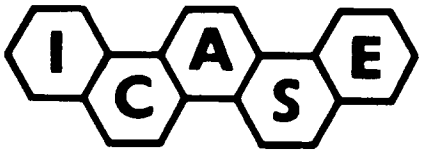
PASTEUR AND MICROBES

A Teacher Resource Book
Commemorating the
100th Year of the
Pasteur Institute
1888 – 1988

INTERNATIONAL COUNCIL OF ASSOCIATIONS FOR SCIENCE EDUCATION



PASTEUR AND MICROBES

A Teacher Resource Book
Commemorating the
100th Year of the
Pasteur Institute
1888 – 1988

ICASE COMMEMORATIVE ISSUE

Compiled by Lucille Gregorio (with assistance from Jack Holbrook)

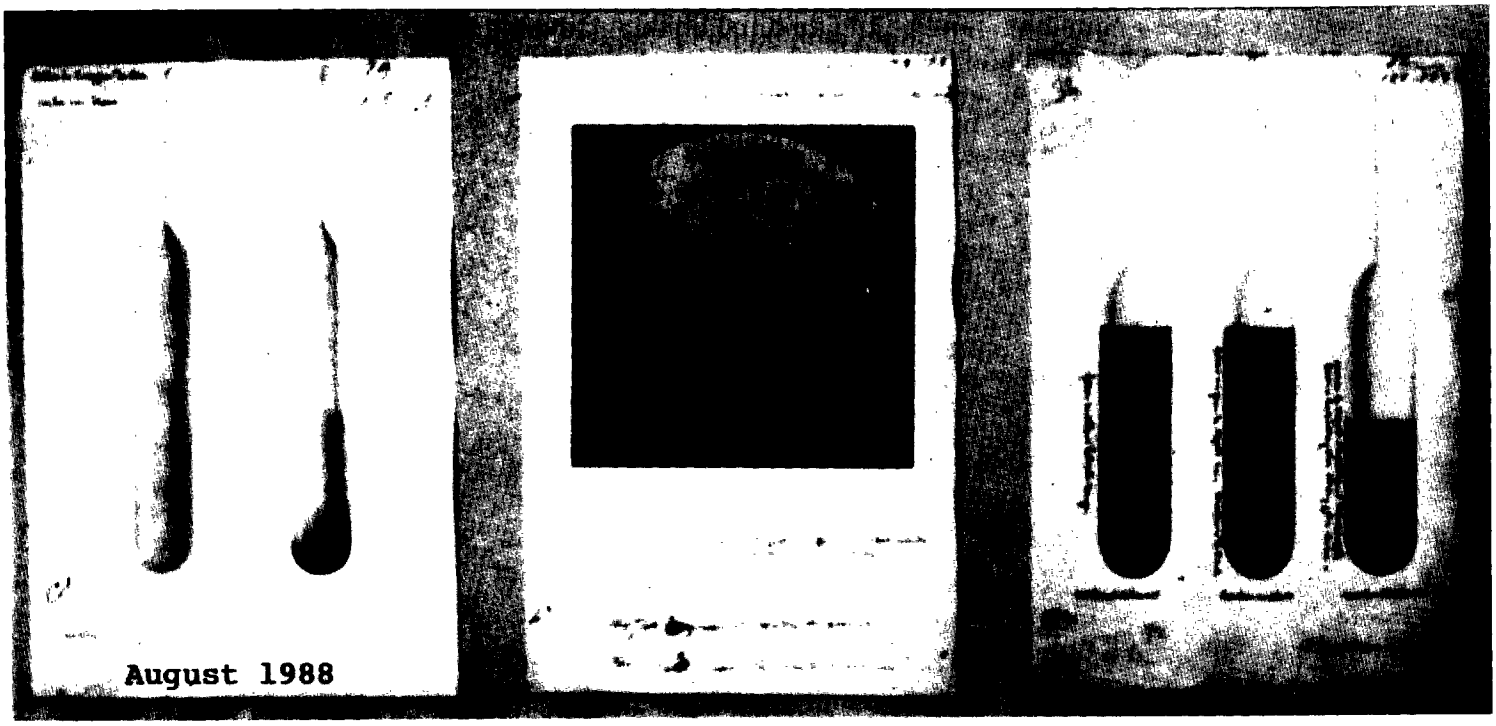
For further information and all correspondence concerning obtaining copies and copyright to :

Dr Jack Holbrook.
ICASE Executive Secretary,
Dept of Professional Studies in Education,
University of Hong Kong,
Hong Kong.

COMMEMORATIVE PUBLICATION

TEACHER RESOURCE MATERIAL

PASTEUR AND MICROBES



FOREWORD

CENTENAIRE DE L'INSTITUT PASTEUR

L'Institut Pasteur célèbre son Centenaire.

Cent ans d'efforts, de recherches, et de succès, lié à l'amélioration de la santé publique.

Créé après la première vaccination contre la rage dont le succès fut extraordinaire, l'Institut Pasteur est devenu pour le monde entier l'un des symboles de la science et de la culture francaises.

Au fil des ans, l'Institut Pasteur est devenu l'un des premiers centres de recherche en biologie au monde.

En effet, dès ses origines, il n'a cessé de contribuer au premier rang, au développement de la recherche biomédicale et à l'amélioration de la santé. C'est en son sein qu'ont été mis au point de nombreux vaccins (notamment contre la rage, la tuberculose et la fièvre jaune); qy'ont été découverts les sulfamides; qu'est née l'immunologie; que s'est développée la biologie moléculaire; qu'a été isolé le virus associé au SIDA.

Depuis toujours, il n'a cessé de s'ouvrir sur le monde. Il a été présent an Afrique et en Asie pour lutter contre less moladies tropicales. En liaison avec tous les grands instituts de recherche du monde, il envoie et recoit chaque année un flux continu de chercheurs.

L'Institut Pasteur rest résolument tourné vers l'avenir et continue de participer à cette grande aventure de l'humanité qu'est la recherche scientifique.

Maxime SCHWARTZ
Deputy Director
Institut Pasteur, Paris

THE CENTENARY OF THE PASTEUR INSTITUTE

The Pasteur Institute is celebrating its centenary. A hundred years of effort, research and success committed to the improvement of public health.

Created after the first vaccination against rabies, which was astonishingly successful, the Pasteur Institute stood for the whole world as one of the symbols of French learning and culture.

Through the years the Institute became one of the world's leading biology research centres.

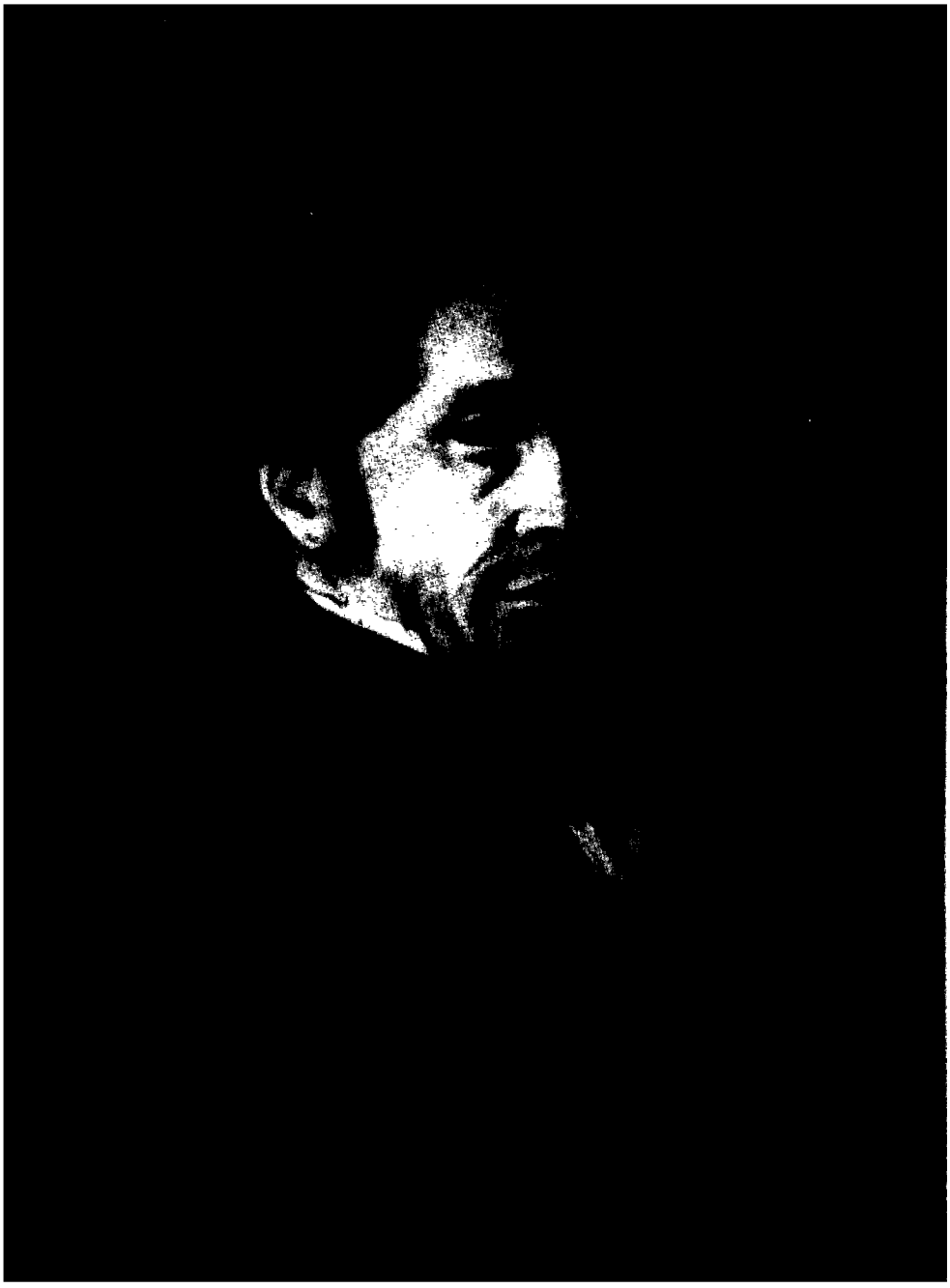
Indeed since its beginning it has not ceased to be a major contributor to the development of biomedical research and to the improvement of health. Through its work numerous vaccinations have been perfected (notably against rabies, TB and yellow fever); sulphuramides have been discovered; immunology was born; molecular biology developed and the virus associated with AIDS has been isolated.

From the very beginning its work has been international. It has worked in Africa and Asia for the fight against tropical diseases. Each year, in cooperation with other major research institutes, it send out and receives a continuous stream of research workers.

The Pasteur Institute is always looking towards the future and continues to take part in humanity's great adventure which is scientific research.

Maxine Schwartz.
Deputy Director,
Paris.

LOUIS PASTEUR



INTRODUCTION

This Resource Material commemorating the 100th year of the Pasteur Institute is a project of the International Council for Associations of Science Education (ICASE). It is dedicated not only to the memories of Louis Pasteur (1822-1895), the First Director of the Institute, the `Father of Modern Bacteriology', and "recognized both in his lifetime and ever since as one of the greatest scientists of history", but also to the science teachers who take the responsibility of teaching the young minds in the acquisition of knowledge, the development of skills and inculcation of proper attitudes, for them, to think critically, solve problems rationally and to make appropriate decisions in life.

The resource material provides ample information about the activities and facilities available at the Pasteur Institute. It also contains science experiments contributed by science teachers and Science Teachers Associations. Emphasis is on useful microorganisms, food production and food preservation. Most of the materials are for secondary level teaching. However, activities which will help acquire livelihood skills are also included, and these are intended for those youths and adults most especially in the developing countries who drop out-of-school because of socio-economic reasons.

Food production and preservation are emphasized because in the developing world lack of food and malnutrition are problems which the people face, not because there are no resources, but because of ignorance on how to make full use of the resources available in the environment.

This resource material is a modest compilation of teaching activities related to Pasteur's works on microbes. Grateful acknowledgement is extended to:

- the Pasteur Institute for the professional and moral support extended to this work,
- the science teachers and the Science Teacher's Associations who contributed the science activities,
- those who in one way or the other helped in the development and publication of this material.

ACKNOWLEDGEMENTS

ICASE gratefully acknowledges all those who helped to make this booklet possible and sincerely hopes this production meets with their approval. In particular thanks go to :

- the Pasteur Institute for the professional and moral support extended to this work, their willingness to supply the foreword and for the many photographs that adorn the front cover and inside pages,

- the science teachers and the Science Teacher's Associations who contributed the science activities, without which this book would not have been possible,

- all those who in one way or the other helped in the development and publication of this material. In particular the ICASE Executive is very grateful to Lucille Gregorio for taking charge of this project and seeing it through to a successful conclusion and to Sheila Haggis of UNESCO for providing the liaison with the Pasteur Institute.

TABLE OF CONTENTS

LA VACCINATION DES MOUTONS

LIST OF TABLES

PASTEUR INSTITUTE

LIST OF FIGURES

PART I. PASTEUR AND THE PASTEUR INSTITUTE

1. LOUIS PASTEUR: FATHER OF MODERN BACTERIOLOGY

Louis Pasteur, recognized both in the lifetime and ever since as one of the greatest scientists in history was born at Dole, France on 27 September, 1822. He came from a poor and uneducated parentage, his father was a tanner, who was a veteran of the Napoleonic Wars.

As a youth, Pasteur was not considered a remarkably good student. His mind worked so carefully, yet his teachers thought he was only a slow to average pupil. His interest was in painting, in fact his ambition was to become a Professor of Fine Arts. In school, he did moderately well in mathematics, but was considered mediocre in Chemistry.

In August 1840, at age 18, he took his "Bachelier es lettres" degree at Besancon, and also worked there to study and teach. His salary was very small. In one of his letters to his parents, he said: "a supplementary master receives board and lodging and 300 francs a year .... I assure you I am not really worth it." He however, did not take advantage of earning extra money by tutoring - because he wanted to use his time in studying mathematics. In August, 1842 he took the "Bachelier es Sciences" degree - then studied and taught at the Barbet Boarding School in Paris. In addition, he attended Chemistry lectures at Sorbonne. In 1843, at age 21, he took the competitive examinations for the Ecole Normale, to become a teacher, and was admitted, being number four in the list of candidates.

Pasteur's decision to enter the Ecole Normale was to have teaching as a career. Candidates of the School committed themselves to teach for ten years after graduation. At Ecole Normale, he established a reputation as a researcher. During those days there already existed a controversy over teaching vs. research, whether instructors should sacrifice their duties to instruct, and have more time to do research. Pasteur was already careful to emphasize that research should never be divorced from teaching, but not much of his time should be devoted to teaching.

After finishing at Ecole Normale, one of Pasteur's Chemistry professors, Balard, took him as his laboratory assistant, an assignment which let Pasteur prepare for his doctor's degree, and which the young scientist finished in 1847.

After his doctorate he was sent to teach at the Secondary School in Dijon. As he was convinced that teaching was a noble vocation which involves a wide responsibility, he set himself to become a good teacher. Despite his own deep knowledge and the ignorance of his pupils, he still took time to carefully prepare his lessons and thought very hard how to improve the way he presented his lessons. He felt that having big classes of about 80 pupils was difficult for a teacher because it was hard to sustain the attention of

the pupils up to the end of the lesson. As a teaching strategy, Pasteur would multiply experiments at the last moment, so everybody would be involved.

In 1848, at age 26, Pasteur was sent to the University of Strasbourg as an assistant. In 1854 he became Professor of Chemistry and Dean of the Faculty of Sciences at Lille, and in 1857 became Administrator and Director of Scientific Studies of Ecole Normale. In 1867, he became a professor at the Sorbonne.

The honours that Pasteur received was because of his hard work. As mentioned earlier, he obtained a succession of professorial appointment and was made a member of the French Legion of Honour. He became famous for his work on stereochemistry; the germ theory of fermentation and disease and the development of vaccines.

Pasteur's doctorate dealt with crystallographic problems and led him to pioneering studies in what is now known as stereochemistry. He studied the crystals of tartrates under the microscope and found that the crystals were not all alike. He obtained his crystals from a solution that did not rotate the plane of polarized light, and he wondered if that was because the effect of one asymmetric crystal was neutralized by the counter effect of its mirror image. Painstakingly, Pasteur managed to separate the crystals into heaps. He dissolved the two heaps separately and found that one solution twisted the plane of polarization clockwise and the other solution twisted it counter clockwise. He discovered the right- and left-handed crystals of tartaric acid.

This was a revolutionary discovery and it took some courage to announce it. Because of this work, Pasteur received the Rumford medals of the Royal Society. Though his achievement in Chemistry was well recognized, Pasteur's accomplishments in biology and medicine could not be surpassed.

Louis Pasteur excelled in all his occupations. As an administrator and Director of Scientific Studies at Ecole Normale, he was responsible for reorganizing the catering for students, the improvement of classroom ventilation and repair of buildings. His work on the curriculum put Normale as a center of instruction in science. He also founded and contributed to the periodical Scientific Annales of the Ecole Normale. As a researcher, he worked on alcoholic fermentation, and as a teacher he continued his meticulous preparation for each classroom work. He also devoted to informal contacts with his students - willing to listen and to advise.

Pasteur was a teacher for the rest of his life. Even when he was asked to work on problems like the silkworm disease and anthrax, graduate students worked with him. When plans were underway for the establishment of the Pasteur Institute for the treatment of hydrophobia, it was planned to be a teaching hospital and laboratory.

As a teacher, Pasteur always advocated laboratory work. He stressed the idea of "learning by doing" as a means of making the minds active. This method of doing work also awakens curiosity and interest. He also organized field trips for his pupils - for them to have contact with the applications of science. It was in one of those units that his advice was sought on the manufacture of beetroot alcohol - an incident that started his investigations in fermentation and led to "pasteurization." This was in 1854 at the University of Lille.

In 1857, Pasteur announced his germ theory of fermentation, which established the role of microorganisms, such as yeast in the economically important industries of alcohol, vinegar, wine and beer production.

Pasteur strongly opposed the doctrine of spontaneous generation of microorganisms. He presented experimental results to support his view that phenomena sometimes attributed to spontaneous generation - that contamination is caused by widely distributed microorganisms.

Other areas of studies which developed from his fermentation work were anaerobic life and Pasteurization. Pasteurization, a process of partial heat sterilization or gentle heating, intended to kill undesirable microscopic organisms is now widely applied to many perishable food substances, the most familiar of which is the pasteurization of milk.

In 1895, Pasteur was asked to study the disease which was attacking silkworms and which devastated the French silk industry. He was able to recommend the control of the disease which saved the industry.

From 1877 until his death in 1895 Pasteur studied diseases notably anthrax, chicken cholera, swine erysipelas, and rabies (hydrophobia). Each study fell broadly into two parts: attempts to isolate the causative organisms and grow it in a suitable medium and devising methods of controlling the infection.

Pasteur introduced a number of vaccines, the one which made him universally famous were the ones for anthrax and rabies (caused by the bite of `mad' dog). His "germ theory of disease" was probably the greatest single medical discovery of all time, for only through an understanding of the nature of infectious disease and the manner of its communication could it be brought under control. Though he was not a medical doctor, he was given due recognition when he was a member of the French Academy of Medicine in 1873. In 1885 he made the first use of his attenuated rabies preparation to prevent a case of rabies in a boy badly mauled and bitten by a mad dog. The treatment worked. Pasteur's discovery of an effective vaccine was the most important scientific achievement in the campaign against rabies and it also marked a milestone in the history of immunization.

In 1888 the Pasteur Institute was established with the financial help of donations from all over the world. Its purpose was to treat cases of rabies, and it has now become one of the most famous centers of biological research in the world. Louis Pasteur became its first Director.

Pasteur died at the height of his glory, recognized both in his lifetime and ever since as one of the greatest scientists in history. The description "Father of Modern Bacteriology" is apt, but his fundamental contribution to stereochemistry, to immunology and to the general application of scientific methods to medicine is worth remembering.

References

1. Asimov, I. (1982). "Pasteur, Louis." Asimov's Biographical Encyclopedia of Science and Technology. Doubleday and Co. Inc. Garden City, New York.

2. Encyclopedia Britannica (1986 edition).

3. McGraw, R. E. (1985). Encyclopedia of Medical History. McGraw Hill Book Co., New York.

4. Moseley, N. (1983). Pasteur; Student and Teacher. **School Science and Mathematics.** 83 (3).

5. Williams T. I. (ed). (1982). **A Biographical Dictionary of Scientists.** John Wiley and Sons, New York.

2. THE PASTEUR INSTITUTE

The Pasteur Institute in Paris, France is a private state-approved Foundation, according to a decree dated 4 June 1987, governed by statutes which have been modified by decrees dated 24 February 1967 and 13 February 1975 and by an order of 29 March 1982.

The activity includes:

. basic research in biology;
. biomedical and biotechnological applications;
. post-university teaching;
. reference centers;
. biological expertise and specialized production;
. a blood transfusion center.

The Institute has about 80 research units, grouped into nine departments:

. bacteriology and mycology
. ecology
. virology
. immunology
. molecular biochemistry and genetics
. molecular biology
. experimental physiopathology
. biotechnology
. medicine

The Pasteur Institute was inaugurated on 4 November 1888 by President Sadi Carnot of France. From the very beginning of the Institute, Pasteur brought together scientists from various disciplines. Since its founding, the Institute has been a great research establishment with a long tradition of service to human health.

The following served as Directors of the Pasteur Institute:

. Louis Pasteur	(1887 - 1895)
. Emile Duclaux	(1895 - 1904)
. Emile Roux	(1904 - 1933)
. Louis Martin	(1933 - 1940)
. Gaston Ramon	(1940 - 1941)
. Jacques Tre'foue'l	(1941 - 1965)
. Charles Gernez - Rieux	(1965 - 1966)
. Preire Mercier	(1966 - 1971)
. Jacques Monod	(1971 - 1976)
. Francois Geos	(1976 - 1982)
. Raymond Dedonder	(1982 - present)

Since the beginning of this century, numerous scientific distinctions have crowned the high level of research pursued at the Pasteur Institute, and the Nobel Prize in Physiology and Medicine has been awarded to eight Pastorians. These were:

(a) Alphonse Laveran - 1907

"For his research on the role of protozoans as disease agents"
(In 1880, he discovered the plasmodium, a parasite responsible for Malaria.)

(b) Elie Metchnikoff - 1908

"For his research on immunity"

(In 1883, he discovered phagocytes and phgocytosis, and cell immunity.)

(c) Jules Bordet - 1919

"For his discoveries in immunity"
(Elucidation of the role of antibodies and complement.)

(d) Charles Nicolle - 1928

"For his work on typhus"
(Discovered the role of the louse in the transmission of infection.)

(e) Daniel Bovet - 1957

"For his discoveries concerning the synthetic compounds which inhibits the action of certain substances in the body especially on their action on the vascular system and the muscles of the skeleton"
(Discovered the synthetic antibiohistamines and curarizing compounds.)

(f) Francois Jacob)
)
(g) Andre Lwoff and) - 1965
)
(h) Jacques Monod)

"For their discoveries concerning genetic regulation of the synthesis of enzymes and viruses"

Great moments have been recognized in the history of the Pasteur Institute. These were as follows:

. 1885 (July 6) - **Pasteur**, administered the rabies vaccine to man for the first time.

. 1888 - **Roux and Yersin**, demonstrated the mode of action of the diptheria bacillus.

. 1894 - **Roux, Martin and Chailloux**, treated diptheria with antitoxin present in the serum of immunized horses. This was the beginning of serotheraphy.

. 1889 to 1900 - **Metchnikoff**, did his research on phagocytes and inflammation.

. 1894 - **Yersin**, identified the plague bacillus.

. 1896 to 1899 - **Bordet**, elucidated the mechanism of action of complement and demonstration of the humoral aspect in immunity.

. 1898 - **Simond**, identified the role of fleas in

plague transmission.

. 1900 - **Bertrand**, discovered the function of oligoelements.

. 1904 - **The Pasteur Institute and Hospital** did the first testing of antiinfectious chemo therapy.

. 1909 - **Nicolle**, identified the role of lice in typhus transmission.

. 1910 - **Levaditi** in collaboration with **Landsteiner** demonstrated that poliomyelitis is caused by filterable virus. Also along with **Borel**, another scientist of the Institute, who hypothesized the viral origin of certain cancers, he may be considered as one of the forerunners in virology.

. 1911 - **Fourneau**, helped in the creation of the Chemotherapy Center, where antibacterial and antiparasitic compounds were developed.

. 1915 - **d'Herelle**, discovered the bacteriophage, a virus which only reproduces in a bacterium.

. 1921 - **Calmette** and **Guerin**, developed the BCG vaccine.

. 1922 to 1926 - **Ramon**, discovered anatoxins and the role of adjuvants in immunity.

. 1932 to 1938 - **Lwoff**, discovered the mechanism of action of growth factors.

. 1936 - **Jacques** and **Therese, Trefouel, Nitti, Bovet**, discovered the antiinfectious action of sulfonamides and sulfones.

. 1937 to 1947 - **Bovet**, discovered synthetic antihistamines and curarizing compounds.

. 1951 - **Lwoff**, demonstrated the existence of proviruses.

. 1955 - **Monod**, studied the enzymatic adaptation and induced biosynthesis of enzymes.

. 1955 - **Lepine**, developed the vaccine against poliomyelitis.

. 1956 - **Wollman** and **Jacob** studied sexuality.

. 1956 - **Oudin**, discovered immunoglobulin allotypy.

. 1960 - **Monod** and **Jacob**, studied the regulatory processes of enzyme biosynthesis and activity and the mechanism of protein biosynthesis.

. 1963 - **Oudin**, discovered antibody idiotypy.

3. FACILITIES AVAILABLE AT THE PASTEUR INSTITUTE

The Pasteur Institute has been a great research establishment since it was founded - with a long tradition of service to the health of mankind. Researches have been done in three sectors - microbiology, developmental biology and immunology. The researches carried out has contributed greatly to the understanding of diseases, the development of diagnostic methods, vaccines and therapeutic tools.
Other facilities available at the Institute are:

(a) a hospital
(b) reference centers
(c) a teaching center
(d) international cooperation programs
(e) a Pastorian scientific community worldwide, and
(f) museums.

(a) A Hospital

The Pasteur Institute Hospital created in 1900 by **Emile Roux** and **Louis Martin** is dedicated to the clinical application of new Pasteur theories. It was the first hospital in the world to individually isolate patients with infectious diseases.

It was in the hospital, where for the first time, sulfa drugs, BCG vaccinations and applications of radium were experimented. Since its beginning, the hospital has been in the front ranks in the study of antibiotics.

The hospital specializes in the treatment of infectious and parasitic diseases, mycoses, and diseases resulting from immune dysfunction (allergies, immunodepressions and autoimmunity). AIDS patients are also admitted in the hospital.

(c) A Teaching Center

In 1889, **Emile Roux** created the first course in technical microbics in 1889, with around 20 `students' from all over the world.

Today the Pasteur Institute welcomes nearly 300 students. They do post-university training in research and diagnosis. The courses are general, specialized or methodological, though the first priority is given to experimentation while theoretical courses provide students with the necessary principles, or help explain certain laboratory experiments.

(d) A Pastorian Scientific Community Worldwide

The Pasteur Institute is at the heart of a network of 27 institutes throughout the world, 19 of which bear the name of Pasteur. These institutes pursue public health activities and conduct research.

In 1891 the first Pasteur Institute abroad was created in Saigon, followed by the creation of a number of other Pasteur Institutes worldwide, dedicated to developing a unique community in the service of public health. Six of these institutes are still entirely dependent upon the Paris Institute, these are:

Pointe - a Pitre
Cayenne
Noumea
Bangui
Dakar
Madagascar

Others are located in Africa, Far East and in Greece, Italy and Iran.

The priority programs of the institutes are for malaria, viral hemorrhagic fevers, hepatitis B, virus-induced diarrhea, rabies and leprosy. Cooperation programs have also been set up to fight AIDS.

(e) International Cooperation Programs

The Institute, since its creation has carried out co-operation programs on an international level, by welcoming a number of students, researches in-training and foreign students. Collaboration agreements have been signed with the:

Weizmann Institute in Israel
National Immunology Institute in New Delhi
Institut Armand Frappier in Canada
Memorial Sloan-Kettering Cancer Center in the U.S.
Riken Institute in Japan

However, the heart of its cooperation is supplies and pragmatic and consists of working with the best foreign laboratories for the advancement of science, and with developing countries to enable them to combat the diseases they are confronted with.

Scientific organizations with seats in the Institute are the:

French Microbiology Society
French Immunology Society
French Society for Electrophoresis
Society of Exotic Pathology

A documentation center also exists at the Institute, as well as the Publication Department, which publishes the Annales of Microbiology, Virology and Immunology and the Bulletin of the Pasteur Institute. There are two photo libraries, one of which is scientific and the other historic.

(f) Museums

The museum is found in the apartments in which Louis Pasteur lived; an entire room is consecrated to scientific memorabilia, including more than a thousand original items representing the embodiment of the great scientists' work. In January 1986, a Museum of Applications of Pastorian Research opened in Marnes-La-Coquette. It is located in an annex of the Institute, near the room in which Louis Pasteur died.
The largest microbiology library in France is found in the Institute. It was created with the very books which belonged to Pasteur. Today, more than 200,000 books are available, some of them dating back to the beginnings of microbiology.

Reference

Pasteur Institute Document. (1987). 1887, 1987, 2087: Towards a New Century. Paris, France.

4. RESEARCHES ON-GOING AT THE PASTEUR INSTITUTE

(A) MICROBIOLOGY

Louis Pasteur was one of the founding fathers of microbiology. From him came the idea that infectious diseases are due to microbes, those microscopic organisms invisible to the naked eye. For the last century, the study of infectious microbes, viruses, bacteria, fungi and parasites as well as diagnosis, prevention and therapy in the treatment of the disease they cause, have constituted one of the principal activities of the Institute.

(a) Discovering Pathogenic Agents

Louis Pasteur and his successors were at the origin of the discovery of several pathogenic agents and their mode of transmission, as in plague and typhus, for example.

This tradition was recently renewed with the discovery of two viruses implicated in AIDS, in 1983 and 1986. These viruses have been designated as HIV-1 and HIV-2. The Pasteur Institute and DIAGNOSTIC PASTEUR have rapidly developed diagnostic tests (ELAVIA I and ELAVIA II) for the detection, in blood of the presence of specific antibodies which are the sign of infection by these viruses.

The determination of the complete structure of the genetic material of these viruses was carried out at the end of 1984 and the beginning of 1987, respectively, at the Pasteur Institute. This has enabled identification of the viral genes and the proteins of why they determine the synthesis. This is an invaluable tool for research and its application; it is especially important in the development of vaccine.

Major progress has also recently been made in non-A and non-B hepatitis, the researches at the Institute have led to the development of the first test for diagnosis of epidemic non-A, non-B hepatitis. Currently, research is being directed towards a diagnostic test to be routinely used by laboratories, as well as towards a better understanding of the virus and the condition of its transmission.

A group of researchers at the Pasteur Institute are also working on the cause of multiple sclerosis in human, which up to now remains unknown.

In addition to the search for unidentified infectious agents, research in progress also involves bacteria or viruses only recently discovered and still poorly understood. This is the case for certain viruses responsible for hemorrhagic fever (H-fever), the study and detection of which has required exceptionally strict security measures because of their extreme virulence.

(b) Understanding Infectious Agents

A group at the Pasteur Institute is studying the genetic mechanism which permits **Shigella,** (a strain of bacteria which possess the capacity to invade the human colonic mucosa and which create abscesses and ulcerations, causing a severe diarrheic disease known as bacillary dysentery) to penetrate into the cells of the intestinal lining, to actively multiply there, and to then kill the cells. The genes responsible have been identified, as have several proteins for which they code, and which intervene in the complex bacteria/cell interactions. All of this work is being pursued with the aim of producing an effective vaccine.

Researches are also done on the parasite **Trypanosoma cruzi**, responsible for Chaga's disease in humans. The disease is selective towards using children. The parasite is a public health problem, as it is estimated that there are 400,000 new cases every year throughout the world, with highly severe forms of the disease in developing countries.

Scientists also pay close attention to the relationship between pathogenic fungi, the hosts in which they develop and immunological aspects of the diseases which they cause, as well as to the development of new diagnostic means and therapeutic procedures. Unusual aspects are also being explored, especially in terms of fungi which occur in drug addicts and persons with lowered immune defenses, such as in AIDS.

(c) Vaccines

At the Pasteur Institute, research on new vaccines and the improvement of existing vaccines is of utmost importance. For example, in 1981, in collaboration with the Weizmann Institute of Science, a group from the Institute developed the first synthetic vaccine against diptheria. Also the first vaccine against hepatitis B was made commercially available in 1981, and which has gone on to show its effectiveness and total harmlessness.

In an effort to obtain a vaccine against malaria, a collaboration is taking place between the Pasteur Institute in Paris, and the Institutes in Cayenne and Dakar.

(d) Diagnostic Tests

The Institute has developed a number of what are referred to as immunoenzymatic tests in order to detect, for example, the viruses responsible for AIDS and hepatitis B. A laboratory has also been created for the development of a new type of reagent which uses monoclonal antibodies, for the diagnosis of bacterial, viral or fungal infections.

Another up-to-date technique being worked on and which is making great strides is that of nucleic probes. The technique enables the detection of the presence or absence of the pathogenic agent sought. In particular, it has been adapted to the diagnosis of hepatitis B. The Institute has also created a `non-radioactive probe' laboratory in which non-radioactive markers for nucleic probes are being developed. The reason for this study is that for the moment, diagnosis using radioactive markers used in this type of probe are limited to use in highly specialized laboratories.

It was at the Pasteur Institute that the groundwork for the antibiotic sensitivity test was laid. The procedure enables measurement of the sensitivity of a given bacteria to various antibiotics. Researchers are exploring the mechanisms of resistance of bacteria to antibiotics, a phenomenon which is becoming a matter of great concern. The advantage of the type of research is two-fold:

- in the choice of antibiotic therapy for a given patient, and
- in epidemiological surveillances, which determines the means of prevention - i.e. hygienic measures and a policy for the use of antibiotics.

At a more general level, the activities at the

Institute involves an evaluation of the effectiveness of antibacterial, antifungal and particularly in the case of AIDS, antiviral products.

A number of units in the Institute, in addition to their own research, also have taken on daily responsibilities at a national and international matters,in matters of public health e.g. collections of microorganism strains and the means to identify them; they practise diagnosis and carry out epidemiological surveillance activities.

(e) Useful Microbes

The Pasteur Institute has not, in any way neglected the study of non-pathogenic species which could have applications in agriculture and industry.

Researches dealing with useful microbes include:

- the genetics of atmospheric nitrogen fixation by bacteria living in symbiosis with graminaceal (**Azospirillium**, for example) or leguminous plants (**Rhizobium**). The findings of the research will hopefully decrease the intensive use of chemically synthesized nitrogen fertilizers.

- the production of methane by bacteria, the bacteria which ferment cellulose into energy products and basic products for synthetic chemistry.

- the biology of the cyanobacteria, which are photosynthetic bacteria still referred to as blue-green algae. The cyanobacteria, which are able to colonize all natural media, constitute a model for the study of photosynthesis in plants.

- the molecular study of biopesticides carried by certain bacteria (**Bacillus thuringiensis**), in this case toxins, and their use in the control of insect vectors of diseases such as malaria, river blindness, some oral encephalitis and against the Lepidoptera caterpillars which destroy crops and forests.

(f) Microbiology and Molecular Biology

Microbiologists at the Pasteur Institute are investigating microbes which are neither dangerous nor useful, such as the collibacillus, acommensal bacteria in humans. The reasoning is that, through the study of an easy-to-manipulate organism, to reach general conclusions concerning the way living beings function. The often quoted phrase of Jacques Monod, "What's true for the collibacillus is true for the elephant" has proven to contain a great deal of truth. The study laid the groundwork for molecular biology , a field in which the aim is to interpret biological processes in terms of interactions between molecules.

(B) DEVELOPMENTAL BIOLOGY

A number of research teams are examining the development of higher organisms. They are attempting to understand how a unique cell formed at fertilization by a union between a sperm and an ovule can result in a complete organism with all its different types of cells, each of them fulfilling a separate function and being precisely localized.

Another subject for investigation in developmental biology is that of the differentiation pathways, and of the specialization of cells, which are constantly being renewed in the adult organism. The objective in this case is to determine why and how stem cells give rise to cell lines which themselves give rise to a given type of cell in blood, skin, or intestine, etc.

The possibilities in this field of study are overwhelming. From the knowledge gained in this field, progress is expected in early detection and control of some developmental abnormalities, such as those affecting the muscles or the nervous system.

Major progress should also come about in the fight against cancer. It has been said that cancer results from disturbances or alterations in the genetic material of the cells, especially at the level of oncogenes, or genes which normally determine the conditions of cell growth. When they are injured due to environmental aggression or chemical or biological agents such as viruses, these oncogenes cause severe disturbances in cell growth and in the activity of cell groups in the process of differentiation; these then begin to proliferate in a disordered manner.

(C) IMMUNOLOGY

The work of Pasteur predated the advent of the science and immunology. Immunology is the science which studies the processes through which the organism reacts against foreign substances, infectious and toxic agents in particular.

Among scientists in the Pasteur Institute who achieved prominence in the evaluation were:

Metchnikoff who worked on phagocytosis;

Roux who developed serotherapy;

Ramon, with the discovery of `detoxified' toxins, the basis for modern vaccines;

Bordet, through elucidation of the role of antibodies and complement, the body of proteins which destroy foreign cells, or those recognized as such;

Oudin, through the discovery of two fundamental concepts, allotypy and idiotypy, which revolutionized the field and created a veritable `Pastorian school of thinking'.

At the Pasteur Institute, more than 100 permanent scientists from 19 research units are devoting themselves to the study of the immune system and the way in which it functions.

A great deal of research at the Institute centers around the genetic basis of immune mechanisms. Also being explored are the structure of the genes of the major histocompatibility complex, their expression in the form of proteins and the regulation of this expression in those phases which are crucial for the defense of the organism.

Other approaches are directed towards the antibodies. The human body can produce antibodies against a virtually unlimited number of different antigens. It is the rearrangement of genes which is at the origin of this extraordinary variety of antibodies, and several teams are investigating these mechanisms.

Elsewhere at the Institute, researchers are analyzing the structure of the antibodies and using highly complicated techniques, in particular X-ray diffraction, as well as powerful calculation methods. These studies are aimed at understanding the way in which antigens and antibodies interact. This group, in 1985, carried out the first study of the structure of an antigen/antibody complex.

Numerous research themes involve cells of the immune system and communications between lymphocytes, either by direct contact between the cells or by chemical messages represented by molecules called lymphokines. Experiments are focusing on identification of lymphokines and receptors of these molecules, as well as on decoding their message.
Research is also taking a look at particular aspects of immune defense, such as inflammation, its mechanisms, mediators and relations between inflammatory processes and various pathologies, including asthma, atherosclerosis, bacterial infections and cancer.

In the Institute, some works are being done involving the identification and study of natural immunostimulants capable of inducing non-specific resistance of the organism to attack from pathogenic forms. The experiments are done with mice. It have been found that certain natural substances which may be produced by microorganisms protect mice against normally fatal bacterial infection and increased their resistance to the evaluation of malignant tumors, which are very severe in these animals.

It is a fact that research in immunology offersfuture therapeutic possibilities, and it has been of immense value in terms of diagnosis.

Allergy, generally considered a less serious manifestation of dysfunction in the immune system, have been studied extensively at the Pasteur Institute, for more than ten years. Current research in this field concerns the mode of action of environment allergens (pollens, acarians) in the process of sensitization in humans and in animals. An organism is considered to be sensitized to a substance when it reacts - sometimes violently (anaphylactic shock) - during the reintroduction of this substance. The allergic mechanism (anaphylaxis) is being explored at different phases.

Research in allergy is thus concentrating on the agents responsible, the mechanisms of immune processes, in particular the use of specific antibodies (immunoglobulin E), the cells affected, the mediators of allergy and their effects, in particular the effect of histamine on bronchospasm in asthma, and upon effective diagnostic procedures, prevention, treatment and follow-up of desensitization treatments.

The Pasteur Institute is, in fact, in charge of the production of allergens, as well as of preparing 'tailor-made' allergenic preparations for a given individual, all of which represent about three million doses for diagnostic and therapeutic use each year.

Their approach to immunology at the Institute has considerably evolved over the last century. Immunology has integrated tools developed by molecular biology for the benefit of the evolution of knowledge about the normal functioning of the immune system, the study of immune dysfunction and the diseases caused by it. The progress accomplished thus far has opened up new horizons in therapy, including direct intervention for modeling immune responses - and is resulting in an optional prevention by vaccination.

Reference

Pasteur Institute Document. (1987). 1887, 1987, 2087: Towards a New Century. Paris.

PART 2. PASTEUR AND HIS WORK

1. THE WORK OF PASTEUR

Everything is connected in Pasteur's work from the work on crystallography to the discovery of virus vaccine and the preventive treatment against rabies.

Pasteur's work began when he read a paper by a German physicist Mitscherlich. This established that two chemical compounds, sodium and ammonium tartrate had the same chemical make up and the same crystalline form although they acted differently towards polarised light. Pasteur decided to search for and discover the cause.

1.1 FIRST PERIOD

1847 - 1862 (Age 25 - 40).

Pasteur essentially worked as a physicist and chemist.

1847 work on asymmetric molecules.

* After several years of work combining crystallography, chemistry and optics, Pasteur established that there was a comparison between the exterior form of a crystal, its molecular constitution and its effect on polarised light.

* Asymmetric crystals refracted polarised light, crystals which were symmetrical did not. He formulated a fundamental law - only products created under the influence of life are asymmetrical. This is because they are made up from cosmic forces which are themselves asymmetrical.

* Asymmetry is the great divide between organic and inorganic worlds.

* Pasteur's work was the origin of a new science called stereochemistry.

* He also developed chemical synthesis.

1855 - 1857 Work on Fermentation

In observing the crystals of paratartrate Pasteur discovered asymmetric molecules and in examining a solution of paratartrate he saw that under the effect of a mould the acid fermented and dissociated. One could no longer find in the fermented liquid the laevo and dextro forms of tartaric acid. Thus a substance inactive towards polarised light (paratartaric acid), had become active laevo tartaric acid under the influence of fermentation.

Therefore since all active substances are derived from living matter, fermentation, instead of being the work of death, such as the Chemist Liebig believed, is the result of life since only life generates substances active towards polarised light. This is the first link which led logically from asymmetric molecules to fermentation and on to contagious diseases.

1857 - 1862

Pasteur studied lactic acid and alcoholic fermentations and demonstrated that :

- all fermentation is due to the presence of microorganisms

- each fermentation corresponds to a particular ferment

Likewise he ascertained that in order to study fermentation one must :

- prepare a sterile environment suitable for the ferment

- grow this with a trace of the ferment.

This is the beginning of all microbiological techniques

In studying the mechanism of fermentation which he carried out to prove the role and specific action of microorganisms, Pasteur was doing the work of a biochemist.

1.2 SECOND PERIOD

1862 - 1877

From the age of 40 to 55, Pasteur became a biologist. He worked on the theory of germs and demolished the doctrine of spontaneous generation.

Following his first discoveries, he asked himself where the microorganisms, the agents of fermentation, originated from. Were they the product of similar germs or did they appear spontaneously in the culture ? It raised the question of whether spontaneous generation occurred.

After many arguments with those opposing his views (Pouchet), he was able, in 1862, to prove through his varied experiments that

a) atmospheric dust contains germs of lower organisms always ready to develop and multiply
b) liquids most likely to putrefy remained uncontaminated if they were protected from these germs.

"Spontaneous generation is just a dream" (Pasteur)

He then asked himself how fermentation worked and how ferments acted.
He discovered, in studying butyric fermentation, a new type of life capable of living screened from air. He suggested the term anaerobic for the ferment which had the property of living without air. The term 'aerobie' was given to microorganisms which needed a free supply of oxygen in order to develop.

Fermentation is the result of life without air. Work on fermentation led Pasteur to apply the methods of the microbiologist to industry abd agriculture.

He studies the make-up of vinegar and the transformation of alcohol to ethanoic acid by a microorganism mycoderma aceti which fixed oxygen from the air onto alcohol. He showed the manufacturers how to obtain vinegar of a consistent quality.

Diseases of Wine

In studying the parasitic ferments of wine, Pasteur showed that each disease is due to a particular ferment. By heating to 50°C it is possible to protect wines from the disease.
This method, applied to all unstable liquids, is known throughout the world as 'Pasteurisation'.

Beer

Changes in beer are the result of microorganisms carried by dust in the air. Pasteur showed the brewers how to protect the wort and heat the beer to 55°C to prevent disease.

Diseases of Silk Worms

In 1865 the raising of silk worms not only in France but also in Italy, Austria and Asia minor was undermined by a disease called the 'pebrine'. Pasteur verified with the microscope that the worms struck down by this disease had bright corpuscles and those corpuscles were responsible for the disease. He showed that the disease is hereditary and contagious.

He discovered another disease 'flacherie' which gave credence to the idea of a particular strain in which the disease occurred. He overcame the disease in practice by the innovation of cellular grains.

The work of Pasteur was of considerable interest; for the first time the problems of heredity and contagiousness and the establishment of prophylaxis were solved scientifically.

This acted as a preface to his studies on contagious diseases and the theory of germs.

1.3 THIRD PERIOD

1877 - 1887

From the age of 55 to 65 Pasteur made microbiology available to the doctor and surgeon.

1877 Study of infectious diseases
- discovered the cause of boils and osteomyelitus ; the stephylococcus
- discovered the pereperal infectious microbe
- discovered pneunococcus

1880 The method of reducing the virulina of microbes
- cholera in hens (by maturing the microbe in contact with oxygen in the air)
- anthrax in sheep (by heating the culture of anthrax bacillus to 43°C)

1881 Vaccinations against cholera, anthrax and swine fever.
By the appreciation of his method
- the study of infectious diseases (microbic agents)
- their prevention (asepsis)
- protection by immunisation (vaccination)

Pasteur founded immunology

1880 - 1885 Rabies

Pasteur had now perfected his experimental methods. He studied rabies. He could isolate the germ but not find it. (Rabies is a disease of the nervous system). He cultivated an invisible microorganism in the bone marrow of a rabbit and fixed it the virus of the disease.

He vaccinated Joseph Meister on July 6, 1885 for the disease caused by the virus of rabies.

14th November 1888 inaugeration of the Pasteur Institue by Sadi Carcot.

Source: Pasteur Institute (1988), Paris, France.

2. PASTEUR 1822 - 1895

CHRONOLOGY

1822	27th December	Pasteur born at Dole (Jura)
1831		Pasteur a pupil at Arbois College
1839		Leaves for Royal College at Besancon
1840		Bachelor of Letters at Besancon Teacher at Besancon
1842		Bachelor of mathematical science at Dijon
1843		Admitted to La Ecole normale superieure
1845		Licentiate of sciences
1846		Nominated Professor of Physics at Lycee de Tournon (Ardeche) but remained at the Ecole normale as a graduate assistance Met Laurent in the Balard laboratory. Studied crystals.
1847		Doctor of science
1848		Research on isomorphism. Historic paper on the separating of sodium and ammonium paratartrates Nominated Professor of Physics at the Lycee in Dijon Nominated temporary Professor of Chemistry at the Faculty of Sciences in Strasbourg
1849		Married Mlle Marie Laurent, daughter of the Rector of Strasbourg university Research on specific properties of the two acids which made up racemic acid
1851		Pasteur's paper on aspartic and malic acids
1852		New research on existing relations between crystalline forms, chemical composition and rotation of polarised light
1853		Pasteur made Chevalier de l'Ordre imperial of the Legion of Honour. He received the prize of the Societe de Pharmacie de Paris for the synthesis of racemic acid Paper on the discovery of the transformation of tartaric acid to racemic acid. Discovered inactive tartaric acid.
1854		Pasteur nominated Dean of Faculty of Sciences, Lille
1855		Beginning of studies on fermentation. Presented a paper on amyl alcohol at Lille.
1856		Beginning of research on alcoholic fermentation
1857		Nominated administrator of the Ecole normale and director of scientific studies Paper on lactic fermentation Paper on alcoholic fermentation

1858	Installed in his laboratory in the attic of the Ecole normale, rue d'Ulm, Paris
	Enquiry by Pasteur on generation said to be 'spontaneous'
1859	Experimental physiology prize from the Academy of Sciences for his work on fermentation
1860	Sampled air at Arbois for his study on the problems of 'spontaneous' generation.
	Examination of the theory of 'spontaneous' generation
1861	Jecker prize from the Academy of Sciences for work on fermentation
	Publication in the bulletin of the chemical society of Paris of the results of his work on vinegar
1862	Elected to the Academy of Sciences (minerology section)
	Alhumbert prize for research on spontaneous generation
	Study on microorganisms and roles these play in acidic fermentation
1863	Napoleon III asked Pasteur to study diseases of wine
	Studies on wines and the influence of oxygen in the air on the acidity of wine
	Nominated Professor of geology, physics and applied chemistry at Ecole des Beaux-Arts
1864	Installed in a labratory at Arbois to carry out research on wine
1865	Practical work on conservation and improvement of wine
	Study on the disease of silk worms amd work on pasteurisation
1866	Publication of 'Studies of wine'.
	Publication of an essay on the scientific work of Claude Bernard
1867	Set up a laboratory of physiological chemistry in the Ecole normale.
	Nominated Professor of organic chemistry at the Sorbonne.
	Grand Prix de l'Exposition universelle for studies on wine
	Resigned from administrative duties at Ecole normale
1868	Diploma of doctor of medicine at Bonn university.
	Pasteur has paralysis (stroke) affecting his left side
	Commander of the Legion of Honour.
	Publication of studies on vinegar
1870	Publication of studies on diseases of silk worms
1871	Studies on beer
1873	Elected member of l'Academie de Medecine
1876	Publication of studies on beer

1877	Paper on the alteration of wine
	Studies on anthrax
	Studies on septicemia
1878	Nominated Grand Officer of the Legion of Honour
	Publication of paper on the theory of germs and its application to medicine and surgery
	Paper on cholera in hens
	Research on gangrene, septicemia and puerperal fever
1879	Paper on the plague.
	Discovered immunisation as a means of halting cultures
1880	Named member of the Societe centrale de la Medicine veterinaire
	Paper on infectious diseases (Pasteur showed for the first time the principle of virus vaccines)
	Start of research on rabies
1881	Nominated Grand-Croix of the Legion of Honour
	Vaccine for anthrax
	Work on yellow fever near Bordeaux
	Elected to Academie francaise
1882	Note on the contagious
	Study of swine fever
1883	Vaccination against swine fever with the help of a culture
1884	New paper on rabies. Papers on pathogenic microbes and virus vaccines at the congress in Copenhagen.
	Pasteur described the general principle of vaccination against virulent diseases
1885	First antirabies vaccination given to a man
1887	Elected permanent secretary of l'Academie de Sciences
	Suffered a second stroke
	First experience of the destruction of rabbits in Australia from the microbe of cholera in hens
1888	Inaugeration of the Pasteur Institute
1895 25th September	Death of Pasteur at Villeneuve-l'Etang

Source : Pasteur Institute (1988), Paris, France.

AT WORK IN THE PASTEUR INSTITUTE

PART III. SOME OF PASTEUR'S EXPERIMENTS

1. LIVING THINGS ARE NOT FORMED SPONTANEOUSLY FROM NON-LIVING SUBSTANCES

In a series of investigations in 1861, Louis Pasteur showed that when microorganisms are excluded from a sterile broth, the broth remained clear, whereas when microorganisms were allowed to enter, the broth became cloudy.

This experiment can easily be repeated by the teacher as a demonstration, or performed as a class investigation where sufficient apparatus is available.

Materials

3 wide mouthed storage jars or conical flasks
3 one-holed bark corks to fit (sterilization spoils rubber)
2 S-shaped glass tubes
1 straight glass tube
Sealing wax
Nutrient broth

Procedure

1. Sterilize the apparatus and medium.
2. Pour freshly prepared nutrient broth into the three bottles.
3. Insert the bark corks carrying the tubes in the bottles (2 are S-shaped tubes and 1 is a straight glass tube).
4. Leave the 3 bottles in a safe place in the laboratory for one or two days.
5. Examine and compare the broths.

Expectation

The media in bottles A and B with S-shaped tubes should be clear while that in C, with a straight tube, will be cloudy.

Procedure

6. Shake bottle B so that the broth comes in contact with the bend of the glass tube.
7. Leave for two days.
8. Examine again.

Expectation

Flask B will be cloudy while Flask A which serves as a control should still be clear.

Procedure

9. Allow the students to look at a drop of the cloudly medium under the microscope to discover the cause of the cloudiness.

In Pasteur's experiment recorded above we saw that in all three bottles air entered the broths freely but in A and B the microorganisms were trapped in the curved parts of the tubes. This was shown by the contamination of the broth in B after it came in contact with the contents of the bent glass tube.

Spontaneous Generation

Until the 18th century people thought that living things arose from non-living materials. This idea is known as the **Theory of Spontaneous Generation.** From his investigations Pasteur observed that organisms do not form on their own. He concluded that living things could arise only from living ancestors (Biogenesis) and could not be formed spontaneously from non-living substances.

2. SPECIFIC ACTIONS OF MICROORGANISMS

In 1857 Louis Pasteur examined under the microscope a greyish slime from the bottom of a jug of milk that had gone sour. He saw a vast number of little rod-shaped cells - bacteria - smaller than yeast cells and without their clear cellulose walls. When Pasteur put a speck of the grey slime into a solution containing sugar, the solution soon contained millions of bacteria. The sugar was actively fermented and some of it was turned into the same acid as that of the sour milk. A drop of this new fermenting liquid made another solution of sugar turn sour as the little red shaped cells multiplied in it. Thus Pasteur showed that definite activities are associated with particular microorganisms. This can be called **functional specificity,** or the specific action of microorganisms. Similarly, certain chemical substances are specific for killing certain germs.

INVESTIGATION 1 - ACTION OF YEAST ON SUGAR

The specific function of a microorganism could be demonstrated in the laboratory by investigating the action of yeast on sugar solution e.g. 10% glucose.

Materials

2 distilling flasks
Yeast
Distilled water
Cork borer
2 conical flasks
Microscope slides
Limewater
Bent glass tube
2 Liebig condensers
Glucose
Corks
Rubber tubing
Microscope
Cover slips
2 beakers
Water bath

Procedure

1. Weight out 100 g of glucose.
2. Dissolve in distilled water and make up to 1 cm^3.
3. Place 500 cm^3 of this solution in each distilling flask.
4. Sterilize the two solutions at 10 lbs/sq. in. for ten minutes.
5. Weight 20 g of yeast.
6. Add the yeast to one flask. Label it `A'.
7. The other flask is the control. Label it `B'.

Note: The students should observe the solution of sugar and yeast under the microscope and record their observations.

8. Set up the apparatus.
9. Leave for two days.
10. Allow the students to observe and compare any changes in the two distilling flasks. Is any gas evolved?

Expectation

Milkiness in the limewater in `A' shows the evolution of carbon dioxide.
No change in the limewater in B.
The evolution of carbon dioxide is due to the action of yeast on sugar.
Note the smell if any of the liquid in A and B. The students should observe and compare the sugar and yeast solution in A and B under the microscope.

Note: Fermentation is due to the action of living yeast.

Distillation

1. Set up flasks A and B for distillation as shown.

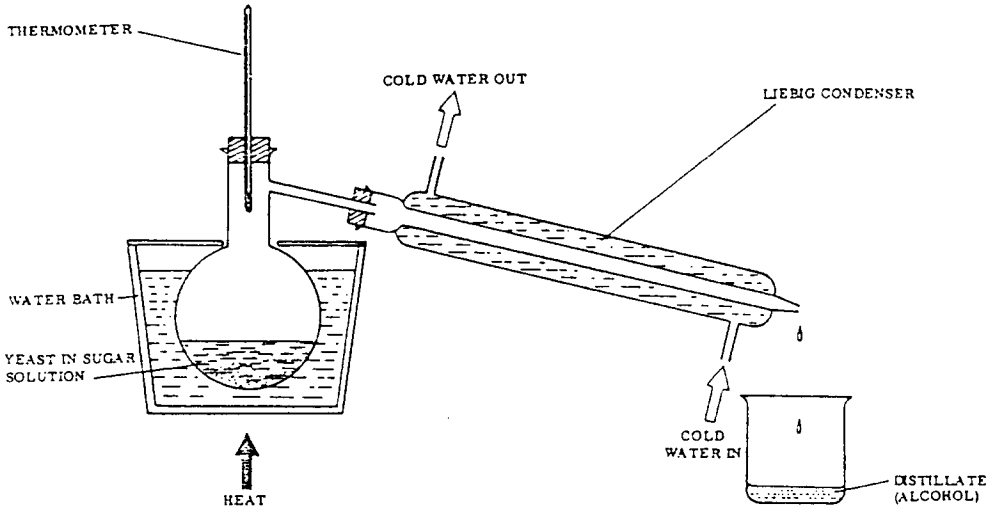


Figure 1. Distillation Set-up

2. Collect the distillate, if any, at 78^{o}C.
3. Smell and taste.

Expectation

A distillate of alcohol is obtained from Flask A while from Flask B no distillate is obtained.

The reaction of yeast on sugar can be summarized simply in the following equation:

$$\underset{\text{Sugar}}{C_6H_{12}O_6} \xrightarrow{\text{Yeast}} \underset{\text{Alcohol}}{2\ C_2H_5OH} + \underset{\text{Carbon dioxide}}{2\ CO_2} + \text{ENERGY}$$

The action of yeast on sugar to produce alcohol, carbon dioxide and energy is known as **Fermentation.** A similar action takes place in one-day-old palm wine.

INVESTIGATION 2 - FERMENTATION IN PALM WINE

Procedure

1. Obtain fresh palm wine.
2. Taste the palm wine.

Note: Fresh palm wine is very sugary.

3. Examine a few drops of the fresh palm wine under a microscope and record your observations.

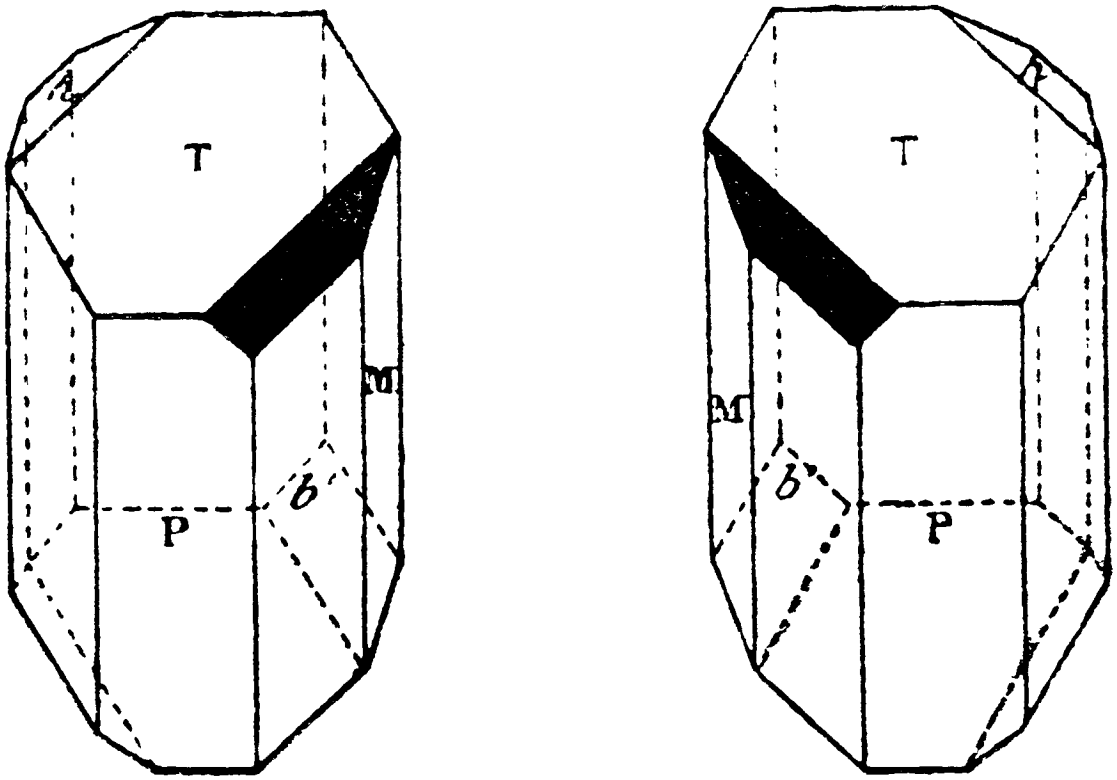
Note: Fresh palm wine contains a few yeast cells.

4. Place the palm wine in a flask and arrange it as in investigation 2.1 but do not add yeast.
5. Leave aside for one day.
6. Observe and note any changes.
7. Distill as in the previous investigation.

These two investigations serve as a good illustration of the specific action of yeast in breaking down sugar to alcohol and carbon dioxide.

Reference

UNESCO Pilot Project for Biology Teaching in Africa (1967-1968). Handbook of Microbiology.



LE GERME DE L'ŒUVRE DE PASTEUR.

PART IV. MICROORGANISMS

1. THE VARIETY OF MICROORGANISMS

There are six major groups of microorganisms:

Bacteria
Viruses
Yeasts (Fungi)
Molds (Fungi)
Protozoa
Some algae

The principal characteristics of these groups are summarized in Table 1. Within these major groups are hundreds of thousands of genera, species, strains and varieties, with many organisms new to science being described each year. There are, for example, well over 30,000 known species of living protozoa; in the 1950's only half this number were known. The number of species described in a particular group is also a reflection of its economic value or potential. The genus **Streptomyces**, for example, was unknown in the 1930's. In the 1940's, after the discovery that **Streptomyces** produces antibiotics, many new species were isolated, so that by the mid 1970's well over 400 species of this genus had been described.

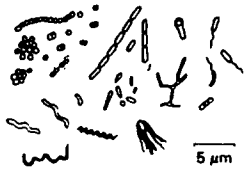
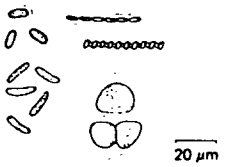
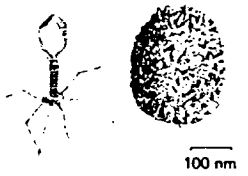
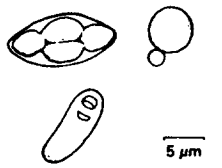
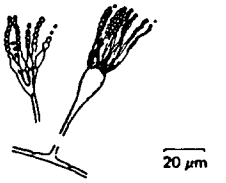
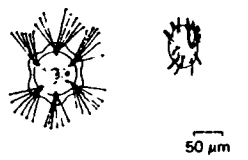
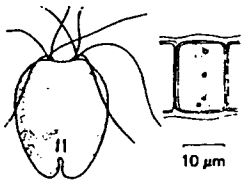
With the advent of genetic engineering, the world-wide search for new and potentially useful microorganisms has become even more important. The potential exists to extract useful genes from any microorganism and insert them into a microorganism and insert them into a microorganism which can already be grown on a commercial scale, to improve its performance or to produce new compounds. The transfer of such genes to higher organisms is also possible.

VERSATILITY OF PHYSIOLOGY AND BIOCHEMISTRY

One important characteristic of microorganisms, concerns the absorption or excretion of materials across the cell membrane. Enzymes associated with extracellular metabolism of substrate may be produced in considerable quantities e.g. in the case of the antibiotic - producing species. The products may therefore be worth exploiting or may be harmful, e.g. in the case of exotoxin causing food poisoning.

The materials which microorganisms utilize in their metabolism are remarkably diverse, and some are at first very surprising e.g. sulphur, heavy metals, oil. In each of these cases, however, the activity has been exploited, respectively to reduce the sulphur content of coal, to remove polluting heavy metals from waste water, and for the breakdown of potentially polluting oil. One of the best known examples is the ability of some microorganisms to digest cellulose, utilized in the production of useful

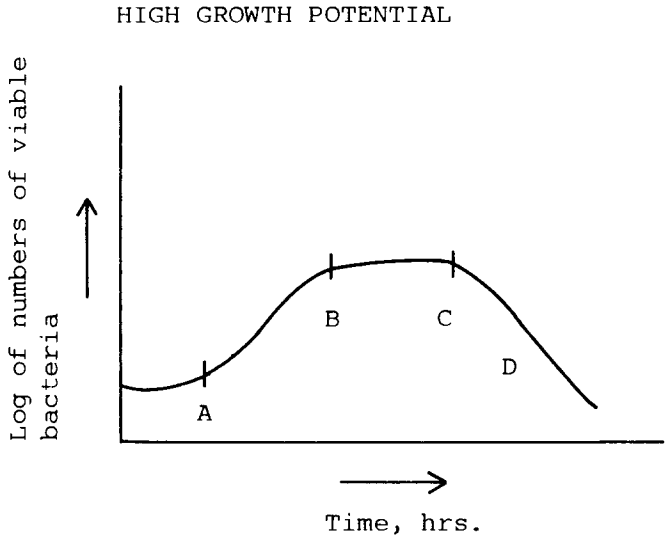
Table 1 Characteristics of major groups of microorganisms (From Pelczar and Chan, 1981)

GROUP	MORPHOLOGY	SIZE	IMPORTANT CHARACTERISTICS	PRACTICAL SIGNIFICANCE
Bacteria		Typical: 0.5 to 1.5 μm by 1.0 to 3.0 μm Range: 0.2 by 100 μm	Procaryotic. Unicellular, simple internal structure. Grow on artificial laboratory media. Reproduction asexual, characteristically by simple cell division.	Some cause disease. Perform important role in natural cycling of elements which contributes to soil fertility. Useful in industry for manufacture of valuable compounds. Some spoil foods; some make foods.
Cyanobacteria		Range: 5.0 to 15 μm	Procaryotic. Unicellular. Cell structure like that of bacteria. Grow on artificial laboratory media. Reproduction asexual by simple cell division or production of spores. Contain chlorophyll and are photosynthetic.	Source of food for aquatic animals. Contribute to soil formation and enrichment.
Viruses		Range: 0.015 to 0.2 μm	Do not grow on artificial laboratory media; require living cells within which they are reproduced. All are obligate parasites. Electron microscopy required to see viruses.	Cause diseases in humans, other animals and plants. Also infect microorganisms.
Fungi: Yeasts		Range: 5.0 to 10.0 μm	Eucaryotic. Unicellular. Laboratory cultivation much like bacteria. Reproduction by asexual cell division, budding, or sexual processes.	Production of alcoholic beverages. Also used as food supplement. Some cause disease.
Fungi: Molds		Range: 2.0 to 10.0 μm by several mm	Eucaryotic. Multicellular with many distinctive structural features. Cultivated in laboratory much like bacteria. Reproduction by asexual and sexual processes.	Responsible for decomposition (deterioration) of many materials. Useful for industrial production of many chemicals including penicillin. Cause diseases of humans, other animals, and plants.
Protozoa		Range: 2.0 to 200 μm	Eucaryotic. Unicellular. Some cultivated in laboratory much like bacteria. Some are intracellular parasites. Reproduction by asexual and sexual processes.	Food for aquatic animals. Some cause disease.
Algae		Range: 1.0 μm to many feet	Eucaryotic. Unicellular and multicellular. Most occur in aquatic environments. Contain chlorophyll and are photosynthetic. Reproduction by asexual and sexual processes.	Important to the production of food in aquatic environments. Used as food supplement and in pharmaceutical preparations. Source of agar for microbiological media. Some produce toxic substances.

substances such as ethanol, and single cell protein which is extracted from the biomass of the cultured organisms. In the case of cellulose digestion, herbivorous ruminant mammals such as the cow must be regarded as pioneer bio-technologists, not only developing compact and efficient bioreactors, but also carrying them around inside the body!

Of course, many microorganisms also compete for the same food sources as man, leading to losses in agriculture, and food spoilage. Others exploit the human body itself and give rise to diseases as a result of the breaking down of molecules, cells and tissues of the body.

The versatility of microorganisms is such that it seems certain that somewhere in the world of these organisms there already exist genes which can produce enzymes for almost every conceivable chemical reaction. These genes represent a remarkable resource, which the geneticists of today are busily exploiting.



A = lag phase

B = logarithmic phase

C = stationary phase

D = decline phase

Figure 2. Typical Bacterial Growth Curve
(From Pelczar and Chan, 1981)

Although microorganisms are found, and are capable of growing, in almost every habitat on earth, every individual species has highly specific requirements for its optimum growth. If these are met, the organism is capable of extremely high rates of growth. This is achieved largely through asexual reproduction including binary fission and fragmentation in bacteria and some species of protozoa, budding in yeast, and spore formation in molds. The well known typical growth curve for microorganisms is shown in Figure 2.

Growth is most rapid in the logarithmic phase where cells undergoing fission do so at their maximum rate, therefore doubling in number at periods of time varying from a few minutes to a few hours. In three hours, a single bacterium dividing at ten minute intervals would have produced a mass of more than 200,000 cells, and in a further hour this would have risen to almost 17 million cells.

Bioreactors, used for example in fermentations, are designed to provide the fermenting organisms with conditions which will optimize their growth rate. This obviously makes for efficient and rapid production, but also the fast growth of the desired organism suppresses other microorganisms which might be present in the substrates for example in the production of beer.

Measures to prevent food spoilage and the spread of diseases via food are of course designed to ensure that the causative microorganisms are deprived of optimum conditions for growth. It can readily be seen that a good understanding of the optimum growth conditions of microorganisms is essential, if their potential for useful production is to be realized, or their potential for harm suppressed.

References

1. Daniel, M. S. (ed.) (1987). Microbiology and Food. Institute of Biology (Hong Kong Branch) Symposium.

2. Pelczar, M. and Chan, E. C. S. (1981). Elements of Microbiology. MaGraw-Hill Book Co. New York.

2. THE VERSATILE MICROBES

Microorganisms live everywhere. Every gram of soil has about 100 million living bacteria. These organisms can also survive and reproduce in all types of water, tolerate freezing or boiling temperature, fresh or salty water, presence or absence of air. Some not only survive high acidity which is normally deadly; they also produce sulfuric acid!

The successful distribution of bacteria lies in their tiny size and way of life. Their size permits a high surface-to-volume ratio. This means they can take in nutrients rapidly and thereby support their high metabolic rate.

Bacteria can adapt to many sources of nutrition: farm wastes, industrial wastes, dead animals and plants, human skin, fruits hanging from trees - just about everything. There are, however, a few things they cannot break down. An example is plastic.

Bacteria can carry on photosynthesis in what we would call darkness. They do this in the absence of oxygen and, therefore, they release no oxygen.

Fungi, on the other hand, lack food-making pigments. They live by absorbing organic matter from waste materials. Many fungi are deadly. A number of them can metabolize sulfur, iron, and even atmospheric nitrogen.

When microbes take up nutrients, some become parts of their cell. Others are broken down. The energy released is used to make new substances like carbohydrates, fats, proteins, minerals, vitamins, and water that they need.

A soil bacterium can make its own cell material from carbon dioxide and ammonium or nitrate. Since its cell contains all the carbohydrates, fats and other nutrients, it is an efficient factory for making protoplasm and generating 79,000 calories of energy.

All nutrients enter the cell by diffusing through the cell wall. Rod-shaped bacteria are more efficient than spherical bacteria in absorbing nutrients.

The strong cell wall of a bacterium prevents the cell membrane from expanding as dissolved nutrients are taken in. But if some chemicals like penicillin are added to the liquid, the wall begins to break down and the bacterium bursts and dies.

Tiny as they are, microbes have cell structures essential for life: an outer selective membrane, inner membrane systems (except bacteria), and structures in making proteins.

Reproduction is mainly through cell division and usually results in two offsprings of the same size. Sometimes, genetic material is exchanged between two microbes through bridges formed out of their cytoplasm, as shown: (Figure 3).

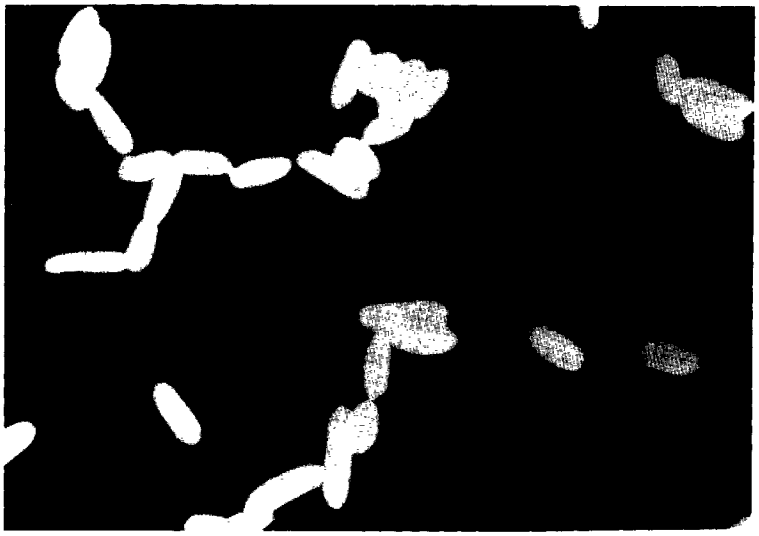


Figure 3. Cell Division in Bacteria

In contrast to bacteria and other microbes, viruses are not cellular. They are composed of only two substances: protein and nucleic acid. They complete their life cycle only inside a living host cell, thereby causing diseases such as colds and polio.

Although some microbes cause diseases, many of them are useful to man. Some bacteria in our intestine help in digestion. Other bacteria help in food-making and processing.

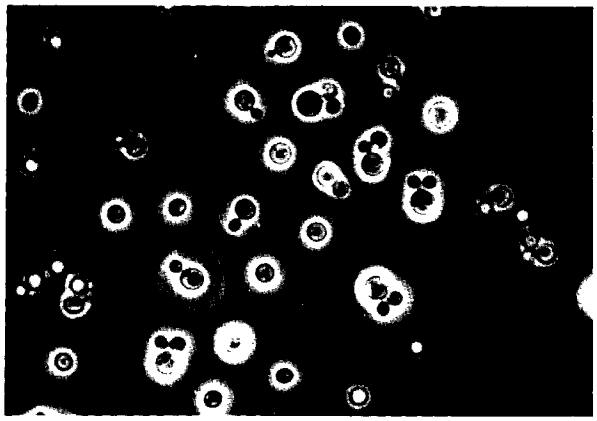


Figure 4. Bacteria and Yeast Magnified

GUIDE QUESTIONS

1. How do salt and vinegar prevent food spoilage?
2. Explain how their tiny size works to the advantage of microbes.
3. Describe one metabolic activity of bacteria that is used in food production.

GLOSSARY

cytoplasm - the protoplasm (jellylike substance) outside the cellular nucleus
metabolism - sum total of all chemical activities in a cell

Source

Baltazar, Victoria. (1985). Bato Balani Publications. Makati, Philippines. (5):1.

3. ISOLATION OF MICROORGANISMS - "PURE" CULTURE

For a better study of shape, size and arrangement of the cells of specific microorganisms and **particularly** for the study of their function a "pure" culture i.e. one containing colonies of one type of microorganism must be obtained.

PREPARATION OF A "PURE" CULTURE

Materials

A prepared agar slope	A prepared culture
Wire loop	Bunsen burner or spirit lamp
Sterile water	Microscope
Microscope slip	Cover slip
Test-tube stand	Stain e.g. Methylene blue

Procedure

1. Sterilize the wire loop by holding it in the flame until it is red hot.
2. Touch one of the colonies on any culture e.g. from air, water, soil, with the sterile loop. The portion of the colony on the loop is called the inoculum. Introducing microorganisms into a medium or inserting them into other organisms is term **inoculation.**
3. Remove the test tube plug and quickly transfer the inoculum to the surface of the sterile agar slope. Apply in zigzag fashion up the slope.
4. Flame the loop and the mouth of the test tube. Pass the cotton plug quickly through the flame, being careful not to burn it unduly. Replace the latter and stand the loop in the rack.

5. Leave the inoculated agar slope in a safe place in the laboratory for one or two days.

Note: If more than one type of colony is present culture is not pure and it will be necessary to repeat the procedure with fresh materials.

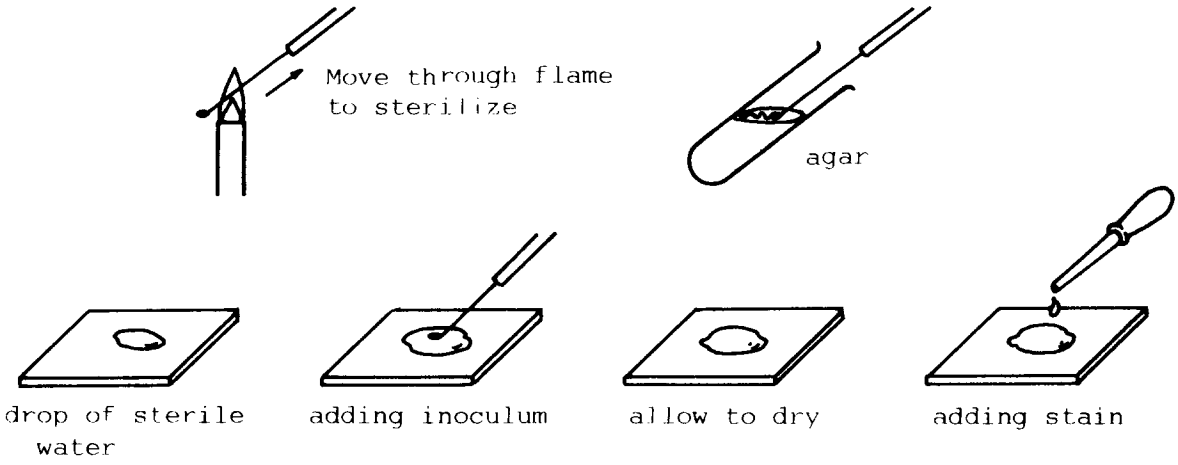


Figure 5. Preparing an Inoculum

Examining the Culture under a Microscope

1. Flame one side of the slide.
2. Place a drop of sterile water on the flamed side of the slide.
3. Using a sterile loop place a little inoculum from the pure culture on the slide, spread and dry. (See Figure 5).
4. Add some stain e.g. Methylene blue.
5. Allow to dry.
6. Examine under the microscope. If possible use an oil immersion lens to show better clarity of structure.

Reference

UNESCO Pilot Project for Biology Teaching in Africa (1967-1968). Handbook of Microbiology.

PART V. LOW-COST EQUIPMENT FOR THE STUDY OF MICROORGANISMS

1. THE CONSTRUCTION OF A STEAM STERILIZER

Microorganisms have become increasingly prominent subjects of biological investigations since the early work of Pasteur, Koch and others. From the information accumulated about the physical characteristics and chemical activities of microorganisms, investigators have been able to utilize some of the unique qualities of these organisms in the study of fundamental biological problems, such as nutrition, growth, genetics, etc.

In order to study microorganisms or use them to investigate biological problems, it is necessary to employ special equipment and techniques. The sterilization of media, and culturing apparatus is essential for most studies involving microorganisms. If specific microbes are to be studied in a state free from possible interaction with others, then all foreign organisms present either on the equipment or in the culture medium must be eliminated.

Sterilization with heat is the most common method of eliminating foreign organisms. Media are commonly sterilized with steam at 15 square inch pressure for 20 minutes in an autoclave or pressure-cooker. The steam sterilizer as shown in Figure 6 will serve as a substitute for a pressure cooker or an autoclave. Although this sterilizer works at normal atmospheric pressure it is satisfactory for laboratory use in schools.

The time required to obtain sterilizing temperatures in a steam sterilizer is dependent upon the rate of energy input of the heat source and the rate of heat loss from the sterilizer.

This improvised steam sterilizer is financially economical, easy to handle, and yet serves the purpose.

Materials Required

	Components	Quantity	Dimensions (mm)
1.	Large tin can with lid	1	150 x 230 (dia. x ht.)
2.	Small tin can	1	130 x 130 (dia. x ht.)
3.	Bolt and nut (bolt with thread on half the stem)	1	6 x 20 (dia. x ht.)
4.	Brass strips	3	2 x 25 x 80

Construction

1. Perforate the small tin can all over (3 mm. in diameter holes).
2. Solder the three brass strips to the base of this tin so as to port it within the larger tin.
3. Drill a hole (8 mm. in diameter) on the lid of larger tin.
4. Fit the hole with the bolt and nut. Assemble parts and test.

2. HOW TO OPERATE THE STEAM STERILIZER

1. Fill the large tin can with water up to a depth of about 1.5 cm.
2. Place the culture media (previously prepared) in the small tin can.
3. Replace the lid and heat the apparatus for about one hour.
4. When steam emerges (through the exhaust) maintain the heat for about 30 minutes more.
5. Turn off the heat, and allow the apparatus to cool before removing the culture media.

Precaution and Maintenance

After each use the sterilizer should be cleaned and thoroughly dried before storing. This procedure will reduce the possibilities of corrosion of the metal and greatly extend the service life of the sterilizer.

Reference

SEAMEO-RECSAM. (1977). Ideas for Making Low-Cost Equipment for Secondary Science and Mathematics. Course DW-Q2. Penang, Malaysia.

(ii) Heat Reflector

Plywood	(F)	1	33x24x0.5 cm
Aluminium	(G)	1	27x28 cm

(iii) Heat Source

Egg Incubator Heat (H) Source		1	See Egg Incubator

(iv) Door

Plywood	(I)	1	35x36x1.0 cm
Hinges	(J)	2	4 cm long
Screws	(K)	8	0.7 cm long
Nails	(L)	6	1 ~ cm long
Rubber Bands	(M)	3	-

Method of Construction

(i) Frames and Trays

Nail three of the wood strips (D) to each of the two plywood pieces (A), as shown in Figure 8, to make the two side pieces of the frame. Nail the bottom edges of the completed side pieces to the wood (B) used as base. Nail the back piece (C) into position as well as the top piece (A). When the frame is finished, the pieces of perforated hardboard (E) which as the trays should slide easily into the frame on the wood strips (d), as shown in Figure 9.

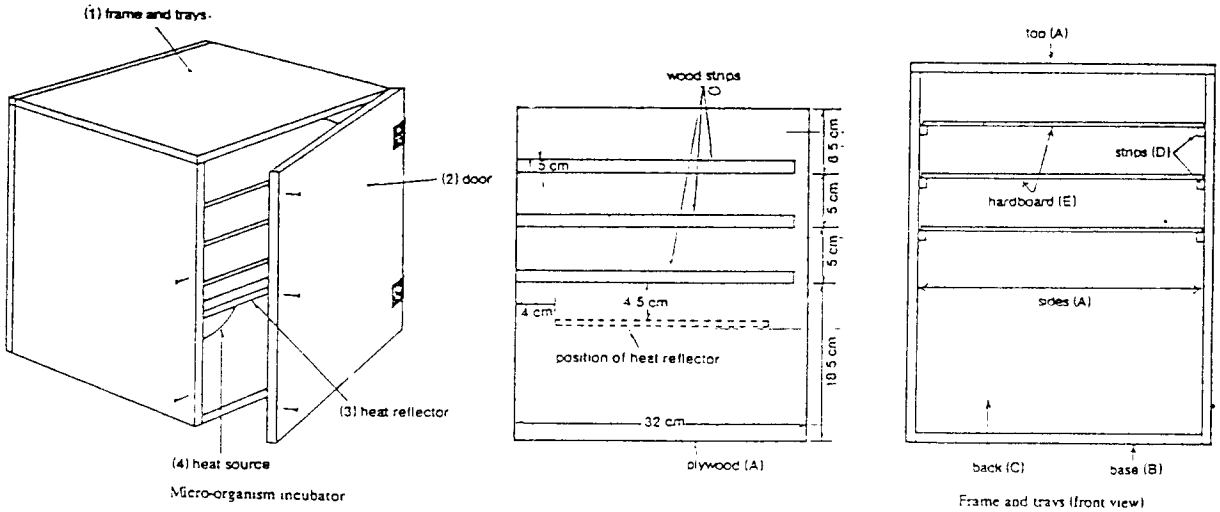


Figure 8 Constructing the Microorganism Incubator

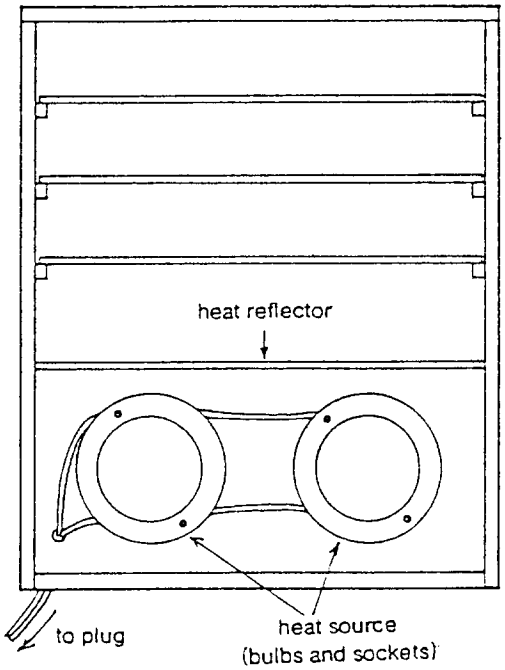


Figure 9 Position of the Trays

(ii) Heat Reflector

Cover one side of the plywood (P) with aluminium foil (G) to make the heat reflector. Nail the reflector into place 13 cm above the base (B) of the frame with a 4 cm gap between the near edge of the reflector and the back (C) of the frame.

(iii) Heat Source

Use light bulbs as the heat source.

(iv) Door

Fasten one edge of the plywood (I) to the side of the frame with the hinges (J) and screws (K) making certain that the door shuts as closely as possible to the frame. The door may be held closed by using rubber bands (M) stretched between nails (L) in the frame and the door as shown in Figure 10.

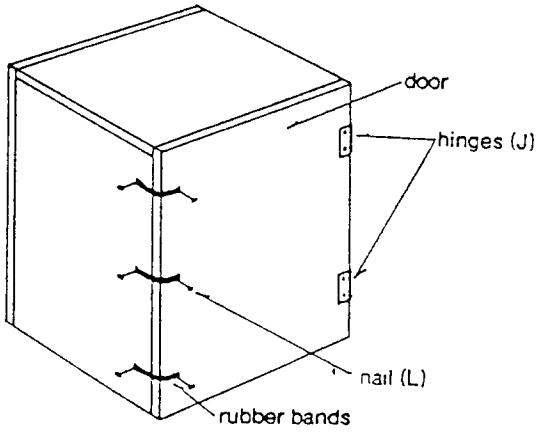


Figure 10 The Improvised Incubator

Source

Bato Balani. (1985). Manila, Philippines. Vol. 5.

PART VI. SIMPLE MICROBIOLOGICAL ACTIVITIES

1. AN EXPERIMENT TO FIND THE EFFECT OF HERBAL GERMICIDES ON MICROORGANISMS

Small enamel basins may be used as petri dishes. The basins and their covers should be sterilized before use. Agar nutrient material can be made in the usual manner. If agar is expensive, moss jelly may be used. The nutrient media may be inoculated with microorganisms from the environment. (See Figure 11.)

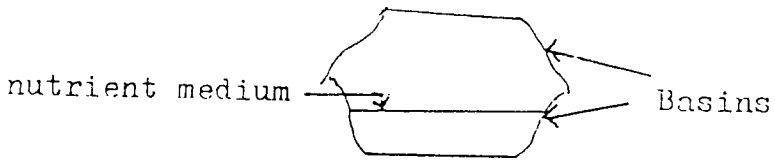


Figure 11. Nutrient Mediums in the Basin

The herbal germicide material may be extracted by grinding the material with water and boiling, and squeezing out the juices. Herbs such as Margosa, saffron which are commonly found can be used. Discs of blotting paper are dipped in each of the extracts and then marked. These discs are then placed in the nutrient media as shown in Figure 12. After some time, pupils are able to see for themselves the germicidal actions of the herbs.

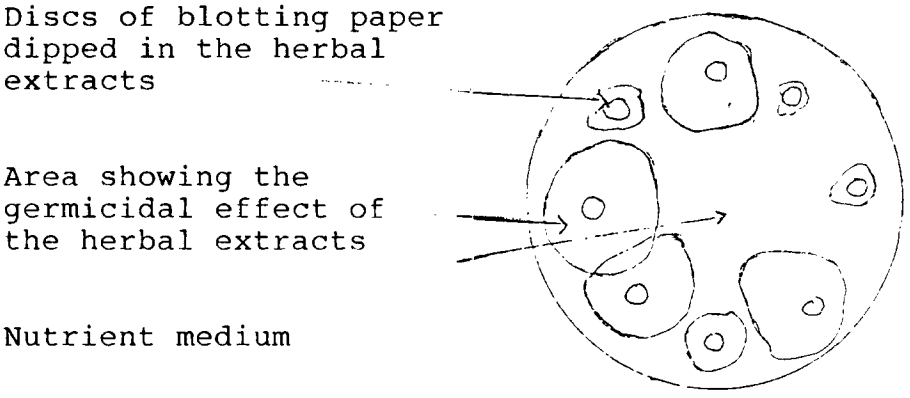


Figure 12. Germicidal Effects of Herbal Extracts

(The same method can be adopted to find the germicidal effects of different soaps and toothpastes.)

2. AN EXPERIMENT TO SHOW THE BENEFICIAL EFFECTS OF WASHING HANDS

A few agar plates are prepared as for activity 1. A pupil is asked to touch 1/3 of the plates before washing the hands. A further one third of the plates are touched after washing his hands. The 1/3 are left alone as controls. After some time, growth of microorganisms are examined and pupils see for themselves the beneficial effects of washing hands.

3. AN EXPERIMENT TO SHOW THE IMPORTANCE OF COVERING THE MOUTH WHEN COUGHING OR SNEEZING

A few agar plates are prepared as for activity 1. A pupil is asked to cough into 1/3 of the plates without covering his mouth. One third of the plates are exposed to the pupil while coughing with his mouth covered. The other plates are left as controls. After some time, the plates are examined and the effects of covering the mouth while coughing can be observed by the pupils.

Contributed by: Mr. Asoka Weerasinghe, Sri Lanka, 1987.

PART VII. MICROORGANISMS IN THE SOIL

1. ARE THERE MICROORGANISMS IN SOIL?

Materials

Soil sample
2 sterile petri dishes containing nutrient agar

Procedure

1. Open one of the petri dishes and sprinkle a little soil on the medium.
2. Quickly replace the cover.
3. Use the second sterile dish containing agar but without soil as the control.
4. Leave both petri dishes in a safe place in the laboratory for two days. Incubate if necessary.
5. Inspect for growth of microorganisms.

Reference

UNESCO Pilot Project for Biology Teaching in Africa (1967-1968). Handbook of Microbiology.

2. USE OF SOIL MICROBES TEACHING MATERIAL

Introduction

Today, environmental problems have attracted international concern.

The following science experiments were designed so that secondary school students may find out about soil microbes, and thus learn about their environment. Furthermore, these activities could lead to an understanding about living soil microbes which survive, like mankind, under severe natural conditions.

EXPERIMENT I - CULTIVATION OF SOIL MICROBES

The object of this experiment is to confirm the existence of soil microbes.

Methods

1. Preparation of the media.

   For molds

   All of the following media (a-c) can be used.

   (a) Mix the following materials, boil them and then pour into a petri dish.

glucose (or maltose)	20 g
peptone	5 g
agar	8 g
water	500 cm^3

   (b) Prepare soup by boiling potatoes and carrots in water, then filter and coagulate by adding agar.

   (c) A piece of bread or cooked rice can itself be used as a medium for molds.

   For bacteria

   (d) Mix the following materials boil and allow to set.

peptone	2 g
meat extract	1.2 g
agar	8 g
water	500 cm^3

   (e) Mix 0.5 g starch with 100 cm^3 water and set by boiling with 0.4 g agar and allow to cool.

2. Put 1 g soil into 9 cm^3 water and shake for a few minutes. After shaking, pipette the water onto the coagulated medium.
3. After a few days, students are able to observe the colony of microbes.

Expected Results

(a) It is possible to confirm the colonies of green molds (penicillium) bread molds (Rhizopus), and black molds (Aspergillus niger).

(b) White, yellow, brown and red coloured colonies of bacteria can be observed.

EXPERIMENT II - MEASUREMENT OF CO_2 CONCENTRATION BY RESPIRATION OF SOIL MICROBES

The object of this experiment is to detect the existence of living things by measurement of CO_2 produced by respiration of soil microbes.

Method

1. 500 g of wet soil from the field is put into a vinyl bag and sealed by inserting a rubber stopper with a glass tube and then sucking out the air.
2. Add 500 cm^3 air into the vinyl bag using a pump.
3. Remove 100 cm^3 air from the bag every 30 minutes and check the CO_2 concentration using a CO_2 detection tube. (See Figure 13.)

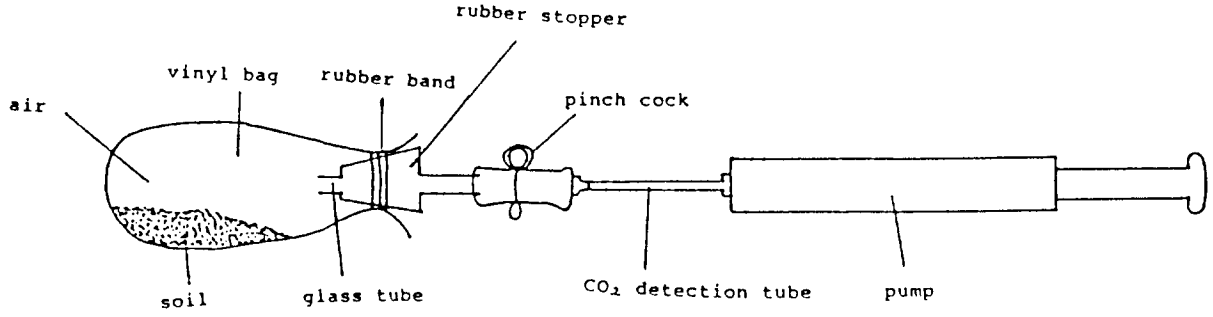


Figure 13. Apparatus for the Measurement of CO_2 Concentration

To confirm CO_2 as the product, air from the vinyl bag can be passed into limewater. A cloud suspension confirms the presence of carbon dioxide. (See Figure 14.)

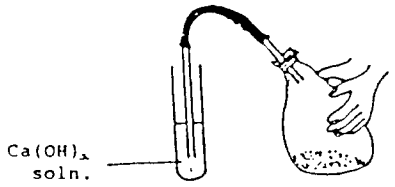


Figure 14. Detection of CO_2

We are able to presume the existence of living things from the fact that CO_2 concentration increases continuously with time. (See Figure 15.)

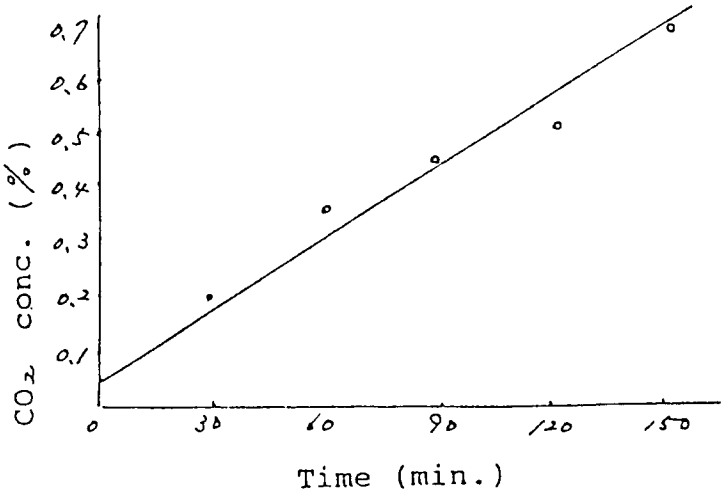


Figure 15. Variation of CO_2 Concentration by the Respiration of Soil Microbes

EXPERIMENT III - MEASUREMENT OF OXYGEN CONSUMPTION FROM THE RESPIRATION OF SOIL MICROBES

The object of this experiment is an examination of the difference in soil microbe activity with content of organic compounds in the soil gathered from different places. Students measure the amount of oxygen which is consumed by the respiration of aerobic soil microbes.

Method

1. The apparatus for listing one type of soil is shown in Figure 16.
2. Different samples of soil are added e.g. upper layer soil from a forest, a rice field, a garden, school grounds.
3. A 10% NaOH solution is in a jar on the soil. A rubber stopper with glass tube seals the jar containing the soil with the other end of the glass tube passing into the coloured water.
4. The jar is cooled by running water to keep the temperature constant.
5. The height of the coloured water rising in the glass tube is measured every 30 minutes for 2 hours and the results plotted on a graph.
6. The experiment is repeated for other types of soil and the results compared.

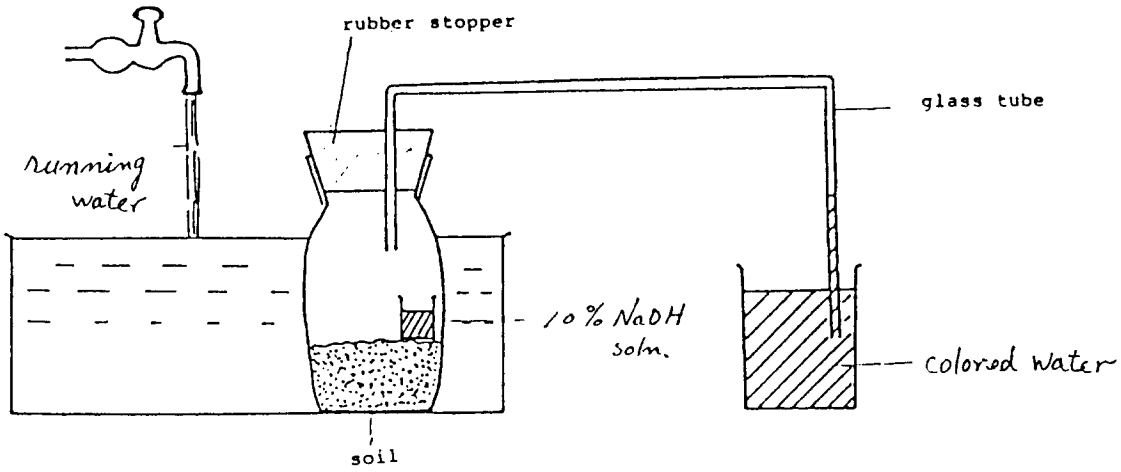


Figure 16. Apparatus for Measurement of Oxygen Concentration Expected Results

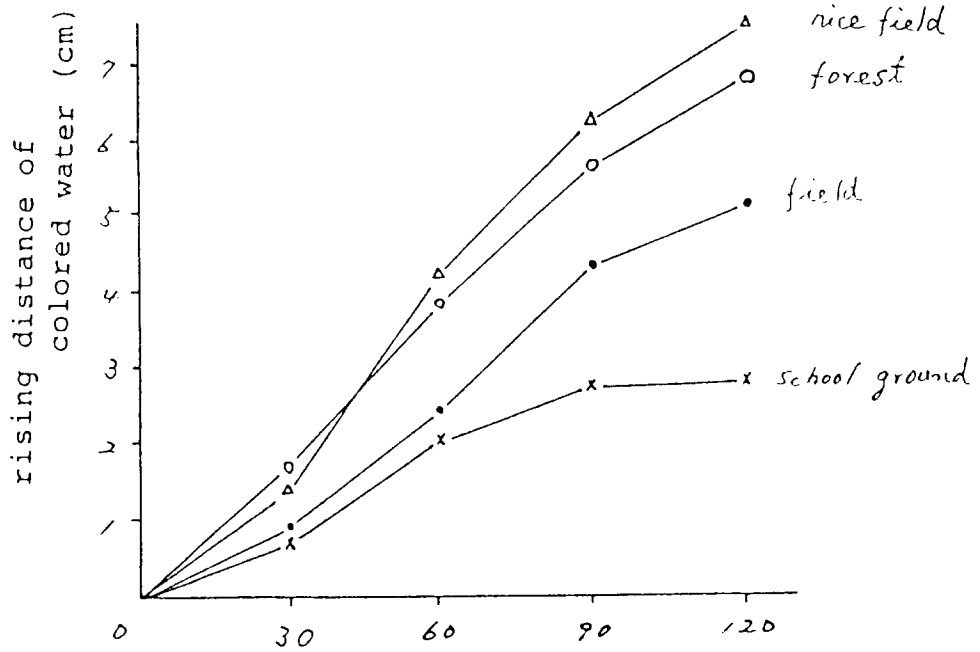


Figure 17. The Difference of O_2 Consumption by Soil Types

From the graph we can see that oxygen consumption for the soil from school grounds as indicated by the height of coloured water is less than the others. As oxygen is consumed by microbes to decompose organic compounds, this suggests that other soils have much more microbes and organic compounds. The more organic nourishment, the greater microbe activity and as a result, plants grow well. That is to say, the organic compounds are decomposed into compounds with lower molecular mass of inorganic compounds which are easily reused by plants.

EXPERIMENT IV - CONDITIONS THAT INCREASE THE RESPIRATION OF SOIL MICROBES AND HENCE THE RATE OF DECOMPOSITION OF ORGANIC MATTER

The object of this experiment is to determine the conditions which affect the rate of respiration of soil microbes. Students measure the requisite oxygen amount about the different respiration substances (glucose, starch, peptone and rape oil).

Method

1. The apparatus for this experiment is same as for Experiment III.
2. 200 g of carbohydrates (glucose, starch), protein (peptone), rape oil are added to the soil and the amount of oxygen consumed is compared by measuring the height of the coloured water in the glass tube.

Expected Results

Figures 18 and 19 show the measures case when (a) 0.5% and (b) 2% respiration substances were added.

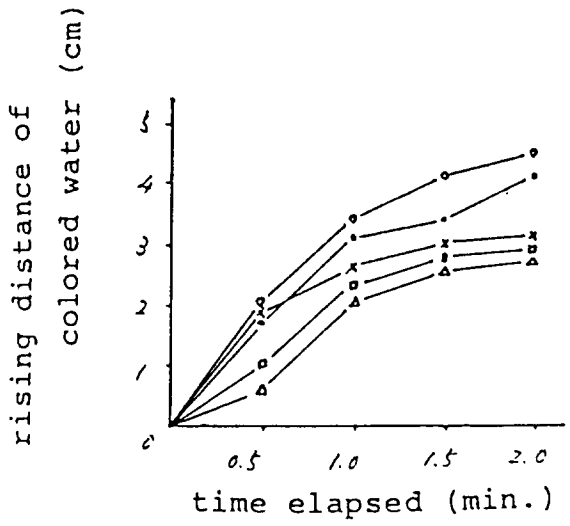


Figure 18. Graph Showing 0.5% Substances Added

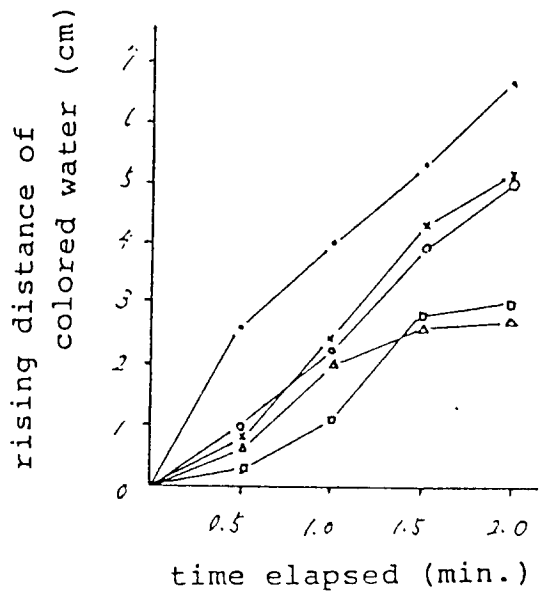


Figure 19. Graph Showing 2% Substances Added

EXPERIMENT V - THE DECOMPOSITION OF ORGANIC COMPOUNDS BY SOIL MICROBES

1. The test for carbohydrate.

Method

(a) Some boiled and mashed potatoes are put into a Erlenmeyer flask together with some muddy water (as shown in Figure 20) and left for 5 days. Perform the following tests and observe the reactions.

i. Starch-iodine reaction
ii. Benedict's reaction
iii. Urine sugar analysis paper

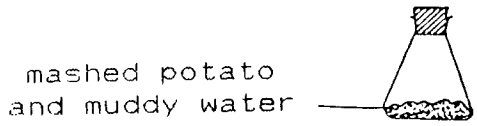


Figure 20. Flask with Muddy Water and Mashed Potatoes

(b) Add a few drops of the muddy water to the medium(e) indicated in Experiment I wait for one day, and then test with the starch-iodine test.

Expected Results

Where the muddy water dropped on the medium, starch-iodine reaction did not appear, as shown in Figure 21.

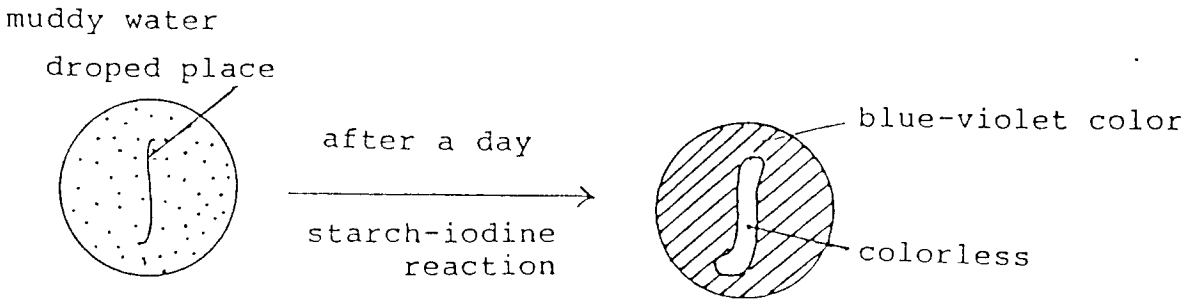


Figure 21. The Expected Effect of the Starch-Iodine Test

(c) Mix 20 cm^3 1% starch solution with 2 g soil and after a few days, test using starch-iodine and Benedict's solution.

Expected Results

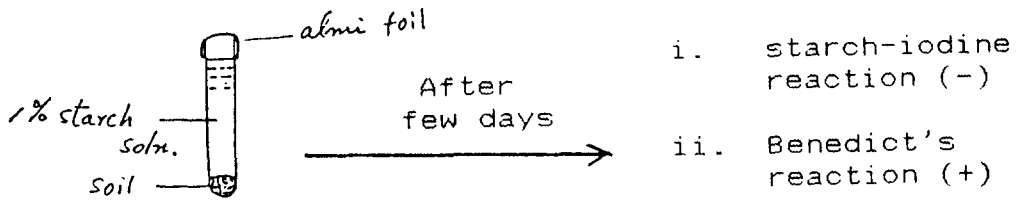


Figure 22. Set-up and Results Showing Starch-Iodine Reaction and Benedict's Reaction

2. The test for protein.

Method

(a) Mix 20 cm^3 of a soup of dried bonito with soil into a test tube and after a few days, test by the Biuret reaction.

Expected Result

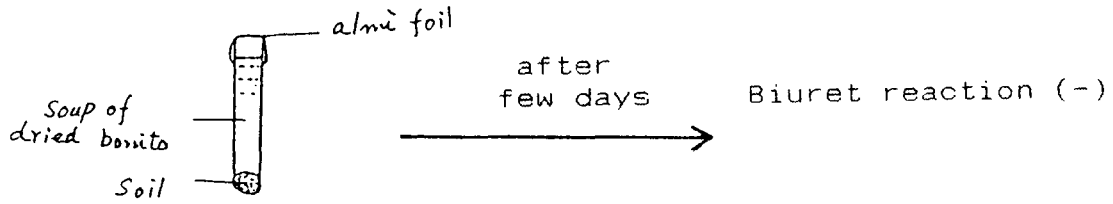


Figure 23. Set-up and Results of Protein Test

(b) (i) Mix the muddy water with fish meat or milk and put into an Erlenmeyer flask. After a few days, filter. Separate the components in the filtrate by paper chromatography. Develop the chromatogram for amino acids by spraying with ninhydrin reagent and identify the amino acid present from the Rf value.

(For reference)

The components of the developing solvent used for this experiment : Butanol 1
Ethanoic acid 3
Water 1

Developing time : 1 hour

Ninhydrin reagent : 0.2% ninhydrin buthyl solution

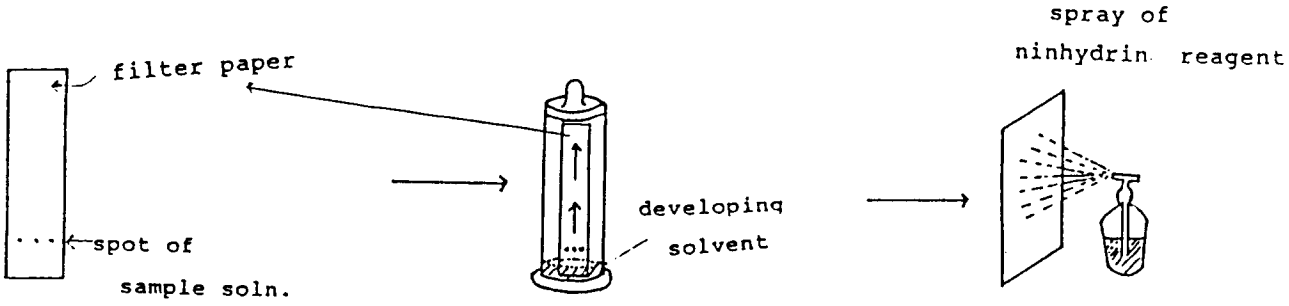


Figure 24. Examination by Paper Chromatography

(ii) If the mixture of above mentioned (i) is left for a few days, ammonia is produced by the decomposition of the protein. The occurrence of ammonia can be detected by Nessler's reagent or concentrated hydrochloric acid.

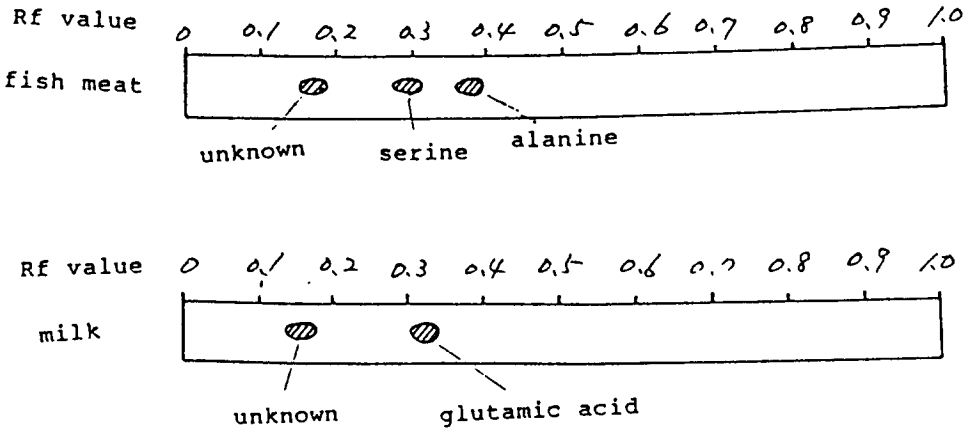
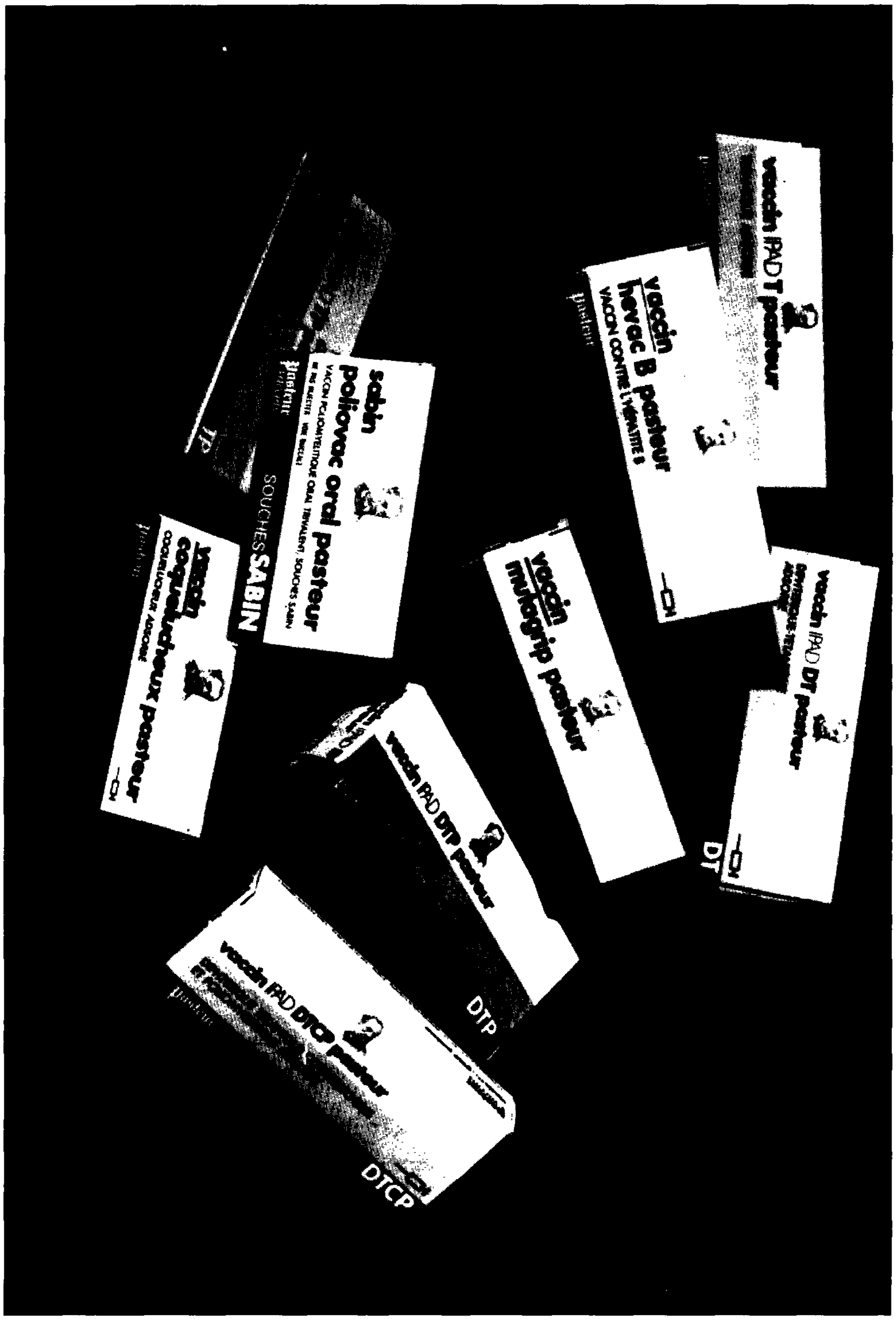


Figure 25. Identification of Amino Acids from Rf Value

Contributor: Mr. Hideharu Sakamoto, Nishigoshi Lower Secondary School Kumamoto Prefecture, Japan, solicited by The Japan Association of Science Education.


vaccin IPAD T pasteur
vaccin hevac B pasteur
sabin poliovac oral pasteur
SOUCHES SABIN
vaccin coquelucheux pasteur
vaccin mutagrip pasteur
vaccin IPAD DT pasteur
DT
vaccin IPAD DTP pasteur
DTP
vaccin IPAD DTCP pasteur
DTCP

PART VIII. MICROORGANISMS AND FOOD MAKING

1. INTRODUCTION

The use of microorganisms in food or as food has been important to man for thousands of years. They help provide him with varied diet. Other microorganisms however, can cause food spoilage and sickness. Activities on this topic which can be performed in the classroom and at home are included in this part of the resource book.

2. YEAST - THE FOOD-MAKING MICROBE

Yeasts are one of the kinds of fungi. They usually exist as single cells. Have you ever seen the powdery `bloom' on grapes or peaches? If you have, you have seen yeast. The `bloom' is made up of millions of yeast cells growing on the fruit. You have probably even eaten yeast - vegemite and marmite are made from yeast.

Yeast's favorite food is sugar. When **oxygen is not present** it uses the sugar for energy and changes it into alcohol. It also gives off a gas - carbon dioxide.

This process is called **FERMENTATION.** This is used in beer and wine making.

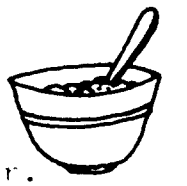
When **oxygen is present** the yeast reacts with sugar to produce carbon dioxide and water.

ACTIVITY 1: YEAST TO MAKE BREAD

Materials

* 100 g of plane flour
* 1 g of salt
* 1.5 g of yeast
* one baking tray
* 3 g of sugar
* 75 cm^3 of warm water
* plastic ice-cream container
* one clean tea-towel

What To Do



1. Put the flour and salt in the container.
2. Mix in the yeast and sugar.
3. Make a well in the mixture and stir in the water.
4. With **clean washed** hands knead the dough thoroughly for about 3 minutes.
5. Leave the dough to stand in a warm place for 20 minutes (or until it has doubled in size) covered with the tea-towel.

What has happened to the dough in this time?

Wonder why that happened?

Knead the dough again for 1 minute and place it on a baking tray - again cover with the tea-towel.

Leave the dough to rise approximately 20 minutes.

Put the dough in an oven at 200^{o}C for about 15-20 minutes. (You may like to do the final rising and baking at home.)

When you cut bread there is always holes in it. What causes that?

In your notebook write out and answer the following questions.

1. When the yeast reacts with sugar in warm moist conditions what is the gas produced?
2. What is fermentation?

ACTIVITY 2: YEAST TO MAKE GINGERBREAD

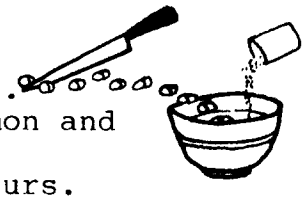
Now let's make a refreshing, bubbly drink to go with our bread. You can make a microorganism drink called ginger beer.

You will need

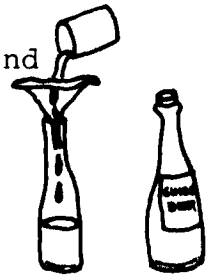
* 1 lemon
* 150 g of root ginger
* 1 kg white sugar
* 7 dm^3 of water
* 1 level teaspoonful of brewer's yeast
* screw cap bottles - clean and sterile

Why sterile?

What To Do



1. Wash and chop up the lemon and ginger.
2. To 1 dm^3 of water add the chopped lemon and ginger plus the sugar.
3. Cover the bowl and leave it for 24 hours.
4. Boil the mixture for 30 minutes.
5. Add the remaining 6 dm^3 of water.
6. Sprinkle the yeast on top.
7. Strain the mixture and put it into bottles and fit the bottle caps.
8. Leave for one week before drinking.



What makes it fizzy?

Write out and answer the following questions in your note-book.

1. What is the gas that bubbles to the surface when the bottles are uncapped?
2. What substance does the yeast use for its food?

Reference

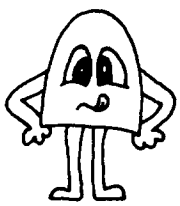
ASMP. (1979). CDC. Canberra ACT 2602, Australia.

3. MICROBES AND MILK

It has been said of milk that it is the `most nearly perfect food' for man. This is because it has nearly all the nutrients in it that we need:

Protein	Sugars
Fats	Minerals
Vitamins	Water

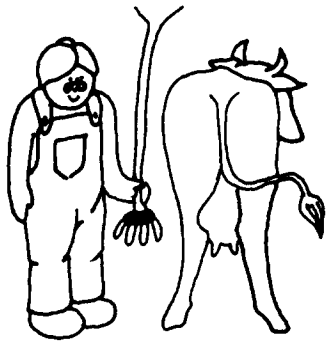
If it is so good for us, why not for microorganisms too?



Why not indeed!

In this section we will look at:

* how microorganisms get into milk
* which microorganisms get in
* how we can try to stop them getting into milk
* how we can destroy microorganisms in milk



Betty's the name.

Dairying's the game.

Hygiene's the aim!

ACTIVITY 1: HOW MICROORGANISMS GET INTO MILK?

The first port of call is obviously the animal that produces the milk.

A sick animal can pass on germs to its milk. Important human diseases such as:

Tuberculosis
Q-fever
Infectious streptococcus
Brucellosis
Infectious staphylococcus

What can I do about these?

In many milk producing states of the USA there are laws which dairy farmers have to follow. They are made to help stop micro- organisms from getting into milk.

Find out about some of these and record them in your note-book.

The second port of call is during milking itself. Micro-organisms we know live in the air, in the soil, in dung and on the animals.

What would happen when any of these got into the milk?

ACTIVITY 2: WHICH MICROORGANISMS LIVE IN MILK?

To check this we will do two small activities.

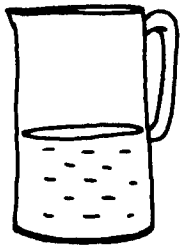
Activity One

You will need

* about 300 cm^3 of milk in an open container

What to Do

Leave the milk out in the room for a couple of days.
What has happened to the milk?
How does it look?
Does it have any unusual smell?
Will it still pour?



Activity Two

You will need

* 1 cm^3 of milk
* 1 nutrient agar plate

What to Do

Carefully spread the milk over the nutrient agar plate.
Leave for a few days at about 37°C.
Examine the plate.
What type of microorganisms do you see growing?
Are they mainly bacterial or fungal colonies?

We can see that milk has many microorganisms growing in it. These microorganisms can quickly send milk `off'.
Tests on agar plates of raw milk show that it can contain from 1,000 to 17,000 microorganisms per cm^3.

Under very bad hygiene conditions this can increase to 398,000 organisms per cm^3. As you can see from Activity Two, 1 mL of milk is a very small amount.

Although milk contains some yeasts and fungi; **bacteria** are the most common microorganisms.

These include **Streptococcus, Lactobacillus, Bacillus** and **Escherichia** to name only a few. You may have grown some of these on your agar plate.

To stop milk going "off" too quickly we must:

1. Have good dairy hygiene.
2. Keep milk refrigerated.

ACTIVITY 3: A TEST FOR MICROORGANISMS IN MILK

As microorganisms grow in milk they use oxygen. This can cause changes in milk if it is kept capped.

We can make use of this knowledge to test milk for micro-organisms.

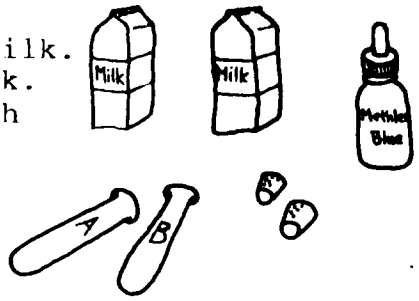
The speed of this change can be used to measure the number of microorganisms in a sample of milk.

You will need

* fresh refrigerated milk
* methylene blue
* 2 corks to fit the test-tubes
* milk a few days old
* 2 sterile test-tubes (marked A and B)

What to Do

In test-tube A put 20 cm^3 of fresh milk.
In test-tube B put 20 cm^3 of old milk.
Add 0.5 cm^3 of methylene blue to both test-tubes.



Leave both test-tubes at the same temperature conditions.

Record the time taken for each tube to de-colourise.
Write out and answer the following questions in your note-book.

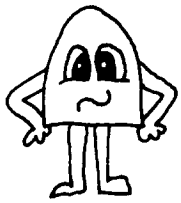
1. Which sample of milk changed colour first?

The rate of change from blue to clear gives a clue to the number of microorganisms present.

2. Which test-tube contains the greater number of micro-organisms?
3. Can you see where this test might be used in food processing?

ACTIVITY 4: HOW WE CAN DESTROY MICROORGANISMS IN MILK?

We know there are some microorganisms that live in milk that can cause us harm.



Can you remember some?

Even with the best hygiene some of these nasty fellows sneak into the milk. Somehow we need to kill them before they get us.

Can you think of some ways that microorganisms can be killed?

It has been found that the best method is HEATING.

Will any temperature do?

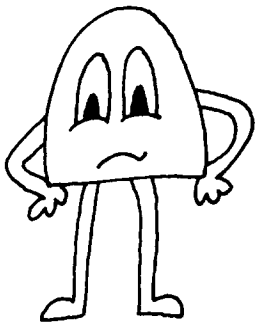
Try this. Take some milk and boil it. What happens to it when it cools? Do you think people would buy this?
We know that such treatment would probably kill the micro-organisms, but the milk is terrible.

Research has shown that there is a better way of `heat-preserving' milk. This can be done by either:

* heating rapidly to 72^{o}C. This is held for 15 seconds. The milk is then rapidly chilled.

* heating rapidly to 65^{o}C. This is held for 30 minutes. The milk is then rapidly chilled.

Such action is called **PASTEURIZATION.** (The process was named after a French scientist, Louis Pasteur, who first used it in experiments with wine.)



Why do you think it is necessary to cool the milk quickly again?

This action doesn't kill all the microorganisms in milk does it? How do you know?

Milk sold in supermarkets must by law be pasteurized. The really good thing about the process is that it doesn't harm the nutritive value of the milk.

Reference

ASMP. (1979). CDC. Canberra ACT 2602, Australia.

4. SOURING OF MILK

The souring of milk is due to bacterial growth on the milk itself. Milk is subject to contamination in many ways. Possibilities of infection by microorganisms are:

(i) the cow or other producing animal,
(ii) the equipment and utensils in which it is processed,
(iii) the people who handle it, and
(iv) the atmosphere.

The milk of all animals consists mainly of 80-90% water. Proteins, milk sugar (lactose), minerals and water soluble vitamins are dissolved or dispersed in it. Fat in milk is distributed as minute globules.

The composition of cow's milk is

87% Water
5% Carbohydrates (sugar)
4% Fat
3.3% Protein
0.7% Ash

Protein, carbohydrate and fat are substrates susceptible to degradation by microorganisms. The most common change occurring in exposed milk is souring resulting from the production of lactic acid. The lactic acid formed will then coagulate the casein (protein) in milk.

Species of Streptococcus and Lactobacillus which are harmless, ferment the carbohydrates of milk, producing lactic acid and so control the growth of other harmful bacteria. The bacteria convert lactose (milk sugar) to lactic acid; the resulting acidity causes the milk proteins to curdle. Mill will taste sour to most people when it contains around 0.20% of acid.

The activities on souring of milk are as follows:

1. The Resazurin Reduction Test to Show Souring of Milk
2. The Methylene Blue Reduction Test to Show Souring of Milk
3. Measuring pH Values of Milk

The materials needed are:

Milk Samples

evaporated milk
sterilized natural whole milk
homogenized pasteurized milk
reconstituted full cream milk - UHT
fresh cow's milk

Equipment

pH meter
water-bath with thermostat
oven with thermostat
refrigerator (storing milk at 10.05°C)
beakers - 50 cm^3, 100 cm^3, 250 cm^3 and 500 cm^3
volumetric flasks - 500 cm^3
measuring cylinders - 50 cm^3
graduated pipettes - 1 cm^3 and 10 cm^3
petri dishes - surface area of 29.13 cm^2, 37.18 cm^2 and 69.70 cm^2
test-tubes
thermometers (-10°C - 110°C)
vernier calipers
cotton wool
pipette filler
test-tube holder

Chemicals

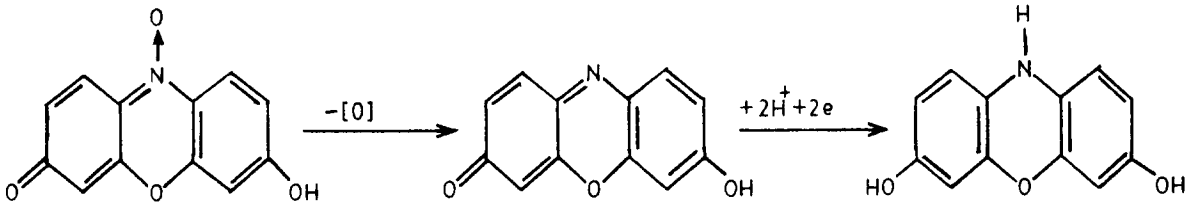
Resazurin
Methylene Blue
Buffer solutions - pH 4 and pH 7

Methods

ACTIVITY 1: RESAZURIN REDUCTION TEST

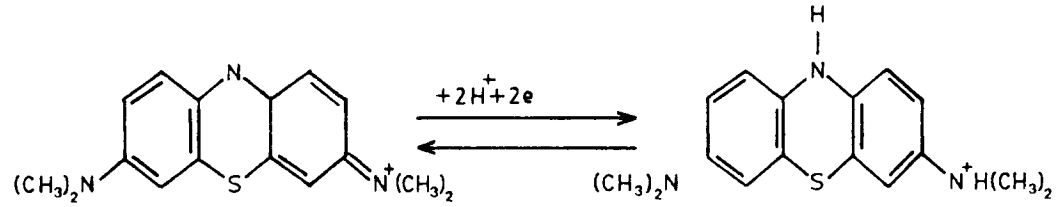
Both resazurin and methylene blue are oxidation-reduction indicators. Resazurin imparts to fresh milk a characteristic blue colour while methylene blue imparts a blue colour to fresh milk.

Reduction of resazurin is indicated by a gradual colour change through various shades of blue and mauve to full pink, at which point the **resazurin** apparently has been completely changed to **resorufin.** In milk, this stage of reduction is irreversible. The second stage, consisting of further reduction of resorufin to dihydroresorufin and characterized by a fading of the pink colour, is reversible.



ACTIVITY 2: METHYLENE BLUE REDUCTION TEST

Reduction of methylene blue is indicated by a colour change from blue to colourless (white in milk).



The removal of the oxygen from milk and the formation of reducing substances during bacterial metabolism causes the colour of both indicators to disappear. The agencies responsible for the oxygen consumption are the bacteria. Basically, the greater the number of bacteria in milk, the quicker will the oxygen be consumed and in turn the sooner will the colour disappear. In general, the reduction time of the indicators is inversely proportional to the initial bacterial content of the milk sample.

ACTIVITY 3: pH MEASUREMENT

Measuring pH values of milk enables us to measure the acidity of the milk. Normal fresh milk has a pH 6.5 to 6.7 which is equivalent to approximately 0.14 to 0.18% acidity. If the milk sours, the pH of the milk will drop as a result of the formation of lactic acid. As soon as the pH value of milk has dropped to pH 4.4 - 4.8 coagulation of casein will take place which will result in the curdling of milk. Both acid odour and flavour will then be detectable. Thus using a pH meter we can measure the pH values of the different types of milk before and after they have been exposed to air.

Preparations for All the Experiments

1. Sterilization

   Wash the glass apparatus with detergent and rinse with tap water. Then place in the oven for 2-3 hours at 120^{o}C. After each set of experiment is completed sterilize all the glassware again before use.

2. Calibrations of the pH Meter with Buffer Solutions

   Use buffer solutions with pH 4 and pH 7 to calibrate the pH meter. When not in use, dip the electrode of the pH meter into distilled water. After use in the milk sample rinse the electrode with distilled water and wipe clean. Dry with soft tissue paper before using in another sample of milk.

3. Pre-tests and Dilution of Indicators

   The resazurin and methylene blue indicators should be available in the laboratory. Transfer into a test-tube 5.0 cm^3 of a sample of milk. Pipette into the test-tube of milk 0.2 cm^3 of resazurin. Do a similar test using methylene blue. If the results are too dark, dilution as indicated.

   Dilute each indicator in the following ways:

   (i) 10.0 cm^3 indicator in 1000 cm^3 distilled water (10:1000)
   (ii) 20.0 cm^3 indicator in 1000 cm^3 distilled water (20:1000)
   (iii) 50.0 cm^3 indicator in 1000 cm^3 distilled water (50:1000)

   Pre-tests were conducted using the diluted resazurin and methylene blue. Observe the gradual colour changes caused by the removal of oxygen from the milk by the bacteria present.

4. Resazurin Reduction Test

(i) Pipette 5.0 cm^3 of each type of milk into test-tubes.
(ii) Drop 0.2 cm^3 of resazurin into each of the test-tubes.
(iii) Expose all test-tubes to air at room temperature.
(iv) Repeat the experiment for all types of milk at different temperatures maintained by the water-baths.

5. Methylene Blue Reduction Test

(i) Pipette 5.0 cm^3 of each type of milk into test-tubes.
(ii) Drop 0.2 cm^3 of methylene blue into each of the test-tubes.
(iii) Expose all test-tubes to air at room temperature.
(iv) Repeat the experiment for all types of milk at different temperatures maintained by the water-baths.

6. Use of Petri Dishes

(i) Pipette 10.0 cm^3 of each type of milk into small sized petri dishes, then another similar volume into medium-sized petri dishes, followed by the same volume into large-sized petri dishes.
(ii) Drop 0.5 cm^3 of resazurin into each petri dish and mix well.
(iii) Expose all petri dishes containing milk to air at room temperature.

(**Note:** Resazurin may be preferred as the indicator because of the gradual and distinct colour changes.)

7. pH Measurement

(i) Measure and pour 50 cm^3 of each type of milk into 50 cm^3 beakers, then another similar volume into 100 cm^3 beakers, followed by the same volume into 250 cm^3 beakers.
(ii) Expose all beakers of milk to air at room temperature.
(iii) Measure at the start of the experiment the pH of each type of milk in each beaker, then at one-hour interval for six hours. Then take the next three readings after 12 hours, 24 hours and finally after 48 hours.

Reference

SEAMEO-RECSAM. (1985). Souring of Milk. Project of Zakaria bin Awang and Au Sau Kheng, Course 3572. Penang, Malaysia.

5. CHEESE MAKING

The process of cheese making usually involves two major steps, curd formation and ripening. Each of these steps is controlled by a specific microorganism. In curd formation, for example, sour milk bacteria are used. The lactic acid that they make from milk sugar (lactose) curdles the milk and the milk curds are separated by pressing. In the ripening process yeast moulds or bacteria are used; the particular variety of cheese produced depends on which microbe is utilized.

VARIETY OF CHEESE	PRIMARY RIPENING MICROORGANISM
Cheddar	Bacteria
Gorgonzola	Fungi
Camembert	Fungi

SOME CHEESE RIPENING MICROORGANISMS

ACTIVITY 1: COTTAGE CHEESE: WHOLE VERSUS SKIM MILK

The basis of all cheese is milk solids, the proteins and fats in milk. Milk solids can be separated from the watery portion of milk in several ways. You can add an acid such as lemon juice or vinegar. You can add an enzyme (a protein that controls chemical reactions in living systems) such as rennet, the milk-coagulating protein derived from the stomachs of calves. It is used to make Junket desserts. And, of course, you can use a lactobacilli culture. When milk is made into cheese, the milk protein is coagulated and then separated as "curds" from the watery portion of the milk, or "whey". Cottage cheese is one of the simplest fresh cheeses to prepare . . .

Most commercial cottage cheese is made from skim milk, milk with the butterfat removed. In this experiment you make cottage cheese from skim milk and compare it with cottage cheese you make from whole milk. The question you'll be trying to answer is: Does butterfat make a difference in the texture and taste of cottage cheese curds?

The procedures for this experiment and for the next one (making soft- or hard-ripened cheese) lend themselves to an almost unlimited series of other experiments in cheese making. (There are specialists called dairy microbiologists who study the making of cheese and other cultured milk products as a career.) The cottage cheese results of this experiment can serve as the raw material for the one that follows. If you intend to go on and make pressed and ripened cheese, read the instructions for Hard-Ripened Cheese and collect all your equipment for both experiments before you start making cottage cheese.

Note: In any cheese-making experiment, there is a possibility that your mixture, instead of ripening, will spoil. This can happen if your equipment isn't perfectly clean, or if the temperature is too high. If your cheese smells bad or looks spoiled, do not eat it.

Materials and Equipment

$2\ dm^3$ skim milk
2 large glass or ceramic bowls or stainless steel pots (do not use aluminum or cast iron)
very large pot and a smaller one (stainless steel) that fits inside it - to be used as a double boiler
heavy cream or creme fraiche (optional)

$2\ dm^3$ whole milk
cultured buttermilk
measuring spoons
plastic wrap
2 small glasses
colander
cheesecloth
2 small bowls
knife
spoons for stirring

Procedure

Allow the cartons of milk to stand, unrefrigerated, for several hours until the milk is at room temperature. Pour the skim milk into one large bowl and the whole milk into the other. Add 3 tablespoons of buttermilk to each and stir well. Cover each bowl with plastic wrap and put the bowls in a warm place overnight.

The next day the milk will be "clabbered", or like a soft custard.

The milk is ready for the next step if the whey is starting to collect around the edges. If this is not happening, it is not "ripe" yet. Give it more time.
Make slices through the curds 2.5 cm apart. Repeat in the other direction to make a crisscross pattern, forming rough cubes.

The next step is to further coagulate the curds and cause them to separate from the whey. This is done by very slow heating to about $100^{\circ}F$ (just warm to the touch). If you heat the curds too quickly or to too high a temperature, they will become tough. Put a bowl containing clabbered milk in a pot containing hot water. (If the bowl doesn't fit into the pot, or if it isn't heat-proof, gently pour the curds and whey into a stainless steel saucepan that does fit in the larger pot.) Heat over a very low flame, stirring occasionally. The heating process should take about 30 minutes. Heat until the milk is just warm to the touch.

Remove the curds and whey from the heat and let them cool for about 20 minutes. Meanwhile, repeat the procedure with the second batch of clabbered milk.

Skim off about 1/4 cup of whey from the first batch of cheese into a small glass. Set aside. Line a colander with two layers of cheesecloth. Pour the curds and whey into the cheesecloth and let drain. From time to time, lift up the cheesecloth and shake the curds to let pockets of trapped whey drain through. Bring the tops of the cheesecloth together, twist, and squeeze out the remaining whey. Put the drained curds into a small bowl. (If you wish, you can rinse the curds under cool water.) Repeat the procedure as precisely as possible for the other batch of cheese.

Observations

Taste the whey samples from both batches. Are they different in appearance and taste? Taste the curds from both batches. Which are more tender? (One standard for judging the quality of cottage cheese is the tenderness of the curd.) Do you think butterfat plays a role in making a more tender curd?

Save the curds from the whole milk for the next experiment, if you wish. If not, salt the curds and mix in heavy cream to taste . . .

Refrigerate the cheese until you are ready to eat it. Cottage cheese is perishable and should be eaten within two or three days.

For Further Study

Design an experiment to observe what happens when clabbered curds are heated above $100^{\circ}F$.

Make cottage cheese from straight cultured buttermilk. You can also try different starter cultures, including yogurt and commercial cottage cheese. Clabber milk with acid such as lemon juice or vinegar. Meat tenderizer (actually an enzyme called papain) also clabbers milk. Try making cheese with this enzyme.

Rennet tablets are used to make commercial cottage cheese. They are, however, almost impossible to find in supermarkets or drugstores. If you do come across them, it might be fun to try using them for the initial clabbering process.

Source

Cobb, V. (1979). More Science Experiments You Can Eat. J. B. Lippincott. New York.

ACTIVITY 2: HARD-RIPENED CHEESE

Cottage cheese is often a stage on the way from milk to what we think of as real cheese - curd and ripened cheese. The ripening process takes anywhere from a few weeks to several years. Some cured and ripened cheese are hard, like cheddar cheese; others are semisoft like Brie or Camembert. The taste and texture of a cheese is determined by a number of factors, including the amounts of butterfat and water in the unripe cheese, whether or not the cheese is cooked, and the type of organism doing the ripening.

One of the simplest hard-ripened cheeses to make is Colby cheese. In this experiment, you make a variety of Colby. The main idea is to learn a technique for making hard cheese that can serve as the basis for any number of experiments.

Materials and Equipment

4 liters coffee can and metal top (save the top when you open the can, or use the new top from a freshly opened can)
whole milk cottage cheese from the last experiment
#2 cans, unopened (1 dm^3 can of minestrone soup can be used)
weights (six very fat cookbooks, or a large C clamp or a handscrew can be used)

hammer	large nail
cheesecloth	scissors
knife	paper towels
rack	paraffin (optional)
disposable aluminum pie pan (optional)	

Procedure

The coffee can is going to be your cheese press. First make drain holes in the bottom. Use the hammer and nail to punch about ten holes in the bottom of the can.

Place freshly made whole milk cottage cheese from the last experiment in cheesecloth and squeeze out as much whey as possible. Line the coffee can with a double layer of fresh cheesecloth. Put the curds into the can and fold the cheesecloth over the top neatly. (You may have to trim away some of the excess cheesecloth.) Slide the top of the can down on top of the cheese. Put the unopened #2 can on the lid of the coffee can. In a real cheese press, the part that serves the same function as the coffee can top is called the "follower".

Now weight the follower. Pile books on top of the #2 can, or use two flat pieces of wood and a C clamp or handscrew. Place the entire apparatus in the sink or the bathtub, as whey will continue to drain out through the holes.

After a few hours, take the weights off and empty out the whey that has collected in the can on top of the cheese. Reassemble. Keep the cheese weighted overnight.

The next day, remove the cheese. Unwrap it and wash the surface. Cut the cheese in quarters. Wipe each piece dry with the paper towels.

Let the cheeses dry on a rack at room temperature for several days. Turn and wipe every day. When the surface has dried out enough, a rind will start to form.

If you wish, you can put a wax rind on the cheese. At the supermarket, buy the paraffin wax used for sealing jelly jars. Ask an adult to help you with the melting; it can be dangerous, since paraffin is highly flammable. Place a block of paraffin in a disposable aluminum pie pan. Set the aluminum pan in a skillet or other pan of water. Heat slowly over a low flame until the paraffin is melted. Do not place the paraffin pan directly over the flame. Dip each piece of cheese in the melted paraffin. Remove, let cool, and dip the other side. It may take several dippings to coat the pieces completely.

Let the cheeses ripen at room temperature in a dry place. It takes about 30 days for Colby cheeses to fully ripen. After the cheeses have ripened for 10 days, sample one of them. Write down your impressions, using words like "chalky", "bland", "sharp", "hard", "crumbly", etc. Each week, eat one of the other pieces and record your impressions of the taste and texture to see what changes occur over time. The **Lactobacilli** continue to grow during the ripening process, forming waste products that make the cheese delicious.

For Further Study

Use this procedure to make cheese beginning with other starter cultures. Compare cheese made from curds started with yogurt to that made from curds started with buttermilk.

Change the butterfat content of the cheese and see how it affects the final flavor. Make cheese from heavy cream, whole milk, and skim milk. Divide each cheese into quarters before coating with paraffin. Taste samples over a period of time.

You might try rinsing the curds of commercial cottage cheese and using them as your starting point. The butterfat content is very low (4 percent). How does this affect the taste and texture of the finished product?

Source

Cobb, V. (1979). More Science Experiments You Can Eat. J. B. Lippincott. New York.

ACTIVITY 3: MOULD RIPENED CHEESE

Certain moulds have been of the utmost importance in making some cheeses world famous. Roquefort, Camembert, and Brie cheeses are all mould-ripened. The moulds involved are different species of a blue mould called **Penicillium.** (Yes, it is in the same genus as the mould from which we got the miracle drug penicillin.)

Moulds, like mushrooms, are fungi. Both are plant types that do not contain the green pigment chlorophyll. Chlorophyll enables green plants to make their own food (sugars and starches) from carbon dioxide and water. Moulds and other fungi cannot do this and must get their food from other substances. That is why they grow on food sources such as trees or bread.

Moulds are made up of many cells, unlike bacteria and yeasts, which are single-celled organisms. Mould cells form thread- like structures called hyphae (pronounced HIGH-fee) that branch and rejoin to make a tangled mass. It is these threads connecting one microscopic plant to another that distinguish moulds from other fungi. Most moulds have two kinds of hyphae. Vegetative hyphae grow into the host material or lie on its surface. Their job is to get water and nutrients for the plant. Fertile hyphae extend into the air and carry the reproductive structures, tiny seedlike items called spores. Fertile hyphae give bread mould its fuzzy appearance.

Mould spores are always floating in the air. Although moulds are often the cause of food spoilage, they are harmless and can be eaten without danger. In fact, Penicillium roqueforti and Penicillium camemberti are considered delicious when eaten in cheese. But when they grow on bread (they are the blue bread mould, not the cottony variety), they are considered very unappetizing. The main danger from eating mouldy food is that other, harmful microorganisms may have entered the food along with the mould.

In this experiment you will try three different methods of transferring mould cultures from one kind of cheese to another. The challenge is to see if you can get the moulds to grow, flourish, and ripen on cheeses normally not the hosts for these moulds. In the process you will discover how moulds ripen cheeses. Again, use your judgment about whether ripening or spoilage is taking place. When in doubt, throw it out.

Materials and Equipment

115 g Muenster cheese
115 g Brie or Camembert
85 g-package cream cheese
115 g Roquefort or blue cheese
pencil with an eraser
magnifying glass
tapestry needle (a sewing needle with a large eye)
knife
straight pin
measuring spoons
shallow dish
waxed paper

Procedure

Cut three cubes of Muenster cheese that are about 3 cm on each side. The first method of transferring mould culture is the simplest. With a knife, scrape a sliver of mould off the outer surface of the Brie or Camembert. Slide the mould off the knife onto a Muenster cube. With a pin, punch a lot of air holds in a piece of waxed paper. Wrap the cheese in it for some protection against airborne organisms.

In cheese-manufacturing plants, unripened curd that will become Brie is spread with a thick mixture of water and mould called a slurry. Next, you will try to transfer mould this way. Make a slurry by mixing about 1/2 teaspoon of mould scrapings with about the same amount of water. In a shallow dish, spread slurry over a cube of Muenster and a piece of cream cheese. Let the water evaporate before wrapping each cube in pinholed wax paper. Keep at room temperature.

The third method of transferring mould culture is by inocula- tion. You will inoculate a Muenster cube and some cream cheese with the blue **Penicillium** mould from Roquefort or blue cheese. Make an inoculation needle by sticking the point of tapestry needle into the eraser of a pencil.

Sterilize the end of your inoculation needle (the eye end) by heating it over the burner of your stove until it is red-hot. (This step is not strictly necessary, as our technique in this experiment is far from being sterile. But it does not hurt to learn some professional techniques. Micro-biologists use heat to sterilize their wire "loops" or inoculation needles.) Make sure the needle is completely cooled before using it. A red-hot needle will burn the cheese and kill the moulds . . . . . You can cool the needle quickly without contaminating it by dipping it in water that has been boiled and then cooled.

Punch some holes in the Muenster cube and in the cream cheese with the eye end of the needle. Sterilize the needle again to burn out the cheese caught in the eye. Now scrape up some blue cheese mould with the eye and insert it into a hole. Repeat until you have transferred mould into all the holes. Put some moulds on the surface also. Wrap the inocu-lated cheese in pinholed waxed paper. Keep at room temperature.

Observations

Observe the growth of the moulds over the next week or so. Use a magnifying glass. Look for softening of the cheese under the moulds and for the appearance of fuzzy fertile hyphae. Smell the ripening cheese. As the mould breaks down the protein in the cheese, amino acids and ammonia form. Ripe mould-ripened cheese has a distinctive ammonia smell.

In the ripening process, moulds actually digest the casein (milk proteins) in the curd. This changes the consistency of the curd from firm to soft, and eventually to runny. Cheese experts consider a Brie or Camembert cheese to be ripe when the entire cheese has a creamy consistency and no chalky curd is found if the cheese is cut.

For Further Study

Next time you see a commercial Camembert or Brie, look closely at the paper in which it has been wrapped. You will see that it has air holes. As an experiment, wrap inoculated cheese cubes in plastic wrap without air holes. Observe the cheese over the next week or so through the plastic. Do not unwrap and let air in. What effects does oxygen supply have on the ripening process? The moulds that produce Roquefort cheese may not need as much oxygen as those that produce Camembert and Brie. Can you design an experiment to test this?

Moulds grow best in moderately warm temperatures. Put some inoculated samples in the refrigerator and keep a duplicate set of samples at room temperature. Compare the rate of mould growth. Do you see why grocery stores keep cheeses under refrigeration?

Salt may also affect the growth of some moulds. Prepare sets of inoculated cheeses using Roquefort, Camembert, and Brie moulds. Salt one set and leave another set unsalted. Which moulds are most affected by salt?

You can test the effect of other factors on the growth of moulds. To be scientific, be sure to change only one variable at a time. Use the same mould for inoculating, the same cheeses as host materials, and the same procedures for transferring moulds.

A French cheese manufacturer moved his factory. The cheese did not ripen properly in the new ripening room. Someone took some cheese made at the old place and rubbed it on the walls in the new room. The problem was solved. Can you think why?

Source

Cobb, V. (1979). More Science Experiments You Can Eat. J. B. Lippincott. New York.

ACTIVITY 4: WHITE-CHEESE

Maintenance of a high level of hygiene and sanitation in the kitchen or cheese house, high quality milk, use of proper utensils, and adoption of proper manufacturing techniques can assure the cheesemaker of superior quality white cheese.

Materials Needed

1. Rennet extract. Rennet is an extract from sun-dried abomasum ruminants of cattle, water buffalo, and goat. Used by cheesemakers to coagulate the milk it is commercially prepared from the abomasum of young ruminants, preferably two weeks of age. Since slaughter of very young calves is not normally done, the common sources of abomasum are mature cows and water buffaloes. The abomasum is available in slaughterhouses.
2. Carabao or buffalo milk. The milk must be fresh and free from undesirable flavor or odor. Poor quality milk will not produce high quality cheese.
3. Table salt. Only salt without dirt or impurities should be used.
4. Cheese moulds. The recommended cheese moulds are round with bottom-free ends and made of aluminum. The diameter may vary from 9 to 10 cm. Clean the cheese moulds immediately after using them, and keep them dry when not in use.
5. Wire-mesh frame. This is used for draining the cheese and recommended when the volume of milk processed a day is 50 liters or less. When the volume of milk exceeds 50 liters per day, a draining table with galvanized top has to be constructed. Each wire mesh should accommodate from 16 to 20 cheese moulds and made in such a way that when the cheese moulds are filled, the frame will not sag in the middle.
6. Aluminum dipper or ladle. Kitchen aluminum dipper or ladle will serve the purpose. This is used to fill the cheese moulds with thin slices of coagulum and also for stirring the milk.
7. Cheesecloth. This is used for filtering the milk and for draining the whey (a liquid coming from the coagulum). Flour bags suit the purpose.
8. Thermometer. This is used to determine the temperature of the milk during pasteurization. The temperature scale of the thermometer should be from $0^{o}C$ to $100^{o}C$. It can be purchased from a drugstore.
9. Wax paper or banana leaves.
10. Two casseroles with different capacities. These will be used for pasteurizing and for "setting" the milk. They can also be used for sterilizing the cheese moulds, ladle and cheesecloth.

Procedure

1. Determine the weight or volume of the milk.
2. Based upon the volume or weight of the milk, calculate and dissolve the required amount of salt. White cheese is usually prepared from water buffalo milk containing 3 percent table salt. This is 3 kg salt for every 100 kg milk or 1 1/2 tablespoonfuls salt per dm^3 of milk.
3. Strain the salted cheese milk. Filter the salted milk into a casserole using clean cheesecloth to remove all impurities of the milk and salt.
4. Pasteurize the cheese milk. By double-boiler method, heat the milk at 72°C and maintain the temperature for 5 minutes. Then cool the milk by replacing the hot water in the double boiler with cold water. The milk may also be cooled by putting the casserole of hot milk under cold running water. If ice water is available, cooling the milk with ice water is better.

   Pasteurization is done to kill any microorganism present which shortens the shelf-life of the cheese. Milk is rendered free from microorganisms when heated to 63°C for 30 minutes, or 72°C for 15 minutes.

   If the milk is to be made into cheese right away stop cooling when its temperature is 40°C. If it is to be processed the following day, the milk must be cooled to a temperature not higher than 15°C.

   Avoid overheating. Salted milk heated to 85°C may curdle or produce weak coagulum with poor draining property. On the other hand, pasteurizing the milk at a temperature below 72°C for 5 minutes will yield cheese with poor quality and shorter shelf-life.

5. Add the rennet extract. When the temperature of the milk is already 40°C, add the rennet extract. With locally prepared rennet, the amount varies from 1 to 2 tablespoonfuls per dm^3 of salted milk. The correct amount can be determined from experience. You can tell the correct amount of rennet when the milk forms a coagulum within 15 to 20 minutes after adding the rennet extract. If the milk does not coagulate add more rennet to double the above rate. Adjust the amount of rennet accordingly next time you prepare the white cheese.

   After adding the rennet, stir the milk slowly for one minute. Then cover the milk to form a coagulum.

6. Prepare the cheese moulds. While waiting for the milk to harden or to form a coagulum, sterilize the cheese moulds, cheesecloth and ladle or dipper by steaming, as follows.

Put 3 to 4 cups of water into the casserole and then place inside the cheese moulds, cheesecloth and dipper or ladle. Cover and keep the water boiling inside for 5 minutes. The steam sterilizes them. In arranging the moulds put the wire mesh frame on a basin or tray. This basin or tray will catch the dripping whey. Cover the frame with cheesecloth and arrange the cheese moulds on it. On a larger scale, cover the top of the draining table with cheesecloth and arrange the cheese moulds on it.

7. Scoop the coagulum and fill the moulds. With a ladle or dipper, transfer the coagulum into the moulds in layers and in thin slices. The slices should be about 1 cm thick. The thinner the slices the faster the whey drains off. When properly done white cheese of fine texture results.

Preparation of Rennet

The following steps are recommended in preparing rennet extract:

1. Wash the abomasum after it has been separated from the rest of the internal organs.
2. With a knife, cut the abomasum laterally on one side.
3. Remove all the adipose tissues that may be found on the outer lining of the abomasum. Just pull them and do not use knife.
4. Wash again and stretch it with 2 to 4 sticks. The sticks should cross each other at the middle of the stretched abomasum.
5. Tie the abomasum at the point where the sticks cross each other and hand it in the sun until it is thoroughly dry.
6. To prepare a rennet extract, but the whole sun-dried abomasum into small pieces (1 square cm or smaller) and soak them in 2 dm^3 of extracting solution for 5 to 7 days at room temperature. The solution is prepared by mixing 1 dm^3 each of vinegar and water, and 4 to 5 tablespoonfuls of table salt.
7. Filter the solution on the 7th day of soaking. Discard the abomasum and place the rennet in bottles. Store the bottles in the refrigerators.

   Although the rennet extract can already be used on the second day after the extraction, it is not advisable to do it because the rennet is still weak.

   Rennet prepared according to the above method can be used at the rate of 2-3 tablespoonfuls per dm^3 of milk. At this rate the milk will show sign of coagula- tion in about 15 minutes after adding the rennet.

   Immediately after transferring the coagulum to the cheese moulds, cover them with another clean cheese-cloth.

8. Drain the whey. Allow the cheese to drain for at least 4 hours. After draining, the curd should have shrunk to about 3/4 to 1/2 of its original volume. By then, the cheese cakes could be handled easily without breaking them.
9. Wrap each cheese cake after draining for about 4 hours. Wrap the cheese cake with either wax paper or clean fresh banana leaves. Handling the cheese by bare hands cannot be avoided, thus it is important that hands be washed thoroughly with soap and water before handling the cheese.

   The wrapped cheese is then ready for the table. Keep the cheese in a cooler or refrigerator. If you follow the above methods carefully, you will get 50-60% cheese yield, depending upon the period allowed for draining the cheese.

Source

Survival Catalogue. (1981). Technology Resource Center. Makati, Philippines.

6. VINEGAR MAKING

Vinegar is a food seasoning made from sugary or starchy materials by an alcoholic fermentation process followed by an acidic one. To be considered as legal vinegar, it should contain 4 percent ethanoic acid.

The manufacture of vinegar involves two steps: (1) the fermentation of sugar to ethanol, and (2) the oxidation of alcohol to ethanoic acid.

The first step is an anaerobic process carried out by yeasts. A simplified equation for the process is:

$$\underset{\text{Glucose}}{C_6H_{12}O_6} \xrightarrow[\text{without air}]{\text{yeast}} 2CO_2 + \underset{\text{Alcohol}}{2C_2H_5OH}$$

The second step, oxidation of the alcohol to ethanoic acid, is an aerobic reaction carried out by the ethanoic acid bacteria.

$$C_2H_5OH + O_2 \xrightarrow[\text{with air}]{\text{bacteria}} CH_3COOH + H_2O$$

Ferment into vinegar or ethanoic acid:

1. When alcoholic fermentation is complete, decant or separate the clear liquid from the sediment.
2. To every 4 parts of the clear liquid, add 1 part of good unpasteurized vinegar, or "mother liquor" (**Acetobacter aceti**), which is found in vinegar.
3. Mix thoroughly.
4. Cover the container with cloth to keep insects out.
5. Allow to ferment until the vinegar is strong enough to use.
6. Decant or separate the vinegar from the sediments and mother liquor by filtration.

Filter:

1. Fold filter paper or cotton flannelette into a cone. Line this in a funnel (glass funnel is preferred; metal funnels can be readily attacked by ethanoic acid) and put over a convenient receptacle such as a bottle or jar.
2. Filter vinegar several times to make it clear or bright. (Juice high in tannin, like cashew or guava juice, are not too easy to clarify.)

Pasteurize:

1. Transfer vinegar to final containers. Seal tightly and pasteurize at 60-72°C for 20 minutes. Use a thermometer to determine the temperature of the water accurately. Place jars or bottles on a rack in a sterilizer. Seal tightly and invert to heat the lids further.
2. Cool containers completely before labeling.

Optional

If accuracy is desired, test the sugar content of the fruit juice to be used with a Brix or Balling hydrometer before fermentation begins.

Source

Survival Catalogue. (1981). Technology Resource Center. Makati, Philippines.

ACTIVITY 3: VINEGAR OUT OF BANANA SKIN

1. Slice up a kilo of banana skins, add 4 cups water, then boil.
2. Extract the juice from the banana skin. Decant and filter through cheesecloth. Add 3/4 cup sugar and 1/4 tea- spoon ammonium sulphate to supplement the nitrogen defi- ciency of banana skins.
3. Cool, then add Baker's yeast. Allow to ferment for 8 days. The alcohol content is 9-10 percent.
4. Add a cup of "mother liquor", or vinegar starter (available at National Institute of Science and Technology, Pedro Gil St., Manila), then set aside or incubate at room temperature.
5. Allow to ferment for 27 days, at which period 4-6 percent acidity is attained.
6. Bottle and age to produce quality vinegar.

Source

Survival Catalogue. (1981). Technology Resource Center. Makati, Philippines.

7. MAKING WINE

The process of making wine is similar to that of making vinegar except that the former stops after the alcoholic fermentation.

Any sugary medium like many fruits and juices containing at least 15 percent sugar or which are as sweet as ripe mangoes may be used. For juices that are less sweet, sugar may be added. If a high ethyl-alcohol content is desired, the fermenting juice is progressively enforced with sugar as this breaks down.

To evaluate the product, note the following:

Appearance: It may be coloured or colourless depending on the kind of fruit based, but the wine should never be hazy or paler than, or too dark for, its natural colour.

Aroma : It should be clean-smelling, with no mouldy odor.

Taste : It should be sweet, not flat with a strong alcoholic taste.

ACTIVITY 1: PINEAPPLE WINE

Wash and peel sound mature fruits. Waste cores and trimmings from the pineapple canning plants can be utilized for wine-making. Crush the peeled fruit and press well to extract the juice. Strain through a cheesecloth or strainer. To one part of the juice, add 2 to 3 parts water. For every 4 cups of diluted juice, add 1 cup sugar. Shake well to dissolve the sugar and heat for 30 minutes to pasteurize. Cool and add 1 tablespoon yeast to every 18 cups of the juice mixture. Pour the mixture into a demijohn or any suitable container. Loosely plug the mouth of the container and store in a safe place. Ferment for two or more weeks until fermentation is complete, i.e., when no bubble is liberated. Pasteurize, then decant the clear liquid into any suitable container and age for 2-3 months or longer.

To the aged wine, add well-beaten egg white (1 for every 12 cups). Heat in a steam bath at $55^{o}C$-$60^{o}C$ for 15-20 minutes to clarify the wine. Filter the clear wine and pasteurize again before bottling.

ACTIVITY 2: RICE WINE

Ingredients

2 cups rice (cooked)
5 g leavened fermented rice available in cake form

Procedure

1. Cook rice; let it cool by transferring it to a clean bowl.
2. Pound the leavened fermented rice finely.
3. Disperse it evenly in the cooked rice with a wooden spoon. Add hot water if cooked rice becomes too dry.
4. Cover and wrap tightly with cloth or doubled waxpaper.

Ferment:

1. Set aside in a place where it will not be disturbed. Let it stand for 4-6 days. This depends on the amount of rice used and the extent of fermentation you want.
2. Squeeze the rice wine. Pour into a glass container. Let it stand for 2-3 days or until clear.

Pasteurize:

1. Filter or decant the wine.
2. Pasteurize in a kettle of simmering water for 20 minutes.
3. Seal completely, label, date and store.

ACTIVITY 3: CASHEW WINE

Ingredients

18-20 big, sound, ripe cashew fruits (make around 1 dm^3 juice)
3/4 cup brown sugar
1/4 teaspoon dry active yeast

Procedure

Prepare the juice:

1. Wash fruit, removing the nuts.
2. Crush the fruit, preferably with a wooden crusher, or extract the juice by pressing. Use no iron utensils.
3. Measure the juice. Add 3/4 cup sugar to every dm^3 of juice.
4. Heat the sweetened juice to $70^{o}C$.
5. Cool to room temperature. This may take at least an hour. Hasten cooling by placing pan of juice in a bowl of tap water.

Ferment the juice for the alcoholic fermentation:

1. Add 1/4 teaspoon of dry active yeast to every dm^3 of juice.
2. Place in a sterile bottle and plug the mouth of the container with cotton.
3. Set aside for at least 1-2 weeks or longer for complete fermentation, especially if progressive amounts of sugar are added to produce a high alcoholic content.

Pasteurize as with vinegar:

1. Decant the clear wine and heat to 50°C to kill any undesirable organisms. If desired, use a double-boiler to prevent excessive heating.
2. When preparing on large scale, age in barrels for a year or longer.

ACTIVITY 4: ORANGE WINE

Ingredients

1 dozen medium-sized oranges
2 mg sodium metabisulphite sugar
1/8 teaspoon yeast

Procedure

Prepare the juice:

1. Wash oranges thoroughly.
2. Cut crosswise and gently squeeze out the juice with an orange squeezer.
3. Strain out coarse pulp and seeds by passing juice through a muslin bag.
4. Test the sugar content of the juice with a hydrometer. (Check sugar content against a composition table.)
5. Add enough sugar to make 22'-24' Balling for a dry wine of medium alcoholic content and to 32'-33' Balling for one that will contain a small amount of sugar after formulation is completed. To prepare: 22' Balling - 1.1 cups sugar/4.4 cups water; 24' Balling - 1.2 cups sugar/4.8 cups water; 32' Balling - 2.1 cups sugar/4.3 cups water; 33' Balling - 2.2 cups sugar/4.4 cups water.

Pasteurize wine:

1. Transfer wine to final containers. Seal and pasteurize at 60°-72°C for 20 minutes. Be sure to place bottles on rack and keep submerged in hot water. Seal tightly and invert to heat the lids further.
2. Cool, label, date and store.

ACTIVITY 5: BLACKBERRY WINE

Wash fruits to remove dirt. Discard spoiled ones. Crush fruits with bare hands or potato crusher without bruising the seeds. Extract the juice by passing through a cheesecloth. To the residue, add water equal to the first extract. Heat to boiling. Strain.

Combine the first and second extracts. To every 3 parts of the extract, add one part of refined sugar and heat to 70°C. Cool. Add one teaspoon of yeast to every 20 dm^3s of the sweetened juice and transfer into wine barrels or demijohns. Set aside to ferment for a month or until no more bubbles or carbon dioxide gas evolves. Set aside for another month. Decant the clear liquid and age in barrels for at least one year.

ACTIVITY 6: PSIDIUM GUAJAVA L. (GUAVA) WINE

Select sound and ripe fruits. Quarter them. To one part of the fruits, add 2 parts water. Boil fruits until soft. Strain and measure the extracts. To every 3 parts of the extract, add one part sugar. Stir and measure the extract. Cool. To every 15-20 dm^3s of the extract, add one tablespoon yeast. Ferment in demijohns for about 2 weeks. When fermentation is complete, transfer into wooden barrels and age for at least a year.

ACTIVITY 7: BANANA PEEL WINE

1. Slice a kilo of banana peels, add 4 cups water then boil.
2. Extract the juice of banana peel. Decant and filter through a cheesecloth. Add 1 cup sugar to extract.
3. Pasteurize, then add 1/4 teaspoon ammonium sulphate to reinforce nitrogen deficiency. Cool, then add wine yeast.
4. Allow to ferment for 8 days. The alcohol content is 11-12 percent.
5. Set aside for a month to age; clarify by adding well-beaten egg white.
6. Filter through clean cheesecloth to produce sparkling wine.

Source

Survival Catalogue. (1981). Technology Resource Center. Makati, Philippines.

8. MAKING DESSERTS

ACTIVITY 1: MAKING NATA

Nata is a white gelatinous growth of cellulose in a fermenting liquid which is made by the fermentation of various sugary juices. The process takes about 10-15 days when a solid layer is formed on the liquid. Cut into desired shapes, boiled and washed, **nata** makes a delicious dessert.

Ingredients

8 cups coconut water or coconut milk (use the grated meat from 1 coconut as desired)
1 cup sugar
3 tablespoons glacial ethanoic acid
2 cups mother liquor (or starter)

Procedure

1. Prepare the culture jars. Wash and thoroughly dry all culture jars and other receptacles to be used in propagating the **nata** organism. Dry the jars in an oven or under the sun. After drying, cover the jars with clean paper or kitchen towel and secure cover on jars with string or rubber band.
2. Prepare the starter. Inoculate 1 dm^3 of the medium with a young and vigorous culture of the **nata** organism. (This can be obtained from the Biological Research Center, National Institute of Science and Technology, Pedro Gil Taft Avenue, Ermita, Manila.) Let it stand in a covered culture jar (any 2 dm^3 jar) for at least one day, if the **nata** is young and vigorous, a thin growth of **nata** will form on the surface of the fermenting liquid after 2 days.
3. Prepare the coconut water medium. Collect coconut water in a clean basin, kettle or sauce-pan by straining it through a piece of clean cheesecloth. Add 1 cup sugar for every 8 cups of coconut water, coconut milk or grated coconut. Stir to dissolve the sugar well. Heat to boiling point to kill undesirable organisms. Cool the mixture to room temperature (27^oC). Add glacial ethanoic acid. Distribute the mixture in culture jars to levels of 5-7.5 cm deep, allowing sufficient air space above the surface of the liquid.
4. Inoculate and incubate. Inoculate every 4 cups of culture medium with 1 cup of the starter. Stir thoroughly. Cover the jars and incubate at 27^oC, the temperature most favorable for the growth of **nata** organisms. Leave the jars undisturbed while the **nata** is forming on the surface of the medium. It is not necessary to put it in a dark place.
5. Harvest the **nata**. After 10-15 days, when the **nata** is above 2.5 cm thick, pick up the **nata** from jars with a clean fork taking care not to contaminate the liquid (called "mother liquor") which can be used as a starter for subsequent growing of **nata**.

Use the following proportions for growing nata: for every dm^3 of coconut water, add 1 cup sugar and 1 1/2 tablespoons glacial ethanoic acid. Inoculate the above mixture with 1 cup mother liquor. Distribute in culture jars. Follow preparation of culture jars and coconut water medium mentioned above.

If a large amount of mother liquor accumulates and will not be used for growing more **nata**, strain off foreign particles and use this to start ethanoic-acid fermentation in vinegar-making.

ACTIVITY 2: NATA DE COCO DESSERT

1. Cut the harvested **nata** into uniform 2.5 sq. cm. piece and boil vigorously in an open pan for one minute. Boil the **nata** in several changes of water until the vinegar odor is removed. If time is not too important, another method used in removing the sour odor from harvested **nata** is to soak it whole in several changes of fresh water for several days until the odor is removed. Expose the **nata** under the sun until it becomes very white. Cut into desired sizes and boil in several changes of water before cooking in syrup.
2. Prepare a thin syrup of 1 part sugar to 1 part water. Use 1 kilo sugar for every kilo of **nata.**
3. Bring syrup to boiling, add drained **nata,** and simmer in an open pan for 5 minutes. colouring may be added at this stage.
4. Let the **nata** stand in syrup overnight. Cover surface with wax paper and weight with a saucer to immerse all pieces in the syrup.
5. Drain the syrup next day. Add sugar to the syrup, using half of the amount originally used. Stir to dissolve the sugar well and boil for 10 minutes.
6. Add the **nata** and simmer for 5 minutes. Repeat the three preceding steps until the **nata** is translucent and well penetrated by the syrup. Any desired flavoring extract (lemon, orange, pineapple) may be added to the syrup at the last boiling. Allow 1 teaspoon of flavoring for every dm^3 of syrup prepared.
7. Drain the syrup when ready to pack the product. Fill sterilized jars with the drained **nata.**
8. Bring syrup to boil and pour into the **nata**-filled jars. Remove trapped air bubbles by inserting a knife along the inside of the jar.
9. Immediately seal jars and process in boiling water bath for 15 minutes.
10. Dry and cool jars completely.
11. Label, date and store.

To evaluate the product, note the following:

Appearance: translucent, uniformly cut **nata**, not grayish white in colour, with an even growth of the culture.

Texture : chewy but tender, not tough or fibrous.

Taste : sweet, not acidic or flat.

Any sugar containing solution, preferably fruit pulp, can be a culture medium for **nata.** Use pulp scraped off from mango seeds and peels after preparing frozen or canned mango, tamarind, guyabano, pineapple, guava, etc., provided the correct procedure is followed. Most fruits contain 15-20 percent sugar which, of course, becomes diluted after water is added for extraction. Hence, adjustment in sugar concentration is necessary if the **nata** organisms are to be supplied with a source of energy to produce the nata growth.

ACTIVITY 3: NATA DE GUAYABANO (Scientific Name: **Anona muricata L.**)

Follow the same basic directions for sanitary handling and preparation of the starter. This time, use guayabano which can be made from guayabano mash obtained after extracting the juice. Allow 1 dm^3 or 4 cups of water per kilogram of guayabano. After preparing the medium, proceed as with **nata de coco.**

ACTIVITY 4: NATA DE PINA (PINEAPPLE)

Follow all steps as that for **nata de coco** except for the preparation of the pineapple medium:

1. Wash pineapple in running water very well.
2. Peel the pineapple.
3. Cut the peelings as thinly as you can without removing the eyes. If desired, use the pulp scraped from the shell or parings of the pineapple, including the cores.
4. Slice the pineapple and grind, preferably in a meat grinder.

Source

Survival Catalogue. (1981). Technology Resource Center. Makati, Philippines.

9. MAKING JAMS

Jams are jellylike products prepared by boiling whole or pieces of fruit pulp with sugar to a moderately thick consistency. Different varieties of fruits can be combined and made into exciting blends.

GENERAL DIRECTIONS

Select ripe firm, fresh fruit

Prefer fresh, slightly underripe fruits to the overripe ones. Also, prefer fruits rich in pectin and acid, such as guava, green tamarind and papaya (**Carica papaya L.**).

Soften the fruit

Prepare the fruit as for other cooking purposes: remove stems and leaves and rinse the fruit in tap water. Fruit with seeds may be left whole or seeded.

Simmer the fruit gently for some time before adding the sugar to soften the skin and to break down the cell walls of the fruit to extract the pectin. Bring the fruit to boiling, then allow to simmer gently until reduced to a pulp. Fruits which readily break down, such as strawberry, do not require the addition of any water, but may require simmering in a covered pan for 15 minutes before adding the sugar.

Add the acid

For fruits low in acid (e.g. papaya, sweet varieties of guava and sweet mango), add citric acid or lime juice before cooking the jelly as it helps to extract the pectin. For every 1 kg fruit, add 4 tablespoons lime juice (approximately 1 average-sized lemon) or 1 1/2 teaspoons of citric acid or tartaric acid.

Test the pectin

Simmer the fruit until the skin is softened. Take 1 teaspoon juice of seeds and skin and place in a custard cup or glass, and cool.

Add 1 tablespoon of denatured alcohol (70 percent). Stir slightly with a fork to mix. **Do not taste.** Determine the quantity of pectin in the fruit. Juices rich in pectin will form a transparent jelly-like lump that can be picked up with a fork. If the fruit has a moderate amount of pectin, the clot of jelly is not very firm and breaks up into two or three lumps.

Add the pectin

Add pectin to fruits deficient in it by blending with pectin-rich fruits (such as papaya) or by adding the juice of fruits rich in pectin.

Add the required amount of sugar, depending upon the pectin content of the fruit. The ratio suggested is 3/4 cup of sugar for every cup of pulp. Add 1 cup of sugar if the fruit is quite rich in pectin.

Add the sugar when the skin of a hard fruit has thoroughly softened, or else the fruit toughens at once and this mistake cannot be corrected. On the other hand, sprinkle sugar on soft fruit like strawberry, overnight to keep it whole if desired. Do not add the sugar too early in the process; if the fruit and sugar are boiled too long together, both the flavor and colour are spoiled and the skins may be toughened. Overboiling may also result in a sticky jam.

Boil rapidly after adding the sugar, observing the jam-making maxim, "Cook slowly before adding the sugar and rapidly and quickly afterward," until the setting point is reached.

If the fruit has been well cooked and broken down before sugar is added, boiling should take only 10-12 minutes for a pint of sweetened pulp, according to the kind and quantity of the fruit.

Test the setting point

The setting point is usually attained when the added sugar is 65 percent of the final weight of the jam. Underboiling results in less than this proportion and may cause fermentation. Overboiling, on the other hand, increases the possibility of the sugar crystallizing out. At this point the sugar concentration is 65 percent (60 percent from the added sugar and the 5 percent from the fruit) so that jam sets with the right ratio of acid and pectin.

Determine the end points by the method discussed in the general directions for jelly-making; the tests for the sugar concentration (weight test, volume test, cold-water test, and temperature test) and the two tests which show when the jam will set, give an indication of the proportion of sugar in it (sheeting or flake test and cold plate test).

Finish the jam

Remove the jam from the hot stove immediately after it reaches setting point.

Quickly remove the scum, if any, with a perforated spoon dipped in boiling water and wiped just before use.

Pour the jam at once into clean, dry and, if possible, warm jars. Fill right to the top with hot jam to allow for the shrinkage which takes place during cooling.

For jams containing whole fruit, prevent the fruit from rising in the jar by allowing the jam to cool in the pan until a thin skin starts to form. Then stir the jam gently and pour into the jars.

Gently press a well-fitted waxed tissue on the surface of the jam in each jar and carefully wipe with a clean cloth wrung out after being dipped in hot water. Tie down jars at once or protect them with a clean cloth or with paper until quite cold before covering.

Label, date and store jams in a dark, cool, airy place. Note that excessive heat or dampness may result in mould growth.

To evaluate the product, note the following qualities:

Appearance: bright-coloured, darkened but not faded or burned; has the characteristic colour of the fruit.

Texture : thick and smooth, not flowing or lumpy; no characteristic fruit shape but not mushy.

Taste : pleasingly acidic, with a characteristic fruit flavour but not astringent or burned.

ACTIVITY 1: MAKING MANGO JAM

Ingredients

2 cups pulp of ripe mangoes
1 cup sugar (increase to 1 1/3 cups if pulp is sour and slightly underripe)
1/2 teaspoon citric acid

Procedure

1. Wash mangoes and blanch.
2. Peel skins and scrape pulp from the seeds by using a stainless-steel spoon.
3. Scoop out pulp and pass through a sieve or blender to obtain a uniform texture. For certain fruit jams the pulp need not be mashed and thus a blender is not necessary.
4. Measure pulp and combine sugar.
5. Cook in a heavy aluminum pan, constantly stirring with a wooden spoon until it is thick enough to be spooned out when lifted from the pan.
6. Place in warm, sterile jars while hot and seal at once.

Note: Do not use iron knives and utensils during the preparation of jam to prevent discolouration of the fruit into a dark-coloured product.

ACTIVITY 2: MAKING PAPAYA JAM

Ingredients

4 cups ripe papaya pulp
3 cups sugar
1/4 cup lime juice or 2 teaspoons citric acid or tartaric acid

Procedure

1. Select fully ripe sound papaya. Remove seeds and fiber and scoop out pulp after running a fork lengthwise and crosswise deep into the pulp to within 1/2 cm from the peel.
2. Boil briskly in a smooth, heavy aluminum pan until thick enough for jam or when jam starts to form a mound on the edge of a spoon.

Source

Survival Catalogue. (1981). Technology Resource Center. Makati, Philippines.

10. MAKING JELLIES

Fruits that are not suitable for canning or freezing can be made into jellies. Jelly, prepared by boiling the fruit, straining the juice and adding sugar until saturated, is a favorite spread for bread.

GENERAL DIRECTIONS

Choose fresh, mature, ripe fruits rich in pectin and, if possible, also acid. Fruits with high pectin content (e.g. guava and tamarind) usually have very distinctive flavors.

Prepare and cook the fruit

1. Wash and sort the fruit.
2. Cut large fruit after removing the stem and leaves.
3. Simmer the fruit in water until tender (about 10-15 minutes) to soften the fruit and thus dissolve the acid and pectin. The amount of water to be used depends on the juiciness of the fruit. In general, the water should be enough to cover the hard fruit in the pan. Remember to cut large pieces of fruit into 5 to 7.5 cm thickness or grind or shred fruits like pared ripe papaya.

Strain the pulp

1. Scald the jelly bag with boiling water.
2. Pour the cooked pulp into the bag and allow it to drain, twisting the open end of the bag tightly but without forcing the pulpy bits to come out. Ideally, it is wise to allow the pulp to strain without squeezing if a clear jelly is required.
3. Extract the juice of pectin-rich fruits a second time. use the same amount of water as in the first extraction.

Test the fruit juice for pectin to determine the proportion of sugar to be added.

1. Mix a teaspoon of the extract with 1 tablespoon denatured alcohol (70 percent). Stir with fork and lift the pectin lump.
2. Stir slightly to mix. Do not taste.
3. Determine the quantity of pectin in the fruit. Juices rich in pectin form a transparent jelly that can be picked with a fork. Juices with a moderate amount of pectin form a jelly that is not very firm and breaks up into two or three lumps.

Add the sugar

Add sugar in proportion to the amount of pectin in the juice. The higher the amount of pectin, the greater the amount of sugar it can hold. Use 3/4 to 1 cup of sugar for every cup of juice. For home-extracted juice, never use more than 1 cup of sugar for every cup of juice.

Boil rapidly to jellying point ($104^{o}C$-$105^{o}C$), above the boiling point of water.

Note: The juice will settle only with the correct pectin-sugar-acid ratio.

To evaluate the product, note the following qualities:

Appearance: transparent, bright, characteristic fruit colour which is clear with the bottom of the container seen clearly.

Shape : follows the contour of the container, can easily be slipped out of the container, and is not sticky.

Texture : smooth, thick, quivery but not runny.

Overall Taste : characteristic fruit flavor with a subacidic taste.

ACTIVITY 1: MAKING GUAVA JELLY

Ingredients

1 kilo mature ripe guava (**Psidium guajava L.**)
1/2 kilo sugar
2 teaspoons citric acid

Procedure

1. Wash the ripe fruits and cut off stems and blossom ends. Boil for 5 minutes, using 5 cups of water for every kilo of guavas.
2. Draw off the water into a bowl and set aside; mash the guavas in the saucepan with a wooden spoon. Return the water and reboil slowly in a covered saucepan for another 15 minutes. Stir occasionally to prevent scorching.
3. Strain the juice with a moistened jelly bag. Twist the open end of the bag using only enough pressure to squeeze the juice but not the pulp. Extract the juice for the second time following the same procedure. Add the same amount of water as in the first extraction. Put the two extractions together.
4. Test the juice for pectin by adding 1 teaspoon of fruit juice to 1 tablespoon of denatured alcohol in a custard cup. Stir slightly with a fork. When done, lift the pectin with the fork. Note character of the pectin. Juice low in pectin forms a stringy precipitate or make the alcohol only cloudy. A large mass will form if a fruit juice is rich in pectin.
5. Test the juice for acidity with a standard acid solution (i.e. 1 teaspoon lime juice with 3 tablespoons water and 1/2 teaspoon sugar). Fruit juices that are less acidic than the standard may be combined with a little fruit acid or commercial acid (citric acid or tartaric acid).
6. Add 3/4 to 1 cup of sugar for every cup of fruit juice. the amount of sugar to use depends on the pectin content of the fruit juice. A fruit juice rich in pectin can be cooked with a higher ratio of sugar.
7. Combine the fruit juice and sugar in a 6 dm^3 saucepan. Boil until the sugar dissolves completely. In another 3 dm^3 saucepan, cook small amounts of this sweetened juice not more than 2 cups at a time.
8. Boil the juice as vigorously as possible until the jelly point is reached. The jelly stage can be detected by the following tests:

   (a) **thermometer test** - end point of jelly cooking ranges from $4^{o}C$-$5^{o}C$ above boiling point of water.

   (b) **sheeting test** - at jellying point the solution falls in sheets; that is, the drops combine at the edge of the spoon to form a sheet before falling.

(c) **cold water** - allow last few drops to drop from the spoon into a glass of tap water. Jelly is done if drops reach the bottom of the glass of water whole; if these drops disperse, then the jelly needs further cooking.

9. Allow the bubbles to subside completely. Pour into sterile jars set on a wad of newspaper. With a fork, lift the scum that formed.
10. Pour melted paraffin before the jelly cools completely. Put on lid and label.

ACTIVITY 2: MAKING PAPAYA JELLY

Ingredients

4 cups pulp from ripe firm papaya grated or sliced thinly 1/4 cm thick
4 tablespoons lime juice
2 teaspoons citric acid
2 cups water

Procedure

1. Combine papaya and 2 cups water, 4 tablespoons lime juice, or 2 teaspoons citric acid.
2. Simmer for 15 minutes in covered saucepan.
3. Tests for pectin and acid, method of cooking, and testing for jellying point are the same as for guava jelly.

Source

Survival Catalogue. (1981). Technology Resource Center. Makati, Philippines.

PART IX. MICROORGANISMS AND FOOD SPOILAGE

1. MICROBIAL FOOD SPOILAGE - PRINCIPLES AND PREVENTION

The term "food spoilage" usually implies decay or decomposition of food which involves changes that renders food unacceptable for human consumption. Food spoilage may be caused by one or more of the following:

(1) Insect/rodent damage
(2) Physical injury due to bruising, pressure, freezing, drying
(3) Activity of naturally occurring enzymes
(4) Chemical changes, particularly involving oxygen
(5) Activity of microorganisms

Spoilage caused by microorganisms is the most important of the above and the discussion to follow is primarily concerned with this aspect.

On the basis of susceptibility to spoilage, foods may be classified as non-perishable (stable), semi-perishable, and perishable foods. Non-perishable foods are foods which do not spoil unless handled carelessly. Sugars, flour and dry beans are examples. Semi-perishable foods are foods like potatoes and apples, which if stored and handled properly, will remain unspoiled for a fairly long period of time. Perishable foods spoil readily unless special preservative methods are used, e.g. meats, fish, most fruits and vegetables, eggs and milk.

Microbial Food Spoilage

Microbial food spoilage is due to the growth and activity of microorganisms - moulds, yeasts and bacteria in foods. All food was once living tissue and is of organic origin and thus is susceptible to deterioration and spoilage by microorganisms. Microorganisms have extremely rapid rates of growth. The microbial growth cycle consists of a lag phase, a log logarithmic (exponential) growth phase, and a stationary phase.

The lag phase is the time taken for the microorganisms to adapt to the food and organize systems for the utilization of available nutrients. In the exponential or logarithmic growth phase, the microorganism is multiplying at its optimal rate under the prevailing conditions and the stationary phase occurs when the new cells are being produced at the same rate that the old cells are dying. Foods are usually spoiled by the time the microbial population proliferates to such an extent as to reach the stationary phase.

Microbial food spoilage is therefore a result of the proliferation of microorganisms, with the accumulation of large number of cells and their metabolic products, causing alterations in the flavor, colour and texture of the food. The souring of milk, the growth of moulds on bread, and the rotting of fruits and vegetables are common examples of microbial food spoilage.

Spoilage Pattern of Foods

The spoilage pattern is rather specific for a given type of food, e.g. fresh meat generally develops a superficial slime during storage, cured meat products tend to spoil by slight souring by bacteria and yeast, vegetables deteriorate due to proliferation of moulds and fungi, fruit juices tend to be fermented by yeasts. The type of spoilage to which a food is subject is largely determined by the initial contamination of the food. This governs the number and kind of microorganisms present. Foods become contaminated from ubiquitous habitats such as soil, surface water, dust, the gastrointestinal and respiratory tracts and skins of animals and man, and the environment where food is manufactured and prepared. These diverse sources provide mixed populations of potentially contaminating microorganisms. However, only a fraction of this initial microflora can grow, under any given conditions of storage. Therefore, those that possess the physiological attributes that allow their survival or growth under the specific conditions encountered can survive and grow. These species become the dominant microflora and are responsible for the typical spoilage pattern. Thus, microbial food spoilage is essentially an ecological phenomenon.

Factors Affecting the Growth of Microorganisms

The selection process which results in a dominant spoilage microflora is governed by such factors as the food itself, its physical state, its chemical composition, its environment as well as the mutual influences among microorganisms. In order to control microbial food spoilage, it is important to survey the basic parameters which affect growth. These can be divided into the following groups:

(1) Intrinsic Factors

These are factors inherent in the food material and relate to the physical properties and chemical composition of the food. Their combined effects will determine the selection of that part of the initial flora capable of growth or survival. Microorganisms unable to compete in a particular environment are gradually eliminated.

(2) Extrinsic Factors

These are environmental factors imposed on the food, e.g. storage temperature, relative humidity and partial pressure of storage gases that select a particular flora.

(3) Microbial Interactions

This relates to the mutual interactions among species in mixed populations.

Intrinsic Factors

(1) pH

pH exerts strong selective influences on microorganisms present in the food. Most microorganisms grow best at pH values around neutrality or just below, whilst relatively few grow below pH 4.0. There are some food bacteria which grow at a low pH, e.g. lactobacilli and some streptococci, but in general, moulds and yeasts are more tolerant of low pHs than bacteria and this effect of pH is reflected in spoilage patterns (Table 2).

Table 2. The Effect of pH on Spoilage Flora

Food	pH	Typical Spoilage Organisms
Wines	2-4	Spoilage uncommon
Fruits	3-5	moulds, yeast
Meats	5-7	Bacteria

The effect of low pH in retarding growth is utilized to stabilize certain foods in which acid-producing micro-organisms ferment carbohydrates to acid, lowering the pH and retarding the growth of other less desirable organisms. Examples of this are fermented milks and vegetables.

(2) Water Activity

The growth and metabolism of microorganisms demands the presence of water in an available form, which is measured by the water activity Aw. The Aw of food is defined as the ratio of the water vapor pressure of the food (P) to that of pure water (Po) at the same temperature, i.e. Aw = P/Po.

For pure water, Aw = 1. The availability of water in a solution is dependent upon the amount and nature of the solute present. The greater the concentration of solute present, the lower will be the water availability. As water is held by the solute, this will be reflected in a decrease of vapor pressure above the solution and Aw will decrease. As water is essential for the growth of microorganisms, a lowering of Aw will retard growth and eventually inhibit it.

Most microorganisms have an optimum Aw of 0.99 for growth. Each type of microorganism has a minimum available water requirement - the Aw below which it cannot grow. As this minimum Aw is approached, growth is progressively retarded. Examples of the minimum Aw values for growth of some groups of organisms are shown in Table 3.

Table 3. Minimum Aw Values for Groups of Food Spoilage Organisms

Microorganisms	Min. Aw	Example of food with such a Aw
Food spoilage bacteria	0.91	Dry ham containing 12% NaCl
Normal yeasts	0.87	Saturated solutions of sucrose, or foods with 15% NaCl
Halophilic bacteria	0.75	Foods containing 26% NaCl, Jams and Marmalade
Xerophilic fungi	0.65	Rolled oats containing 10% water
Osmophilic yeasts	0.60	Dried fruits containing 15-20% water or toffee with approx. 8% water

At high Aw values, where almost all microorganisms thrive well, bacteria rapidly dominate the spoilage pattern because they generally grow much faster than the fungi. However, fungi are more resistant to Aw. As Aw decrease, there is a shift of dominant species from bacteria towards yeasts and moulds. Thus the availability of water will not only control growth, but will select certain tolerant microorganisms which will emerge as the spoilage flora, e.g. in concentrated sugar solutions, osmophilic yeast may become dominant.

(3) REDOX Potential (Oxidation-Reduction Potential)

The redox potential in food governs the type of organisms that grow. The simplest definition of redox potential is the ease with which the substrate loses or gains electrons. This propensity for the transfer of electrons can be measured in terms of electrode potential and is expressed by the symbol Eh, usually in millivolt units. The Eh scale has both positive and negative values, with positive values indicating a more oxidized environment and negative values a more reduced environment. The Eh values of some foods are shown in Table 4.

Table 4. The Redox Potential of Some Foods

Food	Approximate Eh (mV)
Plant juices	+300 to -400
Cheeses	- 20 to -200
Solid meats	-200
Commimuted meats	+200

Microorganisms can be classified as aerobic, anaerobic or facultative based on the critical redox potential required for multiplication and metabolism. Anaerobic bacteria e.g. **Clostridia**, appear to require reduced conditions, i.e. a negative Eh for growth initiation whilst aerobic bacteria e.g. **Bacilli**, require a positive Eh. Between these extremes are the facultative types which grow under either aerobic or anaerobic conditions.

The redox potential in a food is also related to some extent to the oxygen tension prevailing during storage. However, the relationship between the oxygen tension and redox potential in foods is not direct. The partial pressure of oxygen surrounding the food must be greatly changed before the Eh of the food is much affected. This is because most fresh foods possess redox poising capacity i.e. resistance to changes in potential, as they are relatively rich in reducing compounds like thiol-containing amino acids and peptides, reducing sugars and ascorbic acid. This explains why anaerobic growth may take place in a food under apparently aerobic conditions, e.g. meat cuts stored in air may have low interior redox values due to the high redox poising capacity, and may support the growth of anaerobic bacteria to the extent of spoilage.

(4) Nutrients

In order to grow and function normally, microorganisms require not only water, but also an energy source, nitrogen source, certain vitamins and related growth factors, and minerals.

Foods contain varying amounts of carbohydrates, lipids, proteins, minerals and vitamins which can serve as substrates for the growth of microorganisms. Meat products on one hand are highly nutritious, but cabbage, on the other hand, is regarded as poor source of nutrients. Nutrients must be available in a form that is utilizable and degradable by microorganisms. In general, microorganisms normally utilize simple compounds first, e.g. simple sugars, amino acids, alcohols. For more complex compounds like proteins, lipids and higher carbohydrates, they can be degraded only by organisms that have specific enzymes. Therefore, the richer the food in simpler compounds, the wider the range the attacking organisms, and the selective pressure increases as the food becomes more complex towards polymers like proteins and higher molecular weight carbohydrates.

In general, moulds are the least exact species in terms of nutrient requirement, followed by yeast, and bacteria.

(5) Natural Antimicrobial Constituents

Some foods contain natural-occurring substances which have antimicrobial activities. Some examples are shown in Table 5. These antimicrobial factors are often specific in their actions and probably do not affect the growth of more than a small number of organisms.

Table 5. Intrinsic Antimicrobial Constituents of Some Foods

Food Material/Ingredient	Antimicrobial Agent
Fresh milk	Lactenins
Egg white	Lysosome
Cranberries	Benzoic acid
Cinnamon	Cinnimic acid

(6) Biological Structures

The presence of biological barriers of some foods provides excellent protection against the entry and subsequent damage by spoilage organisms, e.g. the shell and shell membrane of the egg, the testa of seeds, the outer covering of fruits, and the shells of nuts.

(7) Previous Processing Factors

Many steps in food processing change the properties of foods and affect the composition of microflora. Thermal processing while inactivating existing normal flora, also softens the tissue and changes the permeability of the food to moisture and oxygen and may release the binding of essential nutrients too. Thus cooked foods are more sensitive to spoilage and if contaminated, the dominant species will easily outgrow the others.

Dehydration of food causes a decrease in Aw which exerts selective influences as discussed earlier. Changes in Aw may also affect the structure of the food in that it generally becomes more accessible to microbial attack.

Foods like fruit beverages, wines and jams preserved by sulphur dioxide, benzoic acid of sorbic acid, if spoiled, may be contaminated by organisms which have developed resistance to these additives.

The pH value may be decreased by the direct addition of an acid such as ethanoic or lactic acid, or by a suitable lactic acid fermentation as in the preparation of many types of fermented sausages, sauerkraut and yoghurt. The pH value so attained is low enough to suppress the less acid tolerant food spoilage agents and so protect effectively against proteolytic spoilage. Finally, during processing the food may gain extra organisms from equipment and the added ingredients.

Thus, as a result of food processing, foods become modified and only those adapted organisms that can grow in the food can cause spoilage.

Extrinsic Factors

(1) Temperature of Storage

The classes of microorganisms that play a role in the spoilage of foods embrace those which grow at temperatures within the range -5 to 70^{o}C but few can grow over the entire temperature range. Each has its minimum, optimum and maximum temperature for growth. Based on this, three main categories of food spoilage organisms may be distinguished (Table 6).

Table 6. Classification of Organisms by Temperature

Group of Organisms	Temperature °C		
	Minimum	Optimum	Maximum
Mesophiles	5 - 15	30 - 45	35 - 47
Psychrophiles	-5 - +5	12 - 15	15 - 20
Thermophiles	40 - 45	55 - 75	60 - 90

The psychrophiles which are found most commonly on foods are gram-negative rods belonging to the genera **Achromobacter, Pseudomonas** and **Alcaligenes.** These organisms will grow well at refrigerator temperatures and will cause spoilage of fish, meat, poultry and other foods normally held at these temperatures. The genera **Bacillus** and **Clostridium** are the most thermo-resistant bacteria of importance in food. Their resistance is due to their thermoresistant spores, not to the vegetative cells. The majority of these thermo-resistant bacteria are species of bacillus, e.g. **B. stearothermophilus, B. thermosaccharolyticum** which are of particular importance in the canning industry. Mesophiles which grow well at moderate temperatures include most food spoilage organisms and their rapid growth rates at these temperatures results in rapid spoilage.

Moulds normally grow over wider ranges of temperature than bacteria. Many moulds are able to grow at refrige- ration temperatures, particularly some strains of **Aspergillus** and **Cladosporium.** Yeast can grow over the psychrophilic and mesophilic temperature ranges but not generally within the thermophilic range.

(2) Relative Humidity

The available water in a food can be a growth control-ling factor, as discussed earlier. The relative humidity of the environment is important both from the viewpoint of maintaining water activity and in controlling growth on the food surface. The equilibrium relative humidity (ERH) is related to Aw by the equation: ERH(%) = Aw x 100%.

An equilibrium exists between the water in the food and that in the surrounding atmosphere. A food of low Aw in an atmosphere of high relative humidity will absorb water until an equilibrium is established and vice-versa. If low Aw is the controlling factor and the food is stored in an atmosphere of high relative humidity, then as water is absorbed the Aw will rise, and control due to restricted water availability will be lost. At high relative humidity water will accumulate on the surface providing a fluid film in which microorganisms spread and slime formation can occur. Beef cuts and whole chicken are liable to this type of surface spoilage before deep spoilage occurs. This is normally due to the high relative humidity in the refrigerator and the fact that meat spoilage is generally aerobic in nature.

(3) Gaseous Atmosphere

Oxygen is required by many organisms for growth. If oxygen is removed by vacuum or replaced by an inert gas like nitrogen, the growth of an aerobic flora will be retarded and to a lesser extent that of facultative aerobes too. For anaerobes, it would seem that they will not grow in the presence of oxygen. However, they may be found growing in conditions which are normally regarded as aerobic. As discussed earlier, the Eh in a food and its redox poising capacity determine whether a microorganism will grow.

Gases like nitrogen and carbon dioxide can have an effect on the growth of microbes by displacing oxygen. Carbon dioxide, in addition, has intrinsic microbiostatic properties, e.g. in gas-packed bacon, reduction of oxygen concentration to 1% of the atmosphere does not delay spoilage appreciably, whilst a certain concentration of carbon dioxide in the presence of oxygen will considerably delay spoilage. Furthermore, the gas-permeabilities of the wrapping packages also play a role in selecting the type of microbes to grow in the food.

Combined Effects of Intrinsic and Extrinsic Factors

The potential for microbial growth resulting in spoilage is a consequence of the combination of intrinsic factors or extrinsic factors that are interdependent. The effect of a given intrinsic or extrinsic parameter is also determined by the values of the many other factors influencing microbial growth mentioned earlier. This can be shown in the relationship of water activity and temperature of incubation. The range of water activity over which growth occurs is greatest at the optimum temperature and most restricted as the temperature varies from the optimum. Another example is the interaction of pH and heat. Canned products of low pH do not require such severe heat treatments to render organisms non-viable as do products of higher pH.

Microbial Interactions

In mixed populations, interaction between microorganisms take place. Most foods have diverse microbial populations and are complex ecological systems, in which one species may affect the survival and growth of another. Two common types of interaction are **symbiosis**, in which one species aids the growth of a second species, and **antagonism**, in which the first species inhibits the growth of the second. These interactions are usually brought about by changes in the growth controlling factors such as pH, Aw, redox potential, nutrient status of the food, and elimination or production of antimicrobial compounds.

One microorganism may enable another to grow by having the enzymatic potential to degrade the otherwise unavailable complex molecules. Organisms may also produce nutrients required by other microorganisms but not naturally occurring in the food substrate, e.g. the synthesis of B-vitamins by moulds and yeasts can initiate the growth of lactic acid bacteria in vitamin B deficient food. The production or metabolism of acid by bacteria can lower or raise the pH value of a food, allowing the more acid-tolerant or less acid-tolerant organisms to become established. The growth of certain facultative anaerobes can lower the redox potential to the level where strict anaerobes can develop. The Aw of foods can also be changed by the metabolism of micro-organisms. This generally involves the release of water and results in a local increase in Aw values of foods. Thus some of the less tolerant organisms previously inhibited are allowed to grow. Furthermore, many microorganisms form metabolites with antimicrobial activity, e.g. methanoic acid, ethanoic acid, propionic acid and alcohol. On the other hand, in some situations, many microorganisms may metabolise an antimicrobial compound to which a second organism is susceptible.

Because of the complex of continuing interactions between the intrinsic, extrinsic factors and microorganisms, a food at any one point in time carries a characteristic flora - its association. The microbial profile changes continuously and one association succeeds another in what is called a succession. Spoilage frequently involves a succession of organisms. Eventually the species which is highly adapted to the particular food will become dominant over the others and is responsible for the ultimate spoilage of the product.

Control and Prevention of Microbial Food Spoilage

The factors that establish the environment in which microbial species will compete for dominance have been reviewed. To control or prevent microbial food spoilage, these factors can be manipulated such that the proliferation and metabolism in microorganisms is arrested. This can be achieved by:

(1) Killing microorganisms by heating, canning, radiation or other physical process.

(2) Inhibiting the growth of microorganisms, i.e. prolonging the lag period in the growth cycle or increasing the time the microbial population takes to double in number in the exponential phase. The methods employed include the following:

   (a) reducing Aw (dehydration, addition of sugar or salt)
   (b) low temperature storage (freezing or refrigeration)
   (c) low pH (acidulants)
   (d) chemical preservatives
   (e) low oxidation-reduction potential (exclusion of oxygen)

(3) Removing microorganisms by filtration or centrifugution, in the case of liquid foods such as fruit juices.

(4) Limiting contamination of foods by maintaining aseptic conditions.

Finally, to prevent post-processing contamination, it is essential that food is properly packaged and stored during its distribution to the consumer.

Source

Kan, M. W. M. (1987). Microbiology and Food Symposium. Institute of Biology, (Hongkong Branch).

2. WHAT MICROORGANISMS DO TO FOOD

Some microorganisms can cause diseases. Illness like food poisoning can be due to the action of microorganisms on food. Microorganisms cause food spoilage and when we eat them we get poisoned. Now let us see how food gets spoiled.

Materials

samples of fresh foods
plastic bag
labels
table swabs
pH indicator paper (1-14)
marker pen
clear bottle
hand lens
beaker or clear bottle

Procedure

1. Choose one food. For example, a corn cob. Place it in a clean plastic bag and tie it. Label with your name and the date. Leave it in a warm place.
2. Choose a second type of food, e.g. coconut water. Place it in a clear container/bottle. Put your name and the date. Leave it in a warm place.

3. After two days, examine the contents of the bag and jar with hand lens.
4. Smell the foods and describe them, if possible, by using familiar descriptions (e.g. rancid, alcoholic, sour, putrid).
5. Measure the pH with the pH paper.
6. Put your observations in the table that follows:

Table 7. Description of Observed Food

Corn Cob	Fresh Food	Spoiled Food
smell		
pH		
type of colony		
colour of colony		
size of colony		

Discussions

1. Why were the food kept in a warm place for 2 days?

_____

_____

2. Which type of foods have the most microorganisms growing on them?

_____

_____

3. Did all the microorganisms growing on the food appear to be of the same type?

_____

_____

4. Explain how the foods might acquire specific smells or flavors due to microbial action.

_____

_____

5. Are the pH values of the fresh and bad food the same? Explain why or why not?

_____

_____

6. Which organism might have been responsible for turning the food alcoholic?

_____

_____

Conclusion

Write a summary describing what you have learned from this experiment.

Source

Science in Action. (1985). Bato Balani Publications. Makati, Philippines.

3. WHAT CAUSES MEAT TO GO BAD?

Materials

2 small pieces sof fresh meat
2 petri dishes with nutrients
droppers
2 conical flasks with stoppers
bunsen burner
culture media

Procedure

1. Put each piece of meat in separate flasks and add enough water to cover the meat.
2. Heat the flasks until the water boils. Keep it boiling for 15 minutes.
3. Close firmly one flask with a stopper and label it "A".
4. Leave the second flask open and label it "B".
5. Leave the flasks to stand 1 to 3 days.
6. Notice any change in odor, and possibly colour, in the two pieces of meat.
7. Shake the flasks.
8. Using a separate dropper in each case, transfer a drop of liquid from each flask to a petri dish with the nutrient medium. You also may want to look at a drop from each flask under the microscope.
9. Cover the petri dishes, and let it stand for 2 to 3 days.

Studying the Results

Q1. In which flask did you observe change in odour? in colour?
Q2. In which flask did you observe microorganisms, if any?
Q3. Illustrate in your notebooks the microorganisms that you see under the microscope.
Q4. What causes meat to go bad?
Q5. How does the fact that you boiled both flasks at the beginning of the investigation provide evidence to support your answer to question 4?
Q6. How does the way you used the two flasks provide evidence to support your answer to question 4?

Source

UNESCO Pilot Project for Biology Teaching in Africa. (1967-68). Handbook of Microbiology.

PART X. FOOD PRESERVATION

1. WHY WE NEED TO PRESERVE FOOD

For thousands of years man has been trying different ways to preserve food so that it won't spoil. Different fruit, vegetables, cereals, animals and fish all become plentiful at certain times of the year. There is usually too much to eat but the remainder must be saved for lean times so that people do not go hungry. The problem of course is how to prevent the microorganisms attacking our reserves of food to ensure a good supply of food all year round.

2. HOW TO PRESERVE FOOD

The very first methods of preservation used heating, cooling, drying or salting of food.

Early man learned to cook his food. This killed many of the microorganisms.

In some areas it was possible to keep food in snow.

For hundreds of years fruit and fish have been dried in the sun. Grain has been dried and turned into flour for probably thousands of years.

Meat and fish have long been preserved by salting.

Early explorers of the sea had to rely on preserved food. Fresh food was not available for months at a time. Salted meat, dried fruit and flour was the basic diet of those early travelers.

These methods were used because they worked. People didn't know about the microorganisms that made it possible.

Our knowledge of microorganisms did not begin till as late as the last century. It began with the work of men like Pasteur, Koch and Jenner.

What did the fellows do
I wonder?

The work of people such as these and many more has shown us that food can be preserved if:

* microorganisms are killed
* food is treated to stop or slow the growth of micro-organisms

Let's look at one of the early ways of preserving food - salting of meat.

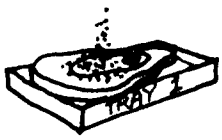
You will need

* two pieces of meat
* two small dishes
* plenty of salt

What to Do

Put one piece of meat in each dish and mark them tray 1 and tray 2.

Rub salt into the meat in tray 1. Make sure the whole surface of the meat has been rubbed. Leave the meat in the dish with the left over it.



The second tray is not unsalted. This is called CONTROL.

What do I need a control for?

Over the next few days look to see what happens to the meat of both trays.

* Record your observations in your record book and answer the following questions.

1 Is there any indication that microorganisms have attacked the meat in either tray?

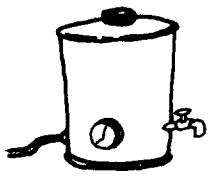
Plain ordinary salt!
What could it do?

2 How could salt help to prevent food spoilage?

Salting is only one method of preserving food. There are many others. Can you think of some of these?

Here are some clues:

Got the preserves on. Had a great crop of pears on the tree this year.



Yes, all these foods have used **HEATING** in the bottling, canning or pasteurizing to kill harmful microorganisms.

What do you think might be one problem with heating some food?

Look carefully at the following graph.

Notice both high and low temperatures stop the growth of microorganisms. Why is this?

x = number of microorganisms

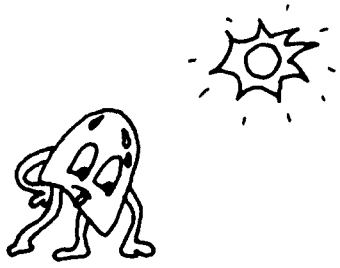
To keep foods at low temperatures where we can see from the graph that few microorganisms can grow we use **REFRIGERATION AND FREEZING.**

Another method of preserving foods like raisins, sultanas and apricots where days are long and hot with low humidity is sun **DRYING.**

Milk, eggs and soup can also be preserved by taking the water out.

Why do you think drying would stop microbes growing?

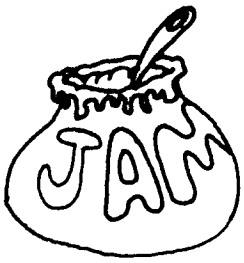
water! water!

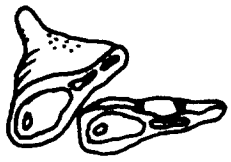


Preservatives

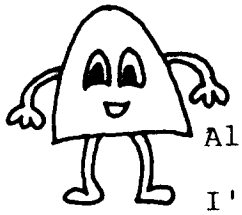
We have already seen that salt can preserve food. When we add things to food to help them last we add what we call PRESERVATIVES.

Can you think of any other preservative besides salt?





Wonder why these preservatives have been used here?



All this preserving.

I'm going to starve!

* Make a list in your notebook, of any preservatives you find marked on prepared foods at home. Are any of these chemicals?

Spotting Food Spoilage

How can we tell if food is spoiled?

Some ways that we usually use are:

* smell
* colour
* taste
* sight

Can you think of any more?

Always check food before eating it!
You have perhaps had a case of food poisoning. It is not very nice is it? The body usually reacts violently resulting in vomiting or diarrhoea.

Such attacks are usually caused by one of two BACTERIA with long names:

Staphylococcus aureus

and

Salmonella typhimurium

Fortunately, such attacks usually only last a few hours to a day.

The worst kind of food poisoning is called BOTULISM. It is caused by another bacterium **Clostridium botulinum.**

The poison from this bacterium is one of the most powerful poisons known.

Death often results from such poisoning.

Boiling food for 30 minutes at 80^{o}C can destroy this poison if it is suspected.

Pickled or canned meat and vegetables which show any signs of bulging or gas bubbles should be avoided. This is a sign of BOTULISM.

Old saying from my Grandma.

<u>If in doubt throw it out</u>!

Very good advice too!

Hygiene and Food Spoilage

Many cases of food poisoning may be stopped by simply taking care when food is prepared.

We are always told to wash our hands before working with food.

We often hate washing and wiping up. Why can't we just keep on using the same plates over and over again?

They tell us not to re-freeze food once it is thawed. Why do you it is the case?

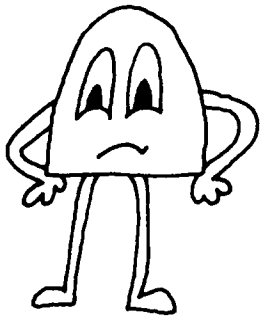
See if you can find out some of the strict rules that are put on cleanliness at abattoirs and canneries.

Remember

* cleanliness
* check food before eating
* do not keep food too long

These simple things all help reduce our chances of food poisoning attacks.

Meanies!



Source

ASMP. (1979). CDC. Canberra ACT 2602, Australia.

3. FOOD PRESERVATION BY HEATING

Procedures

1. Put small pieces of pineapple into 2 test-tubes and plug test-tubes with cotton wool.
2. Put the test-tube into a can of 15 cm height. Fill the can with about 100 cm^3 of water, and then cover the can.

3. Boil the water in the can for approximately 15 minutes. Take the test-tube out of the can and put a mark on the tube.

   Be sure that the test-tube is firmly plugged during the experiment.

4. Compare the result of the two test-tubes for 7 days and make a record.

Questions

1. Are pieces of the pineapple in the two test-tubes changed? If yes, how and what is the cause?
2. Why is it necessary to firmly plug the test-tubes during the experiment?

There are two methods of food preservation by heating i.e., sterilization and pasteurization. Sterilization is the method to free the foods from microorganisms by extreme heating. This method can be done using a pressure-cooker at the temperature of 120^{o}C under the pressure of 1 atmosphere, or using any other cooking utensil at the temperature of 180^{o}C under the pressure of 1 atmosphere. By applying this method, all microorganisms will be destroyed. However, owing to the fact that some types of food are easily degenerated in high temperature, pasteurization is recommended for maintaining the quality of these kinds of food because they will be treated at the temperature of only about 60^{o}-70^{o}C for about 15-30 minutes.

Since this method can destroy only some types of microbe pasteurized foods e.g. fresh milk, have to be kept at the temperature of lower than 5^{o}C in a proper sealed container for a long time preservation.

3. Should sterilization or pasteurization be applied in the production of canned food? Explain.

Source

IPST. General Science Textbook for Grade 9. Bangkok, Thailand. (Translated from Thai language.)

4. HEALTH HAZARDS OF MICROBIAL FOOD CONTAMINATION

From the beginning of life until death, a person is subjected to potentially hazardous environments. During one's lifetime, some disappear and others take their place so that the problems of safety are not static. The ingestion of food is no exception. Food may serve as a carrier of chemical and biological substances, either added or acquired as contaminants from soil, water, air, food handlers, equipment and other sources.

If illness follows shortly after the consumption of food, then it is customary for the victim to claim that he or she has **food poisoning**. Adopting such a general definition, Bryan (1973) listed approximately 200 diseases that can be transmitted to man by food. The causative agents of these diseases are: bacteria, viruses, fungi, parasites, chemicals and plant and animal toxins. This paper will only deal with health hazards associated with microbial contamination of food.

Causative Organisms

The microbial flora of food consists of the microorganisms associated with the raw material, those acquired during handling and processing and those surviving any preservation treatment and storage. Since these organisms do not arise spontaneously, they must contaminate the food at some stage of production, harvesting, handling, processing storage, distribution and/or preparation for consumption. The investigation of an outbreak of food poisoning invariably involves examination of all of these different stages. However it must be remembered that not all microorganisms in food produce illness but others do and some of these are listed in Table 8. For some of these diseases causing organisms (e.g. viruses, protozoa) the food merely acts as a carrier whereas for others (e.g. bacteria and fungi) there is rapid multiplication in the food and the heavy growth which results, induces illness by one means or another after ingestion of the contaminated food. Table 9 shows the clinical features of illnesses produced by some of the two types of bacterial food poisoning.

Table 8. Microbial Causes of Food Poisoning

MICROORGANISM	EXAMPLES*
1. BACTERIA	
Bacteria grow in food and form populations large enough to:	
(a) colonize the gut of a susceptible person.	**Salmonella spp.** **scherichia coli** **Campylobacter jejuni** **Yersinia enterocolitica**
(b) intoxicate the gut when eaten by susceptible person.	**Clostridium perfringens** **Vibrio cholerae** **Vibrio parahaemolyticus**
(c) produce sufficient toxin in food to cause illness in susceptible person.	**Staphylococcus aureus** **Bacillus cereus** **Clostridium botulinum**
2. VIRUSES	
Viruses are unable to multiply in food which acts merely as a carrier. Penetrate cells of gut and replicate before spread, in some cases, to other parts of the body.	Hepatitis A Poliomyelitis Gastroenteritis viruses
3. FUNGI	
(a) Toxic fungal fruiting bodies eaten as food.	Death cap fungus
(b) Mycotoxins produced when fungi grow in food.	Aspergillus (aflatoxin) Claviceps (ergot alkaloid)
4. PROTOZOA	
Presence of resistant cyst in food (no multiplication) infects gut when ingested.	**Entamoeba histolytica** **Toxoplasma gondii**

*This is not a comprehensive list.

Table 9. Clinical Features of the Illnesses Produced by Some Food Poisoning Bacteria

Bacteria	Illness
Salmonella spp.	INCUBATION PERIOD, 6-48 h; usually 12-36 h DURATION, 1-7 d Diarrhoea, abdominal pain and vomiting Fever nearly always present
Vibrio parahaemolyticus	INCUBATION PERIOD, 2-48 h; usually 12-18 h DURATION, 2-5 d Diarrhoea, profuse and often leading to dehydration; abdominal pain and fever
Escherichia coli	INCUBATION PERIOD, 12-72 h DURATION, 1-7 d (a) Cholera-like illness with watery diarrhoea and pain (b) Prolonged diarrhoea with stools containing blood and mucus
Campylobacter jejuni	INCUBATION PERIOD, 2-10 d DURATION, 5-7+ d Flu-like symptoms with abdominal pain and fever followed by diarrhoea, often severe
Clostridium perfringens	INCUBATION PERIOD, 8-12 h DURATION, 12-14 h Diarrhoea, abdominal pain, nausea but rarely vomiting; no fever
Staphylococcus aureus	INCUBATION PERIOD, 2-6 h DURATION, 6-24 h Nausea, vomiting, diarrhoea and abdominal pain; no fever Collapse and dehydration in severe cases
Bacillus cereus	INCUBATION PERIOD, 8-16 h DURATION, 12-24 h Abdominal pain, diarrhoea and occasionally nausea INCUBATION PERIOD, 1-5 h DURATION, 6-24 h Nausea and vomiting, and occasionally diarrhoea
Clostridium botulinum	INCUBATION PERIOD, usually 18-36 h Death in 1-8 d or slow convalescence over 6-8 months Symptoms variable: disturbance of vision, difficulties speaking and swallowing. Mucous membranes of mouth, tongue and pharynx usually dry. Progressive weakness and respiratory failure.

Pathological Changes in Food Poisoning

The illnesses produced by disease causing microorganisms result from damage to the intestinal lining either due to microbial penetration and multiplication (e.g. some bacterial, viral and protozoal infections) or as a result of toxins produced in the food or in the gut lumen: some bacterial and fungal infections). We have already seen from Table 8 that some bacteria multiply to produce large numbers (10^6) in the food and then colonize the gut after ingestion. These bacteria on reaching the intestine attach to and penetrate epithelial cells (possibly by producing toxins inside the cells) and induce diarrhoea by destroying the epithelial lining.

Other bacteria multiply in the lumen of the intestine, but do not invade the tissues, and produce enterotoxins which act on the intestinal cells causing disease. Much attention has been directed towards understanding the mechanism of action of these toxins and in particular to the one produced by **Vibrio cholerae.** This enterotoxin reacts rapidly with a ganglioside receptor on the epithelial cells. It does not damage the cells but activates an enzyme, adenyl cyclase and thereby raises the intracellular level of cyclic adenosine monophosphate (GAMP). As a result of this, water and electrolytes (Na, Cl) are lost through the intact epithelial cells into the small intestine. As the multiplying **Vibrio cholerae** increase in number more and more epithelial cells are affected, the absorptive power of the colon is overwhelmed and there is profuse watery diarrhoea. As a consequence of this fluid loss the patient becomes seriously dehydrated and death may follow if fluid is not replaced. A large amount of fluid loss also occurs in **Clostridium perfringens** food poisoning. However, the precise mode of action is not known but it is believed that it results from interaction of the toxin with the membranes of cells of the intestinal epithelium.

The final group of bacteria listed in Table 8 are those which produce toxins in the food and illness results from ingestion of this toxin. These latter food poisonings are often referred to as food intoxications to distinguish them from the previous two types of food infections. In **Staphylococcus aureus** and possible **Bacillus cereus** intoxications, the toxin is absorbed from the intestine and acts directly on the vomiting center in the central nervous system. However, in the case of botulism (**Clostridium botulinum** intoxication) the absorbed toxin acts directly on the peripheral nervous system, interfering with the release of acetyl choline at cholinergic synapses and the neuromuscular junctions, producing the acute neurological symptoms.

Bacterial Food Poisoning

It is not possible within the scope of this paper to provide details of all of the different microbial food poisoning organisms. Many different organisms are involved and different foods are commonly implicated in outbreaks (Table 10). These foods being infected by a range of organisms whose sources are detailed in Table 11. It is proposed to briefly discuss one example of each of the two types of bacterial food poisonings since these represent the main causative agents.

Table 10. Foods Commonly Associated with Outbreaks of Particular Forms of Bacterial Food Poisoning

	Meat*	Poultry*	Fish*	Milk*	Eggs*	Sweets (Puddings)	Vegetables*	Canned Foods
Salmonella spp.	++	++		+	+	+	–	±
Vibrio parahaemolyticus	–	–	+	–	–	–	–	–
Escherichia coli	+	+	+	–	–	–	–	–
Yersinia enterocolitica	–	–	–	+	–	–	–	–
Staphylococcus aureus	+	x	±	±	±	+	–	±
Clostridium botulinum	±	±	±	–	–	–	(+	+)
Bacillus cereus	–	–	–	–	–	–	+	–
Clostridium perfringens	+	+	–	–	–	–	–	±

* and their products

++ very common

\+ common

± occasional

– uncommon

Table 11. The Reservoirs of Bacteria that Cause Food Poisoning

Infectious Types	Toxin Types (toxin produced in the gut or in the food)
Salmonella spp. Intensively managed farm animals, poultry and their environs. Humans, especially carriers	**Staphylococcus aureus** Humans, farm and domestic animals
Vibrio parahaemolyticus A marine organism of world-wide distribution, especially common in coastal and estuarine waters during summer months. Probably overwinters in sediments.	**Clostridium botulinum** The majority of serotypes occur in soil; the psychrotrophic serotype E appears to be a common contaminant of water, sea sediments and mud.
Escherichia coli Alimentary tract of man, farm animals and domestic pets.	**Clostridium perfringens** The alimentary canal of man, farm animals and domestic pets; present also in soil, dust, etc.
Campylobacter jejuni Primary depot not identified	**Bacillus cereus** Common in soil and on vegetation
Yersinia enterocolitica Primary reservoir not identified.	

Salmonellosis

Salmonella are the most common cause of infective food poisoning. The transmission of the disease is usually from animals to humans by the ingestion of food of animal origin (meat and poultry). Also, there is direct transmission from humans to humans, from humans to animals and from animals to humans. Following the introduction of the bacteria to the gut, the most frequent syndrome encountered is acute gastroenteritis (Table 9), occasionally with loss of human life. Apart from this the economic consequences of this infection are far reaching and result from cost of medical care, hospitalization, lost time and income through absence from work, death of animals, decreased production of animals, loss or reduced value of contaminated products, recall of products from market outlets and testing and control procedures.

Statistics indicate that cases of salmonellosis are increasing and thus control of salmonella is of major importance. The control measures needed are far reaching and will only be mentioned briefly. Some of the recommendations are long term objectives and may be almost impossible to achieve but if salmonellosis is to be reduced every effort should be made to:

1. Ensure animal feeding stuffs are salmonella-free and imported feeds are suitably heat treated.
2. Eliminate salmonellas in poultry breeding stocks.
3. Improve hygiene standards in abattoirs and broiler houses.
4. Avoid cross-contamination risks, particularly of cooked by raw foods, in processing factories and kitchens.
5. Ensure adequate heating of foods, followed by rapid cooling where foods are to be stored.
6. Refrigerate foods where possible and avoid leaving foods at room temperature for lengthy periods.
7. Ensure food handlers are not salmonella carriers.
8. Control rodents, birds and pests in and around factory premises.
9. Increase salmonella surveillance, particularly of cooked foods.

Staphylococcus Poisonings

The most common form of toxic food poisoning is that produced by **Staphylococcus aureus.** Although this organism is found widespread in nature, the most important source is probably the human body, the principal reservoir being the nose. Between 30 and 40% of healthy individuals carry **Staphylococcus aureus** and many of these nasal carriers inevitably also harbor the organism on their hands and other parts of their body. Animals may also be important sources. Dairy cows commonly carry the organism on the udder and teats. This close association with the udder inevitably means that milk becomes infected but **Staphylococcus aureus** can also be spread from the infected region to milking equipment, other utensils and the hands of workers. Food handlers infected with this organism are thus the vehicles of transmission to the food. Cooked foods handled by a **Staphylococcus aureus** carrier, and subsequently stored under warm conditions for lengthy periods are the main cause of this form of food poisoning. Cured cooked meats, especially hams, which are made up into sandwiches or otherwise eaten cold are commonly implicated as are other meats and poultry. Outbreaks from raw or pasteurized milk are rare but raw milk products such as cream and cheese have given rise to outbreaks. Control measures are therefore important and should be aimed at limiting the contamination and subsequent growth of the organisms in food.

Vital control measures recommended are:

1. Keep handling of cooked foods to a minimum.
2. Personnel with septic lesions should not handle foods; because of the high nasal carriage rate in humans it would be impracticable to prohibit such carriers from handling foods but disposable gloves should be worn by all operatives.
3. Adequate heat treatment of the food is essential followed by prompt cooling to $10^{o}C$ or below where foods are to be stored.
4. Minimize cross-contamination from raw to cooked foods and from dirty working surfaces, equipment and utensils.

Failure to observe correct food hygiene has indeed led to many outbreaks with inevitable distress following the consumption of the food. In one of Mar Khayyam's poems;

> "Here with a loaf of bread beneath the bough
> A flask of wine, A book of verse - and thou
> beside me singing in the wilderness
> And wilderness is paradise now",

food features as a definite requirement for happiness. How different it would have been if the food contained food poisoning organisms.

Source

Egglestone, S. I. (1985). Microbiology and Food Symposium. Institute of Biology, (Hongkong Branch).

PART XI. MICROORGANISMS AND CROP PRODUCTION

The population of the world increases at the rate of 78 million people per year. The pressure to feed this growing population is indeed great. More food is now produced from the world's agricultural systems than ever before. However, because of the increasing world population, the per capita food increase has been relatively small. In the case of grain, for example, the annual world production thirty years ago was 250 kg per person, the figure today is approximately 320 kg per person. Furthermore, the increase in production is by no means evenly distributed. In the 1930's most parts of the world except Western Europe were self-sufficient in term of grain, but in the 1980's only North America, Australia and New Zealand are producing enough grain to feed their populations (Table 12).

Table 12. Changing Pattern of World Grain Production - Surpluses (+) and Deficits (-) in Various Regions and countries

Region/Country	1934 - 1938 tonne x 10^6	1980 tonne x 10^6
North America	+ 5	+130
Latin America	+ 9	- 10
Western Europe	-24	- 16
East Europe and Soviet Union	+ 5	- 46
Africa	+ 1	- 15
Asia	+ 2	- 63
Australia and New Zealand	+ 3	+ 19

Adapted from Introduction to Environmental Science by J. Turk 2nd Ed., 1985.

Inorganic Fertilizers

Most of the increase in crop production in recent years is the result of the increased usage of commercial inorganic fertilizers. There are, however, several disadvantages associated with their intensive use. Firstly, inorganic fertilizers, particularly nitrogen fertilizers, require large quantities of oil or coal to produce. As the world's fossil fuel reserve is both diminishing and non-renewable, we are actually trading one form of stored energy with another. The second problem is decreasing humus content of soils. Most inorganic fertilizers are applied in a soluble form and easily washed away by rain except those that are bound by organic derived humus. Unless plant materials and animal waste, and remains are constantly added to

soil, more inorganic fertilizers will be wasted. The third disadvantage relates to the washed away fertilizers which find their way into surface and ground waters, these form sources of pollution, promoting eutrophication of rivers and lakes, and also contaminating drinking water supplies.

Biological Fertilizers

Man has used plant compost, bone meal, animal and human wastes to supply nutrients to food crops for thousands of years. In the soil, these complex organic materials are transformed by a variety of heterotrophic microorganisms including bacteria, actinomycetes and fungi into simple, inorganic nutrients which can be absorbed and utilized by crops. The microbial biomass formed also supports a viable community of protozoans, invertebrates and vertebrates which also contribute to the fertility of the soil.

Role of Biological Nitrogen Fixation

Aside from microbial processes that occur naturally, man also unknowingly used microorganisms to increase crop production even in the early days of agriculture. It was well known in Roman time that alternate planting of beans, peanuts and alfafa with regular grains could enhance crop production. We now know that this is due to the nitrogen-fixing bacteria which live symbiotically within the roots of these plants. Nitrogen is the most important and very often the growth limiting nutrient in crop yield. Nearly six million tonnes of nitrogen fertilizer are utilized annually in the world today and this amount is expected to double by the end of the century. With the ever increasing price of commercial nitrogen fertilizer and man's greater awareness of his own natural environment, it is envisaged that biological nitrogen fixation will exert a greater importance than it does at present, and vigorous research and development work is going in this area.

Nitrogen-Fixing Organisms

The ability to immobilize atmospheric nitrogen is found in a variety of free-living and symbiotic bacteria including: **Rhizobium** living within the root nodules of legumes; photosynthetic cyanobacteria also known as the blue green algae; **Frankia**, a filamentous actinomycete, the anaerobic spore forming **Clostridium**, the ubiquitous **Klebsiella**, and groups of methane utilizing, acid and heat tolerating archeobacteria. An estimated 175 million tonnes of nitrogen are fixed biologically on earth each year, with around 70% of this taking place in agricultural lands (Table 13). **Rhizobium** is the most important genus of nitrogen-fixing bacteria, accounting for half of the world total.

Table 13. Nitrogen Fixed in Various Habitats of the World

Source	tonne x 10^6yr	Percentage
Arable agriculture		
Grain legume	35	20
Non legume	9	5
Non-arable agriculture		
Permanent pasture and grassland (mostly by forest legumes)	45	26
Forest and woodland (partly by tree legumes)	40	22
Non-agriculture		
Land	10	6
Sea	36	20
Total:	175	

From Nitrogen Fixation in Plants by R.O.D. Dixon and C.T. Wheeler 1986.

Root Nodule Formation in Rhizobium

Symbiotic growth of **Rhizobium** within legume plants starts with the infection of the root hairs of the plant by the bacteria which bind with lectin (a kind of protein) secreted by a particular species of host (Figure 27). This is followed by lengthening, curling or branching of the root hair and dissolution of the cell wall to allow the entrance of bacteria. Within the root hair, the **Rhizobium** divides and moves in a file toward the cortex of the root where further bacterial multiplication and tissue enlargement results in nodule formation (Figure 28). At this point the bacteria break away from the plasmalemma, or membrane surrounding the infection thread and enlarge to form Y or pear-shaped bacteriods before active nitrogen fixation takes place.

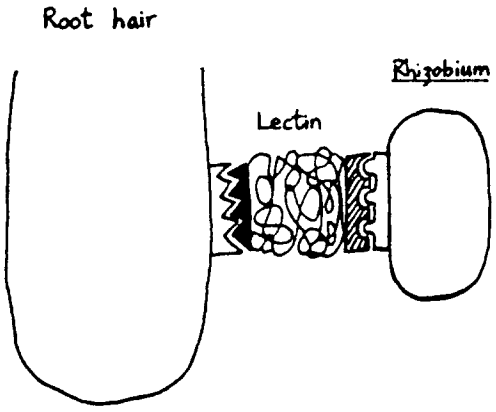


Figure 27. The Attachment of Rhizobium to Root Hair (Modified from Dixon and Wheeler, 1986)

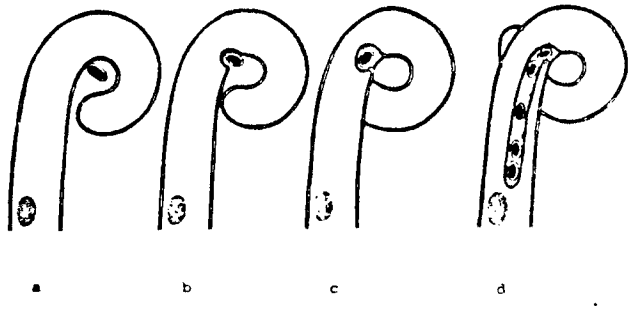


Figure 28. The Invasion of Legume Root Hair by Rhizobium (From Dixon and Wheeler, 1986)

Biochemistry of Nitrogen Fixation

The following chemical equation of nitrogen fixation appears remarkably simple but a tremendous amount of energy is required for the reaction to proceed.

$$N_2 + 3H_2 \text{ --------} \rightarrow 2NH_3 \qquad \Delta G = -53 \text{ KJ}$$

It is estimated that 12 g of glucose is required to fix 1 g of atmospheric nitrogen. Energy in the form of ATP is used by the enzyme nitrogenase to transfer electrons from ferridoxin, a molecule with unusually low reducing potential, to hydrogen atoms. Nitrogenase includes two non-haem iron proteins with sulphur and molebdenum groups. It is estimated that only a few kilograms of enzymes in microorganisms are responsible for the total nitrogen-fixing activity on earth today. Nitrogen fixation is inhibited by the presence of O_2, therefore the host legume plant, aside from supplying ATP, also synthesizes leghaemoglobin, a molecule which binds with O_2 readily so that as a result it does not interfere with the reaction. The NH_3 produced by the bacteria is utilized by the host for the production of its proteins, enzymes and nucleic acids.

Nitrogen Fixation in Rice

Apart from being the most important item in the Asian diet, rice also constitutes half of the calorie intake of the overall world population. Over 50% of the world's rice is produced in Asia with China being responsible for more than one third of global production.

Table 14. Yield of Rice in Various Parts of the World

Region/Country	Yield (tonne hectare^{-1})
East Asia	4.3
China	4.1
Taiwan	4.5
Japan	5.8
Southeast Asia	2.2
Indonesia	3.1
Thailand	1.8
Kampuchea	1.0
South Asia	1.9
Sub-Sahara Africa	1.4
Latin America	1.8
Australia	6.3
U.S.	5.0

Data extracted from `Rice' by M.S. Swaminathan, Scientific American, January 1984.

As shown in Table 14, the yield of rice ranges from 1-2 tonnes per hectare in poorer countries in Asia, Africa and Latin America to around 6 tonnes in Australia and Japan. This difference can largely be attributed to the amount of nitrogen fertilizers used in these countries. Even when commercial fertilizers are not available, each hectare of rice paddy is still able to obtain between 20 and 40 Kg of nitrogen from the air annually by biological nitrogen fixation and produce one or two tonnes of grains. This is partly due to the activities of heterotrophic bacteria which colonize the surface and interior of rots, and the base of shoots, and can reach as much as 10^{10} cells per gram of dry root (Figure 29).

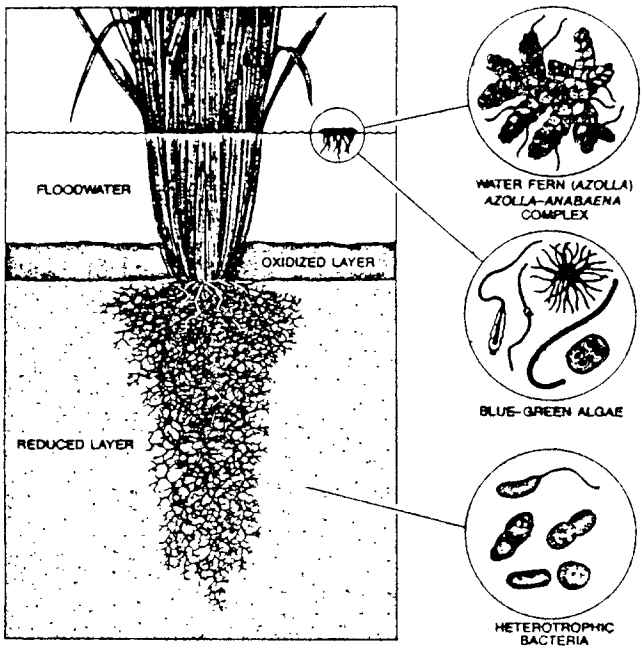


Figure 29. Nitrogen Fixation in Rice Paddies by Microorganisms (From Swaminathan, 1981)

Free living nitrogen fixing cyanobacteria are most active in neutral or alkaline rice paddies. Associated with the water fern **Azolla** is the cyanobacterium **Anabaena** which can fix up to 3 Kg $\text{nitrogen}\,\text{ha}^{-1}\text{day}^{-1}$. The **Azolla-Anabaena** system has been intensively studied in the rice growing part of the United States and is utilized as `green manure' in Asian countries such as China, Vietnam and India.

Field Application of Cyanobacteria Culture in China

In the 1970's, China developed a successful nitrogen fertilizer utilizing mass cultures of cyanobacteria spread directly onto rice paddies. The system involves isolating and culturing, in the laboratory, **Anabaena, Nostoc** and other species which have high nitrogen fixing activities. The stock culture is then seeded into small ponds and subsequently grown in larger ponds. Control of environmental temperature and pests are important during the growth phase. The harvested cells can be dried, stored in powder form, and applied directly to rice paddies along with other crop nutrients such as phosphate, magnesium and calcium. Cultured cyanobacteria can inhibit the growth of weeds and are able to fix about 300-400 Kg of nitrogen per hectare annually. An average of 10-20% increase in rice yield in some parts of China has been attributed to this method.

Other Uses of Microorganisms

Examples of using microorganisms to enhance crop production are numerous. It has been shown that adding a culture of **Pseudomonas putida** (a common soil inhabitant) to sugar beet or potato fields, will enhance crop yield significantly. This is mainly attributed to the organism's ability to sequester iron near the root of the plant so that iron in the soil is preferentially available for the plants and not for the growth of harmful bacteria and fungi. Mutualistic growth of soil fungi and actinomycetes with crop roots are called mycorhizae or actinorhizae. These lead to the enlargement and solubilization of nutrients such as phosphates and for the greater uptake of water by root hairs.

Intensive research on insect bacteria and viruses in the last ten years has proved that it is possible to use microorganisms as effective pest control agents for crop plants. The bacterium **Bacillus thuringensis** has been cultured in mass quantity and used to kill larvae of some crop eating insects. A crystalline protein from the spore of this organism was found to be specifically toxic to the gut of Lepidoptera insects. Microbiological control of insects can minimize the environmental hazards caused by using chemical pesticides.

What Lies Ahead

In the mid 1960's, as a result of the development of short-stalked, fertilizer-receptive grains, and research work done at the International Rice Institute in the Philippines and other agencies in the world, high yield varieties were made available to many countries. This results in a 2-6 fold increase in grain production which not only fed millions of people but also paved the way for rapid industrial development in the 1970's. This was the start of what is known as the Green Revolution. Agriculturalists predict that with the recent developments in plant breeding and genetic manipulation techniques using micro-organisms, we are about to witness a second Green Revolution. Laboratories in many countries are carrying out research in this area. For example in Japan, as part of a long term search for biological resources, the government plans, in the next few years, to acquire a large collection of plant genetic stock. During the 1990's, they will concentrate research on efforts designed to increase the nitrogen-fixing capability of micro-organisms. By the end of the century the Japanese aim to produce crop plants which have incorporated N-fixing genes from these microorganisms and be able to fix nitrogen themselves.

Techniques and Technologies

In vitro cell and tissue culture was first developed to grow animal and plant viruses which cannot survive outside living cells, now the technique has become central to modern plant breeding methods. Cells from many plants including vegetables, fruit trees, and ornamental plants can now be grown in sterile test tubes or large fermenters and can produce useful metabolites in great quantities. They can also transform into a mass of cells, termed a callus, and with the aid of appropriate hormones develop into whole plants. One gram of cultured cells can give rise to around 1000 new plants. Even for the highly priced ginseng, we can obtain as many as 500 ginseng plants this way.

Traditional methods for obtaining new plan varieties such as grafting, cross pollination and irradiation are now being superseded by methods arising from advances in molecular biology.

These include protoplast fusion and genetic recombination. Protoplast fusion involves the removal of the cell wall by enzymes such as pectinase and the union of 2 plant cells and their genetic components. Some of the resulting cells give rise to new plants with desirable characters from both parents. Protoplast fusion has been used on molds and yeasts in the brewing and dairy industries to obtain new improved strains. New plant varieties have been obtained between different species of tobacco, carrot, petunia and also between plants of different genera. The technique, of course, is not without its problems. For example fusion of tomato and potato cells yields `pomato', a new plant which unfortunately bears no fruit.

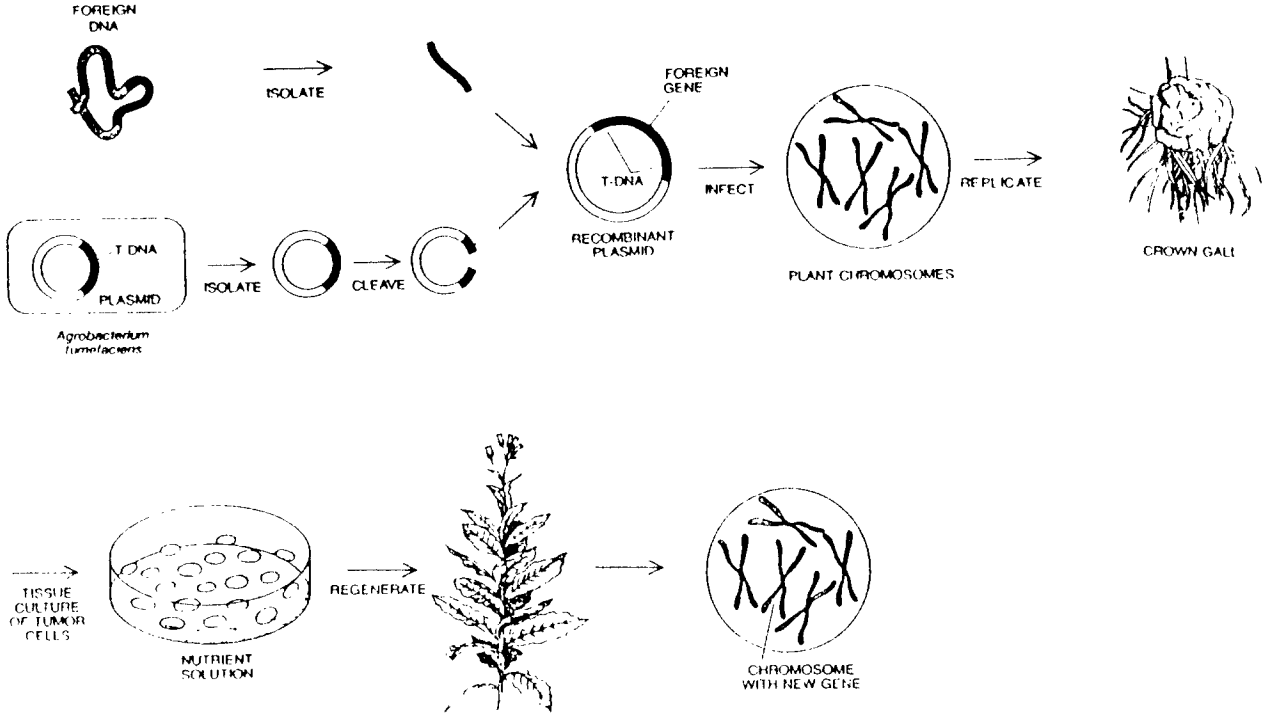


Figure 30. Introduction of Foreign Gene into Plant Cells (From Brill, 1981)

Genetic recombination technology has achieved considerable commercial success in many areas and will be the hope of plant breeders in the future. Several ways of introducing new genes into plant cells have been developed. These include making use of **Agrobacterium tumefaciens**, a bacterium known to infect many dicot plants and cause a tumor-like growth called crown gall (Figure 30). It contains a plasmid (circular DNA outside bacterial chromosome) with a T-DNA section which can split and incorporate a foreign gene segment, such as that which codes for the synthesis of nitrogen fixation enzymes. Tumor cells from the infected plants can give rise to plants which contain the foreign gene. The T-DNA system has its shortcomings as inaccurate insertion of the gene would disrupt the normal growth cycle of the plant, and so far it has now worked for monocots, the important grain producing group. Another method under investigation is using cauliflower mosaic virus (CAMV) which contains genes responsible for infecting cauliflower. The genes can be spliced (cut) and inserted to the bacterium **E. coli** and amplified many times as it multiplies. The amplified DNA can be used as a vehicle to introduce new genes into cauliflower and other plants. The third method is using the free living bacterium

Klebsiella pneumoniae which has a chromosome containing a set of 17 genes for nitrogen fixation (nif genes). The nif genes can be inserted into **E. coli**, or **Azotobacter**, a common soil bacterium, which in turn acquire the ability to fix nitrogen. The same genes have also been inserted into a eukaryotic yeast cell (Figure 31). However, yeasts treated in this way have so far failed to express the gene and are therefore unable to fix atmospheric nitrogen.

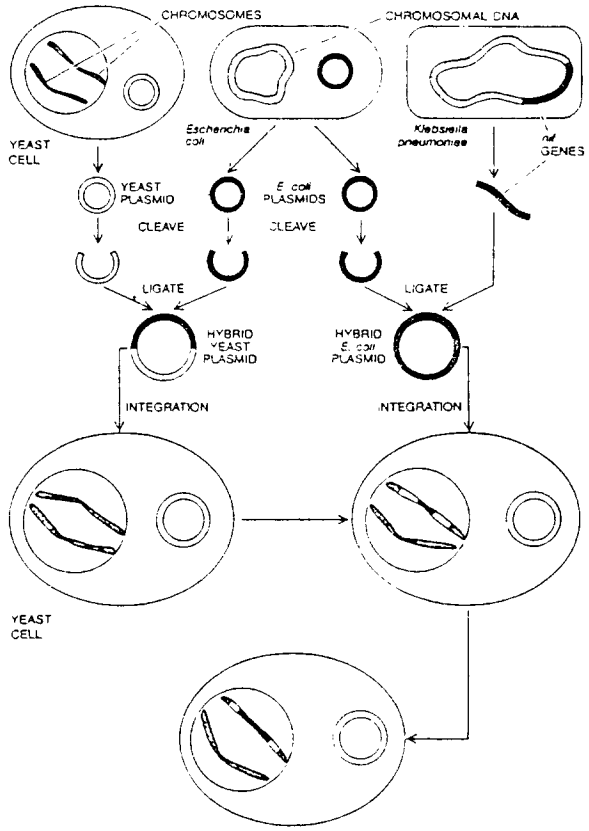


Figure 31. Insertion of Nitrogen-Fixing (NIF) Gene (From Brill, 1981)

In terms of research, microbial and plant genetics are areas of high priority world-wide. Substantial funding is being provided by government agencies as well as large corporations such as ICI, Shell, Dow Chemical and Campbell Soup who are fully aware of the economic potential of such work. By the turn of the century, it should be possible to tailor-make genetic engineered plants such as nitrogen-fixing wheat, blight free potato, drought resistant rice, high-protein grains, and crops which can grow in saline and acid soil and flourish in the harshest of natural environments.

Source

Kueh, C. S. W. (1985). Use of Microorganisms in Increasing Crop Production. Proceedings of the Microbiology and Food Symposium. Institute of Biology, (Hongkong Branch).

PART XII. ADDITIONAL ACTIVITIES

1. PHYSICAL AND BIOLOGICAL SCIENCE

EXPERIMENT 1: LIVING THINGS COME FROM LIVING THINGS

1. Pour about 30 cm^3 of canal water into 2 test tubes, about 30 cm^3 each.
2. Plug the tubes with cotton wool. Boil the test tubes for 5-10 minutes, and wait until the water is cool.
3. Put some living organisms e.g. paramecium into one of the test tubes and replug the tube.
4. Observe what happens in the test tubes; use a magnifying glass for close observation of any living organisms in the two test tubes. Make a record of observation for 7 days.

Questions

1. What is the purpose of boiling the canal water?
2. Are the changes that occurred in the two test tubes the same or different? How?
3. If the two test tubes were unplugged, what would happen in the two test tubes?
4. In what cases could you apply the principles that you learned from the experiment?

Translated from **Physical and Biological Science Textbook for upper secondary school level on "Life and Development"** P. 3. (Institute for Promotion of Science Teaching, Bangkok, Thailand).

2. FOR BIOLOGY

Pasteurization

The method of destruction of harmful germs by heating at approximately 62°C for 30 minutes and immediately cooling down the temperature, was discovered by Louis Pasteur. It is thus called pasteurization after his family name. Pasteurization is used to destroy infection diseases particularly those spread among human e.g. tuberculosis.

Translated from **Biology Textbook for Grade 10 Students** (Upper Secondary School Level) P. 80. (IPST, Bangkok, Thailand).

3. FOR CHEMISTRY

Pasteurization

Pateurization can destroy only some diseases and greatly reduce the number of bacteria. Pasteurized milk can therefore be kept for a longer time. The method is to heat the milk at the temperature of 62^{o}C for 30 minutes.

The pasteurized milk must be kept in the place where the temperature is low and must be sold within 3 days after the packing date.

Translated from a **Reading Material for Students under the Development and Promotion of Science and Technology Talents Project (DPST) on "Milk and Milk Products"**, P. 2. (IPST, Bangkok, Thailand).

4. FOR BIOLOGY

Germ Theory of Disease

In the 19th century, Louis Pasteur, a French Biologist discovered that yeast and bacteria were the main factors which caused fermentation. Besides, he made an observation that wine kept for a long time became sour because of one type of bacteria which produced acetic acid in the wine. He further thought that since bacteria could spoil the wine, it might bring disease to human and animals. From this hypothesis, he later developed a theory called "Germ Theory of Disease".

Translated from **Biology Textbook for Grade 10 Students** (Upper Secondary School Level) p. 71. (IPST, Bangkok, Thailand).

CONCLUDING STATEMENTS

This Resource Book is a modest contribution of ICASE to the Centennial Celebration of the Pasteur Institute. Although the Institute in Paris and those found in other parts of the world are more concerned with health and the prevention and diagnosis of disease, this book is focused on other roles of microorganisms.

There is a very large list of beneficial types of microorganisms, but only a few are mentioned here. Some examples of microbial fermentation and its applications are given emphasis.

The use of microbes in industry is not extensively covered in this book. There are many other industries which are dependent on microorganisms. Examples are the curing of leather, the curing of tobacco, the flavouring of coffee and cocoa, the retting of flax, and on a large scale the manufacture of alcohol, glycerol, propanol, and other related products.

Students who may want to look into other aspects of microorganisms may wish to refer to materials found in libraries. Local practices or indigenous technology which are not widely publicized could also be examined.

A minority of microorganisms are pathogenic and each pathogenic organism causes a particular disease. This resource book does not attempt to discuss the activities of pathogens because of the danger in handling such microorganisms, and the lack of precautionary and preventive measures available in the primary and secondary science classrooms of many countries. Experiments in this area carried out by primary and secondary students **are not to be recommended.**

ICASE Secretariat.

SOURCES AND AUTHORS OF INCLUDED ARTICLES

(Reproduced with permission)

1. ASIMOV, I. (1982). `Pasteur, Louis'. Asimov's Biographical Encyclopedia of Science and Technology. Doubleday and Co. Inc. Garden City, New York.

2. ASMP. (1979). CDC. Canberra ACT 2602, Australia.

3. Baltazar, V. (1985). The Versatile Microbes. **Bato Balani**. Makati, Philippines. 5(1).

4. Banwart, G.J. (1979). Basic Food Microbiology. AVI Publishing Co. Connecticut, U.S.A.

5. Bato Balani. (1985). Manila, Philippines. Vol. 5.

6. Brill, W.J. (1981). `Agricultural Microbiology'. **Scientific American'**. September.

7. Bryan, F.L. (1973). Diseases Transmitted by Food. Publication No. (HSM) 73-8237. U.S. Department of Health, Education and Welfare. Atlanta, Georgia, U.S.A.

8. Clegg, A.G. and Clegg, P.C. (1973). Man Against Disease. Heinemann Educational Books.

9. Cobb, V. (1979). More Scientific Experiments You Can Eat. J.B.Lippincott. New York.

10. Daniels, M.S. (ed). (1987). Microbiology and Food. Institute of Biology (Hongkong Branch) Symposium.

11. Dixon, R.O.D. and Wheeler, C.T. (1986). Nitrogen Fixation in Plants. Blackie.

12. Egglestone, S.I. (1985). Microbiology and Food. Institute of Biology (Hongkong Branch) Symposium.

13. Encyclopedia Britannica (1986 edition).

14. Frazier, W.C. (1978). General Principles Underlying Food Spoilage. In: Food Microbiology, 3rd ed. McGraw Hill, N.Y.

15. Hayes, P.R. (1985). Food Microbiology and Hygiene. Elsevier Applied Sciences Publishers. London.

16. Hodgkiss,I.J. (ed.) (1986). Biotechnology. Institute of Biology (Hongkong Branch).

17. International Commission of Microbiological Specifications for Foods. (1980). Microbial Ecology of Foods. Vol. 1. Academic Press. London.

18. IPST. (no date). Biology Textbooks for Grade 10 Students for Upper Secondary School Level. Bangkok, Thailand.

19. IPST. (no date). General Science Textbook for Grade 9. Bangkok, Thailand. (Translated from Thai Language).

20. IPST. (no date). Physical and Biological Science Textbook for Upper Secondary School Level on `Life and Development'. Bangkok, Thailand.

21. IPST. (no date). Reading Material for Students Under the Development and Promotion of Science and Technology Talent Project (DPST) on `Milk and Milk Products'. Bangkok, Thailand.

22. Jay, J.M. (1978). Intrinsic and Extrinsic Parameters of Foods that Affect Microbial Growth. In: Modern Food Microbiology. 2nd ed. Litton Educational Publishing. N.Y.

23. Jensen, W. et. al. (1979). Biology. Wadsworth. California.

24. Kan, M.W.M. (1978). Microbiology and Food. Institute of Biology (Hongkong Branch) Symposium.

25. Kueh, C..S.W. (1985). Use of Microorganisms in Increasing Crop Production. Proceedings of the Microbiology and Food Symposium. Institute of Biology (Hongkong Branch).

26. Litchfield, J.H. (1976). Food Microbiology. In: Industrial Microbiology. Miller, B.M. and W.Litsky. McGraw Hill. N.Y.

27. McGraw, R.E. (1985). Encyclopedia of Medical History. McGraw Hill. N.Y.

28. Moseley, N. (1983). Pasteur: Student and Teacher. **School Science and Mathematics**. 83(3).

29. Moser, P.W. (1987). It Must Have Been Something You Eat. **Discover**. 8(2).

30. Noble, W.C. and Naidoo, J. (1979). Microorganisms and Man, Studies in Biology III. Arnold.

31. Parry, T. and Pawsey, R. (1979). Principles of Microbiology. Hutchinson.

32. Parry, T.J. and Pawsey, R. (1981). Principles of Microbial Spoilage. In; Principles of Microbiology for Students of Microbiology. Hutchinson Publishing. London.

33. Pasteur Institute Document. (1987). 1887, 1987, 2087: Towards a New Century. Paris, France.

34. Pelczar, M.J. and Chan E.C.S. (1981). Elements of Microbiology. McGraw Hill.

35. Pelczar, M. J. Jr. and Reid, R.D. (1958). Microbiology (2nd ed.). McGraw Hill Book Co. New York.

36. Sakamoto, H. (1988). Use of Microbes as Teaching Materials. Nushigoshi Lower Secondary School. Kumamoto Prefecture, Japan.

37. SEAMEO-RECSAM. (1977). Ideas for Making Low-Cost Equipment for Secondary Science and Mathematics. Course DW-Q2. Penang, Malaysia.

38. Science in Action. (1985). Bato Balani Publications. Makati, Philippines.

39. Student Laboratory Guide to Biological Science. (1963). Harcourt, Washington.

40. Survival Catalogue. (1981). Technology Resource Center. Makati, Philippines.

41. Swaminathan, M.S. (1984). `Rice'. **Scientific American.** January.

42. UNESCO Pilot Project for Biology Teaching Africa. (1967-1968). Handbook of Microbiology.

43. Weerasinghe, Asoka. (1987). Trinity College. Kandy, Sri Lanka.

44. Williams, T.I. (ed.). (1982). A Biographical Dictionary of Scientists. John Wiley and Sons, New York.

45. Wiseman, A. (ed.). (1983). Principles of Biotechnology. Surrey University Press. United Kingdom.

46. Wyss, O. and Eklund, C. (1971). Microorganisms and Man. John Wiley and Sons, Inc. New York.

THE INTERNATIONAL COUNCIL OF ASSOCIATIONS FOR SCIENCE EDUCATION (ICASE)

Aim

The International Council of Associations for Science Education (ICASE) was established in 1973 to extend and improve education in science for all children and youth throughout the world by assisting member associations. It is particularly concerned to provide a means of communication among individual science teachers' associations and to foster co-operation efforts to improve science education.

Activities

ICASE activities include
- publishing a newsletter and a handbook for science teachers.
- issuing a directory of science teachers' associations worldwide.
- disseminating information about activities of national and regional groups.
- arranging regional activities in association with other organisations such as UNESCO.
- promoting exchanges of science teaching personnel.
- using its endeavours to promote research in science education.

Membership

Membership is available to :
- National associations for the promotion of science education.
- National associations for the promotion of education through separate disciplines.
- Science education sections of national scientific associations or national educational associations.

Associate membership is available to :
- Sub-national groups concerned with science education.
- Multi-national associations concerned with regional or international activities in science education.
- Companies and Foundations with interests in science education.

Constitution

The Governing Body of ICASE is the General Assembly consisting of one delegate from each member association together with any members of the Executive Committee who are not delegates.

The Executive Committee comprises of President, Past-President, Vice-President and up to eight members elected on a geographical basis. The Executive Secretary, Treasurer and Editor are appointed by the Executive Committee. ICASE is closely linked with the Committee on Science Teaching of the International Council of Scientific Unions (ICSU) and thereby enjoys full recognition by UNESCO and other international and national organisations.

Executive Committee

9. ICASE AND OTHER PUBLICATIONS FOR SALE

The following conference reports and books are available from the ICASE secretariat or from the ICASE treasurer. Please add 25% for postage and packing when ordering any books.

Specials rates are applicable for bulk orders and thus member associations are strongly requested to place orders in preference to individuals (although individual orders will also be processed).

9.1 ICASE PUBLICATIONS

	Prices US$	L
ICASE 1987 Yearbook - Adv in Sc and Tech Ed.	5.00	3.50
ICASE 1988 Yearbook - What Res. says to Sc Teach	5.00	3.50
Primary Science - Report of Conf. Bangalore 1985	3.50	2.00
Science Resource Notes No 2 - Chromatography	2.00	1.50
Science Resource Notes No 1 - Uses of Gas Syringe	2.00	1.50
Notes for Teachers - Halley's Comet	2.00	1.50
Junior Secondary Science - Some Resource Notes	2.00	1.50
Science Teachers influencing Education Worldwide - symposium report (ASE meeting 1983)	1.30	1.00
European seminar - Energy and Environment (Monte Carlo 1981)	12.00	9.50
5th ICASE-ASIAN symposium - Science for the Informed Citizen	5.00	3.50
4th ICASE-ASIAN symposium - Updating and Retraining of Science Teachers (Hong Kong 1982)	4.50	3.25
3rd ICASE-ASIAN symposium - Out-of-School Activities (Singapore 1981)	3.25	2.50
2nd ICASE-ASIAN symposium - Low Cost Equipment (Philippines 1979)	2.00	1.50
1st ICASE-ASIAN symposium - Environmental Integrated Science Education (India 1977)	1.25	1.00
African Regional Conference (FASE) - How relevant is Science Education ? (Zimbabwe 1982)	2.00	1.50
Nijmegen Conference report on Integrated Science Education Worldwide (Netherlands 1978)	3.00	2.25
200th Anniversary of Man's First Flight - Notes for Teachers (1983)	2.00	1.50
Commemorative issue - Resource notes on Newton	6.00	4.50
Physics of Road Traffic Accidents (1987)	5.00	3.50

9.2 UNESCO PUBLICATIONS

New Trends in Primary School Science Education	9.00	6.75
New Trends in Integrated Science Teaching, Vol V (1980)	7.50	5.40
New Trends in Chemistry Teaching Vol V (1981)	8.50	6.25
New Trends in Biology Teaching Vol IV (1977)	10.00	7.50
New Trends in Laboratory Equipment	10.00	7.50
New Trends in Home Economics Education	4.50	3.60
UNESCO Handbook for Science Teachers (1980)	10.50	7.95

	Price US$	L
New UNESCO Source Book for Science Teaching	7.50	5.40
Innovations in Science and Technology Education	10.00	7.50
Out-of-School Science & Tech. Education	10.00	7.50
New Trends in Physics Teaching - vol IV (1984)	13.50	9.00

9.3 COMMONWEALTH SECRETARIAT AND CASTME PUBLICATIONS

CASTME Caribbean Regional Seminar (1982)	4.00	3.00
Low Cost Science Teaching Equipment - Report of Seminar in Papua New Guinea (1979)	2.00	1.50
Development and Production of School Science Equipment	7.50	5.40

9.4 ICSU CTS PUBLICATIONS

A series of book evolving from a conference on Science and Technology Education and Future Human Needs held in Bangalore, India in 1985

Vol. 1	Science and Technology Education and Future Human Needs - the introductory book to the series	(price on application)
Vol. 2	Ethics and Social Responsibility in Science Education.	
Vol. 3	Education, Industry and Technology.	
Vol. 4	Land, Water and Mineral Resources in Science Education.	
Vol. 5	Education and Health.	
Vol. 6	Food, Agriculture and Education.	
Vol. 7	Energy Resources in Science Education.	
Vol. 8	The Environment and Science and Technology Education.	
Vol. 9	Science Education and Information Transfer	

9.5 MISCELLANEOUS

Report of a Regional Seminar on Mathematics and Science Education in Botswana, Lesotho and Swaziland	2.00	1.50