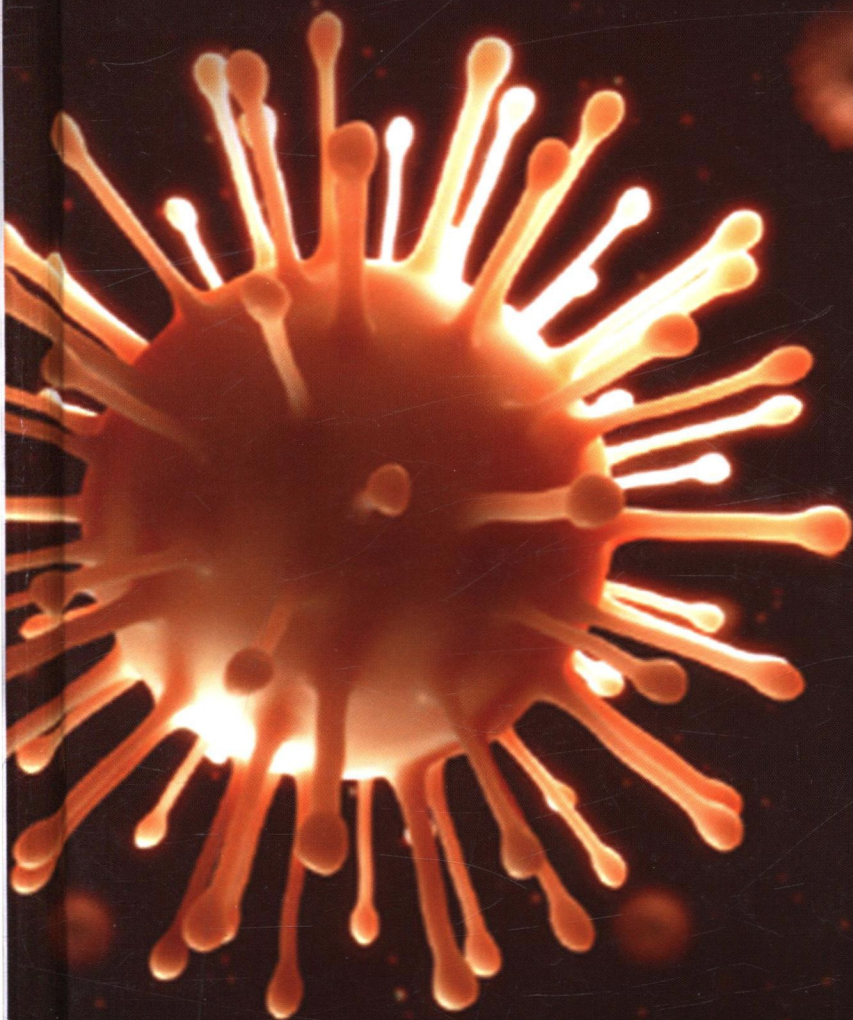


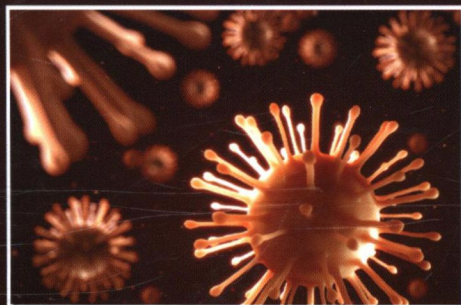
Ameer Khusro | J.P. Preetam Raj
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PRACTICAL BACTERIOLOGY



Alpha Science

PRACTICAL BACTERIOLOGY



Ameer Khusro | J.P. Preetam Raj
P. Agastian S. Theoder

PRACTICAL BACTERIOLOGY contains impressive experiments on Bacteriology Research Ideas (Chapter 15) that have not included in any practical book so far. The book has been written according to the application of bacteria in various fields of biotechnology. The experiments have been written in brief and the procedures summarized in an appealing and uncomplicated language. Appendices of the book provide a broad knowledge in bacteriology to the readers. The subject matter in *Practical Bacteriology* contains meaningful and essential experiments in order to carry out novel and competent research.

Salient features

- The book summarizes the basic imperative techniques of bacteriology
- Most of the experiments included in the book guide the readers to develop novel ideas
- The book describes fundamental as well as application aspects of bacteria
- An up-to-date concise and adequate background information in order to understand the experimental procedure effectively.



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PRACTICAL BACTERIOLOGY

Khusró | Pretam Raj | Agastian

Practical Bacteriology

Ameer Khusro
J. P. Preetam Raj
P. Agastian S. Theoder



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Preface

“Nothing is more conservative than a bacterium”

—Nick Lane

The main purpose of Practical Bacteriology is to condense and summarise the basic technique of bacteriology experiments. The book is written in such a form that it can serve undergraduate students, postgraduate students and research scholars in the fields related to bacteriology. An attempt has been made to summarise all the necessary experiments required during bacteriology practical and research projects. Although many practical microbiology books have been published but to the best of my knowledge this is the first book which contains all the basic experiments related to bacteriology for helping students in order to begin their research career in the field of bacteriology. The book contains some new experiments which are not included in any practical book so far. This book can become a milestone for the students and researchers whose focus is towards only bacteriology in the field of Microbiology. The book is written in such a way that it can help students to understand the concept and procedure of a particular experiment. The standard procedure has been referred from the reputed text books and journals and the same has been modified in a simple way. The major benefit of this book is that the contents of the experiments have been written in a nutshell and the procedures are summarised in simple and easy language. The summarising references can help students to consult only a limited number of sources. The subject matter in *Practical Bacteriology* contains all the basic and necessary experiments to carry out novel researches.

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Finally, but most important, I wish to extend appreciation to my family for the support, patience and encouragement.

Ameer Khusro

Introduction

"If you don't like bacteria, you're on the wrong planet."

—Stewart Brand

Bacteriology is the branch of science and a subdivision of Microbiology which deals with the study of bacteria. Bacteriology discusses about the isolation, identification, classification and characterization of bacterial species. Bacteria are unicellular microorganisms that lack a nuclear membrane. They can be useful or harmful, pathogenic or non-pathogenic, Gram (+) or Gram (–) and aerobic or anaerobic. Bacteria are one of the major causes of human diseases such as Tuberculosis, Cholera, Plague, Dysentery, Diphtheria and Tetanus. Major advances in bacteriology over the last century were the development of vaccines such as Tetanus toxoid, typhoid, cholera vaccines etc. Discovery of antibiotics was the major breakthrough in the field of bacteriology. Antibiotics helped to eradicate many bacterial diseases. But now development of antibiotic resistant bacteria is one of the major concerns in the current scenario. Bacteria resistant to antibiotics resulted in multidrug resistant bacteria. In recent years scientists have concentrated on the isolation of novel potential antibacterial compounds from herbal medicinal plants. But still eradication of diseases from the society is a major issue.

On the other hand bacteria have played a very important role at the industrial level for large scale production of enzymes and proteins which is a milestone for mankind. Bacteria are an important source of various industrially important enzymes such as amylase, protease, cellulase, lipase, pectinase, xylanase, chitinase etc. Bacterial enzymes are potentially useful in the pharmaceutical and chemical industries.

Bacterial cellular responses to different stress conditions have attracted current interest in Microbiology. Bacteria launch stress responses under hostile and challenging environmental conditions like during depletion of nutrients and oxygen, changes in temperature and presence of antimicrobial agents. In unfavourable conditions, bacteria have developed special physiological mechanisms. Hence the production of enzymes is higher than the normal

conditions. Bacteria under stress “switch on” a catalogue of genes responsible for the enhanced production of enzymes. Once the bacteria enter into the stationary phase, they produce an enhanced amount of enzymes and proteins. Environmental changes often occur suddenly and therefore, a quick response is crucial to assure cell survival. In such context, translational regulation of pre-existing mRNAs provides a fast and efficient way to control gene expression.

A range of bacteria, including *E. coli*, *Bacillus* sp. and *Pseudomonas* sp. have been reported to elicit an array of survival strategies in response to such drastic changes. Bacteria have developed special physiological mechanisms including the production and secretion of specific proteins in unfavourable and stress conditions. Hence, the production of proteins is higher than in normal condition. The stress responses are very specific and the mechanisms include metabolic alterations, activation of chaperones and signal transduction cascade dedicated for sensing and responding to various stress.

A broad attention has been kept to control the environmental pollution from the last two decades due to hazardous materials. Heavy metals and their compounds are major environmental pollutants. Presence of these heavy metals in the marine environment may cause a serious threat to the environment because of their ability to persist for several decades. Heavy metals exist both in bioavailable and non-bioavailable forms. Their mobility depends on two factors; the metallic element that precipitates as positively charged ions and the one which makes up negatively charged components of salt. Heavy metals are recognized to be powerful inhibitor of biodegradation activities. These heavy metals can not be degraded, and are indestructible. The toxic effects of heavy metals result mainly from the interaction of metals with proteins and inhibition of metabolic processes. Heavy metals like Cu, Cd, Pb, Zn, Ni and Hg accumulated in soils and water bodies at higher concentration can be toxic to plants, animals, humans and aquatic life. The presence of non-biodegradable heavy metals in such effluents is responsible for its persistence in the food chain. Bacteria play a major role in the biogeochemical cycling of toxic heavy metals. Higher concentration of these heavy metals has deleterious impact on the microbial communities in the environment. Bacteria inhabiting in metal polluted soils have evolved various strategies to resist themselves against metal resist. Heavy metals play a crucial role in all metabolic process of the bacteria. Such metal resistance microorganisms can be used as bioremediation agents. Bacteria have the capacity to survive under toxicological stress condition which is sometimes hazardous to human beings and animals. Bacterial genes determine transport system for maintaining equilibrium, intracellular concentration balancing needs and toxicity, and for elimination of purely toxic elements such as Hg, Pb, As, Cr, Cd and Ag. Several bacterial species utilize intracellular and extracellular binding mechanisms to avoid toxicity to heavy

metals. Various metal resistant bacteria have been isolated from contaminated soils, waters and sediments. Many bacteria adapt to the environmental stress through synthesis of proteins, especially by exposure to toxic heavy metals. Heavy metals influence the microbial population by affecting their growth, morphology, biochemical activities and ultimately resulting in decreased biomass and diversity. Metabolic activities were induced when the bacterial cells were incubated with sub-inhibitory concentrations of certain toxic heavy metals. Bacteria utilize different strategies to adapt to varying environmental situations including exposures to high concentrations of heavy metals.

Bacteriological studies have been subsequently divided into a number of specializations such as soil bacteriology, marine bacteriology, clinical bacteriology and industrial bacteriology. This particular branch of science is going to be exploited more in future because of the wide range of industrial applications of bacteria and lack of endangered bacterial species.

Quotations

1. *"Happiness and bacteria have one thing in common; they multiply by dividing."*
—Rutvik Oza
2. *"There are bred certain minute creatures which cannot be seen by the eyes, which float in the air and enter the body through the mouth and nose and there cause serious diseases."*
—Marcus Terentius Varro
3. *"What's great about bacteria is you have a surprise every day waiting for you because they're so fast, they grow overnight."*
—Bonnie Bassler
4. *"We mostly don't get sick. Most often, bacteria are keeping us well."*
—Bonnie Bassler
5. *"For the first half of geological time our ancestors were bacteria. Most creatures still are bacteria, and each one of our trillions of cells is a colony of bacteria."*
—Richard Dawkins
6. *"Biology will relate every human gene to the genes of other animals and bacteria, to this great chain of being."*
—Walter Gilbert
7. *"Always trust a microbiologist because they have the best chance of predicting when the world will end"*
—Teddie O. Rahube
8. *"Microbes are doing things we didn't even know they could do 10 years ago."*
—Robert Jackson
9. *"You can find bacteria everywhere. They're invisible to us. I've never seen a bacterium, except under a microscope. They're so small, we don't see them, but they are everywhere."*
—Bonnie Bassler
10. *"Support bacteria, it's the only culture some people have."*
—Bumper Sticker

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Glasswares

1. **Erlenmeyer flask:** It is a type of laboratory glass flask commonly known as conical flask. Erlenmeyer flask is a conical body structure with flat bottom and cylindrical neck named after Erlenmeyer in 1860. Laboratory flasks vary in their sizes ranging from millilitres to litres. The mouth of Erlenmeyer flask can be stoppered using cotton wools (absorbent and non-absorbent).

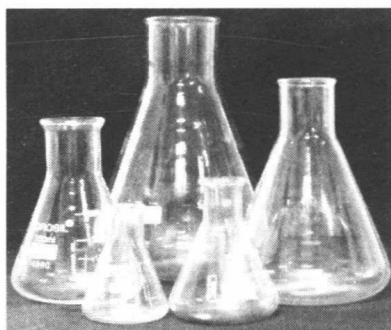


Fig. 1.1. Erlenmeyer flask

Flasks can be used for making solutions, chemicals, media etc. in the laboratory. Erlenmeyer flasks are also used in bacteriology for the preparation of various media. The media required for the growth of bacteria are mixed by swirling the content of the flask.

2. **Culture tube:** Culture tube or test tube is a common glassware in bacteriology laboratory. It is a long glass tube structure ranging up to 180 mm with U-shaped bottom. It is used in the laboratory for handling and culturing all kinds of microorganisms. It is mainly used during Serial dilution technique. The test tubes have the capacity to resist high temperature even above 250°C.

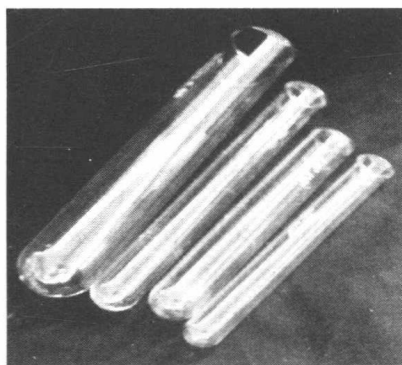


Fig. 1.2. Culture tubes

Heating of the chemicals and solutions present in the test tubes is done by holding the test tubes with test tube holders. The bacterial culture can be preserved for long time by making agar slants in the test tubes.

3. **Measuring cylinder:** Measuring cylinder is also called as graduated cylinder or mixing cylinder. It is used to measure the accurate volume of liquid required for the experiments. It should be noted that while measuring the accurate volume of the liquid in the measuring cylinder, the lower meniscus of the liquid is checked. The size of the measuring cylinder varies from millilitres to several litres.

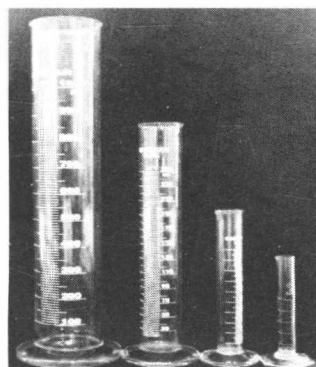


Fig. 1.3. *Measuring cylinder*

4. **Beaker:** A beaker is a simple flat bottomed glassware used for heating solutions and mixing media in bacteriology laboratory. Beakers are also available in various sizes ranging from millilitres to several litres. Because of the presence of a spout in the beaker it cannot be covered with a lid.

In view of this, aluminium foils are commonly used as a covering material in order to prevent the contamination of the liquid present in beaker. It is used to prepare bacterial growth media by appropriate mixing of ingredients.

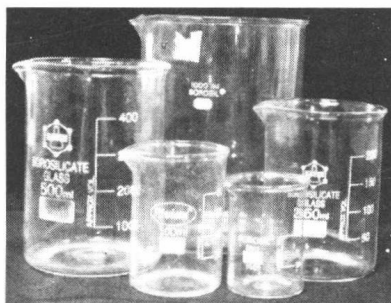


Fig. 1.4. *Beaker*

5. **Petri dish:** A petri dish is also called as Petri plate, named after German bacteriologist Petri. Petri dishes are most often used to culture bacterial cells. There are two types of petri plates available in the laboratory- Glass petri plate and Disposable petri plate. Glass plates can be reused after sterilization while disposable plates are discarded. Disposable petri dishes are made up of plastics which are discarded after a single use. Petri plates are often used to make agar plates in the bacteriology laboratory.

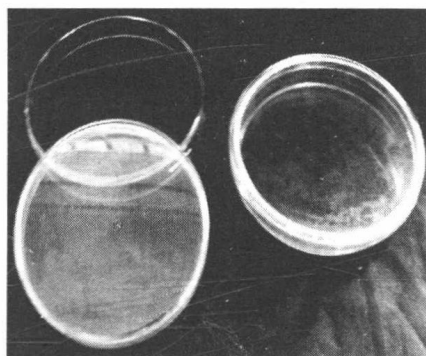


Fig. 1.5. *Petri dish*

Petri plates are incubated in inverted position in order to prevent water condensation inside the plate that may result in contamination. Once the media is poured and the inoculum is streaked, petri plates are sealed with Para film in order to prevent the entry of foreign microbes into the plates.