, Brno, Czechoslovakia
Kazuo Komagata	Tokyo University of Agriculture, Tokyo, Japan
Yoshimasa Kosako	Japan Collection of Microorganisms, RIKEN, Saitama, Japan
Reiner M. Kroppenstedt	Deutche Sammlung von Mikroorganismen, Braunschweig, F.R.G.
T. Kudo	Japan Collection of Microorganisms, RIKEN, Saitama, Japan
Cletus P. Kurtzman	Northern Regional Research Center, Peoria, U.S.A.
David Labeda	Northern Regional Research Center, Peoria, U.S.A.
Joon Lew	Lew Institute for Bio-medical Research, Seoul, Korea
Kazuo Matsumoto	National Institute Agrobiological Resources, Tsukuba, Japan
Katsumi Mori	National Institute Agrobiological Resources, Tsukuba, Japan
R.G.E. Murray	Department of Microbiology and Immunology, University of Western Ontario, London, Canada
T. Timothy Myoda	University of Delaware, Rockland, U.S.A.
Takasi Nakase	Japan Collection of Microorganisms, RIKEN, Saitama, Japan
Jiro Nishikawa	Science University of Tokyo, Noda, Japan

Jin-Sook Park	Department of Microbiology, Ham Nam University, Taejon, Korea
J. Schindler	Institute of Hygiene and Epidemiology, Prague, Czechoslovakia
Faustino Sineriz	PROIMI-MICEN-UNT, Tucuman, Argentina
V.B.D. Skerman	Department of Microbiology, University of Queensland, Brisbane, Australia
Lindsay Sly	Australian Collection of Microorganisms, Department of Microbiology, University of Queensland, Brisbane, Australia
Da-Kang Song	Institute of Microbiolgy, Chinese Academy of Sciences, Beijing, China
Erko Stackebrandt	Department of Microbiology, University of Queensland, Brisbane, Australia
Inga-Maj Stenström	Applied Microbiolgy, Chemical Center, University of Lund, Lund, Sweden
Hideaki Sugawara	World Data Center, RIKEN, Saitama, Japan
Junta Sugiyama	Institute of Applied Microbiology, University of Tokyo, Tokyo, Japan
Sung-Oui Suh	Institute of Applied Microbiology, University of Tokyo, Tokyo, Japan
Ken-ichiro Suzuki	Japan Collection of Microorganisms, RIKEN, Saitama, Japan
Jin Tamaoka	RIKEN Institute, Saitama, Japan
Tao Tian-Shen	China Center for Type Culture Collection, Wuhan University, Wuhan, China
Lourdes M. Tapay	Microbial Culture Collection, University of the Philippines, Los Banos, Philippines
Hans G. Trüper	Institute für Mikrobiologie und Biotechnologie, Universitat Bonn, Bonn, Germany
Jan Ursing	University of Lund, Malmo, Sweden
Antonio Ventosa	Department of Microbiology, Universtiy of Sevilla, Spain
Junko Watanabe	Nippon Roche Research Center, Department of Microbiology, Kanagawa, Japan
K. Yamasato	Institute of Applied Microbiology, University of Tokyo, Tokyo, Japan
Yuji Yamauchi	Pfizer Central Research, Aichi, Japan
Myonsun Yoh	Laboratory for Culture Collection, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan
Akira Yokota	Institute for Fermentation, Osaka, Japan
Ju-Hyun Yu	Korean Culture Collection of Microorganisms, Department of Food Engineering, Yonsei University, Seoul, Korea