Biyani's Think Tank

Concept based notes

Microbiology
B.Sc. (Practical)

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Preface

I am glad to present this book, especially designed to serve the needs of the students. The book has been written keeping in mind the general weakness in understanding the fundamental concepts of the topics. The book is self-explanatory and adopts the "Teach Yourself" style. It is based on question-answer pattern. The language of book is quite easy and understandable based on scientific approach.

This book covers basic concepts related to the microbial understandings about diversity, structure, economic aspects, bacterial and viral reproduction etc.

Any further improvement in the contents of the book by making corrections, omission and inclusion is keen to be achieved based on suggestions from the readers for which the author shall be obliged.

I acknowledge special thanks to Mr. Rajeev Biyani, *Chairman* & Dr. Sanjay Biyani, *Director* (*Acad.*) Biyani Group of Colleges, who are the backbones and main concept provider and also have been constant source of motivation throughout this Endeavour. They played an active role in coordinating the various stages of this Endeavour and spearheaded the publishing work.

I look forward to receiving valuable suggestions from professors of various educational institutions, other faculty members and students for improvement of the quality of the book. The reader may feel free to send in their comments and suggestions to the under mentioned address.

Author

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MICROBIOLOGY
(PRACTICAL)

Introduction of Microbiology

Q.1. What is Microbiology?

Ans.: Microbiology is a branch of science that deals with the study of various morphological, physiological as well as genetic aspects of microorganisms along with their activities. This field is divided in number of diversities as **Bacteriology, Virology, Protozoology, Physiology, and Mycology.** It can be employed in various fields as pharmaceutical, food, agriculture, waste treatment, industrial microbiology etc. There are many applied aspect of microbiology that convey the variety and significance of microbial activities.

Microbiology : The study of microscopic organism its name is derived from three Greek words – ***Mikros*** Means small, ***Bio*** means life and ***Logos*** mean to study.

Q.2. Who is the father of Industrial Microbiology?

Ans.: Louis Pasteur

Q.3 Who is the father of Microbiology?

Ans.: Anton Van Leeuwenhoek

Q.4 What is Disinfection?

Ans.: It is complete destruction of pathogenic organisms or organisms capable of giving rise to infection.

Q.5. What is Pasteurization?

Ans.: It is a technique of sterilizing milk by heating at specific temperature for specific duration and then followed by cooling.

Q.6. What is Incineration?

Ans.: It involves killing of micro-organisms by using flame.

Q.7. What is Clone?

Ans.: Clone is referring to as a colony that is derived from single parent cell; all the cells of a clone are identical genetically and constitute a homogeneous population.

Q.8. What is Contamination?

Ans.: The contamination is unwanted growth of microbes alongwith useful microbes.

Q.9. What is Inoculums and Inoculation?

Ans.: **Inoculums** is the minimum amount of micro-organism required for its propagation.

Inoculation is the transfer of inoculation on the fresh medium.

Q.10. What is Incubation?

Ans.: Allowing the micro-organism to grow in the specific set of optimal conditions as temperature, light, humidity, duration etc.

Q.11. What is Lyophillization?

Ans.: In this process, water is removed by evaporation, sublimation from the per frozen sample. After drying the strains are stared under vacuum in in dividual vialsor Ampoules. This process is widely used to prescribe bacteria tests, fungi and some viruses.

Q.12. What is Buffer Solution?

Ans.: Buffer solution is a solution that is resistant to change in pH on the addition of small amounts of acid/alkali.

Q.13. What is Floatation Method?

Ans.: In these method different concentrations of sucrose is used in which VAM stores float. The spores can be separated out and examined under the microscope.

Q.14 What is Micrometry?

Ans.: Micrometry is the measurement of micro-organisms. Micro-organism are small. The dimensions of these are usually expressed in units smaller than millimeters. The micrometer (μm) is 10^{-3} of a millimeter, one millionth (10^{-6}) of a meter and 1/25400 of an inch.

A Nanometer – 10^{-9} of a meter.

An Angstrom – 10^{-10} of a meter.

Q.15. Explain the following :

(i) Protozoology

(ii) Algology or Physiology

(iii) Microbial Ecology

(iv) Microbial Taxonomy

(v) Air Microbiology

(vi) Exomicrobiology

(vii) Immunology

(viii) Bio-technology

Ans.: (i) **Protozoology :** It is the study of protozoan–animal-like and mostly single-celled, encaryotic organisms.

(ii) **Algology or Psychology :** It is the study of algae–simple aquatic organisms ranging from single-celled forms to large sea weeds.

(iii) **Microbial Ecology :** It is the study of inter-relationships between microbes and environment.

(iv) **Microbial Taxonomy :** It is concerned with the classification, naming and identification of microorganism.

(v) **Air Microbiology :** It deals with the role of aerospora in contamination and spoilage of food and dissemation of plant and animal disease through air

(v) **Exomicrobiology :** It deals with the exploration for microbial life in outer space.

(i) **Immunology :** It deals with the immune system that protects against infections and helps us to

understand the phenomena for both acquired and innate, immunity, in addition to the study of antibody–anti-gen reactions in the laboratory.

(ii) **Biotechnology :** It is the scientific manipulation of living organisms especially at the monocular and genetic level to produce useful products.

Q.16. What is the use of Hanging Drop Technique?

Ans.: Hanging drop preparation is useful for microscopic examination of living micro-organisms, especially bacteria without staining them to see their mobility due to flagella.

Q.17. What is Viruses?

Ans.: Viruses are non cellular biological entities compounds solely of a nucleic acid surrounded by a protein coat, the cap Sid.

Q.18. What means the Microphotograph?

Ans.: The microphotograph means a small photograph, the details of which can be seen clearly only with the help of optical enlargement.

Q.19. Why we store micro-organism in liquid nitrogen?

Ans.: Liquid nitrogen is the only suitable method for long term preservation of micro-organism that do not survive freeze-drying. Some microbes do not show the effect of cooling and warming. Such loss is reduced by the use of cryoprotectant such as glycerol and dim ethyl suphoxide (DMSO) and the adjustment of growth conditions, rate of cooling and warming.

Q.20. What are the basic requirements of a Microbiological Laboratory?

Ans.: Basic requirements of a Microbiological Laboratory is :

(1) **Common Glassware :** Test Tubes, Culture Tubes, Petridis, Pipette, Erlenmeyer Flasks (Conical, Round Bottom etc.), Volumetric Flask, Beaker, Measuring Cylinder, Stirrer/Glass Rod, Funnel, Dropping Bottle, Microscopic Slide, Cavity Slide, Cover Slip etc.

(2) **Tools/Apparatus :** Glass Spreader, Inoculation Needle & Inoculation Loop, Bunsen Burner, Spirit Lamp), Water Bath, Autoclave, Laminar, Airflow, Incubator, Hot Air Oven, Quebec Colony Counter, pH Meter, Spectrophotometer (Colorimeter), Centrifuges, Haemocytometer & Microscope.

Instruments

Q.1 What is Glass Spreader & its use.

Ans.: Bending L shaped glass rod called spreader. It is used to spread evenly the microorganism on agar surface present in liquid medium.

Q.2. What is Inoculation Needle & Inoculation Loop?

Ans.: Inoculation needle loop is made up of a long platinum wire fixed in to a metallic rod. A wire loop has a handle with steel screw shaft in which nichrome or platinum wire is to be fitted. The loop should be such so as to retain a small circular film in it by dipping in solution for this proper size (5-7cm) of the wire is recommended.

The loop and wire used for picking small quantities of solid materials from a microbial colony, and can be used to inoculated either a liquid or a solid medium. Both the loop and straight wire must be flamed immediately after uswed so that contamination is avoided.

Q.3. What is Water Bath & its use?

Ans.: Water bath is an instrument that is used to provide constant temperature to a sample. It consists of an insulating box made up of steel fitted with electrode heating coil. The temperature is controlled through a thermostat. The main use of water bath is the incubation of samples at a desired & constant temperature.

Q.4. What is Autoclave?

Ans.: The killing action of heat on the organisms can be done by using increase in the steam in a closed system. The water molecules become aggregated resulting in increase in their penetration. The water boils at 100°C and the steam accumulates in a closed container result ion in increase in pressure.

The autoclave is usually of pressure cooker type made up of gunmetal sheet that is supported in an iron case. It is based on moist heat sterilization. It usually operates at 15 lb/inch^2 steam pressure and 121°C kmp for 30 min. This temperature for 30 minutes is enough to kill all the spores and call of microorganisms.

Q.5. What is Laminar Airflow.

Ans.: Laminar flow is an apparatus consists of an air blower in the rear side of the chamber that can produce airflow with uniform velocity along patroller flow lines.

There is a special filter system of high efficiency particulate air filter HEPA that can remove particles as small as 0.3mm. It uses is all the work related to pouring, plating streaking etc. are to be carried out in the flame zone of the burner or spirit lamp.

Q.6. Write name of Filter present in Laminar Air Flow?

Ans.: High Efficiency Particulate Air (HEPA) filters are present in laminer air flow.

Q.7. What is Haemocytometer?

Ans.: Heamocytometer is the name given to a device used to measure the blood cells. This is also used for counting the other cells viz. spores, bacteria etc.

Q.8. What is pH Meter?

Ans.: The electrode system for measurement of pH is called pH meter. A standard pH meter has two electrodes - one glass electrode and the second Mercury Miraculous Chloride (Calomel) or silver – silver chloride reference electrode. The reference electrode is emerging in saturated KCl solution.

P^H can be defined as a negative log of hydrogen ions.

(H^+) concentration pH = -log10H^+=7.

pH is the degree of acidity alkalinity of a solution on a scale 1 to 14.

Q9. How many types of Centrifuge are there?

Ans.: There are three types of centrifuge :

(1) **Small Bench Centrifuge :** The maximum speed of these centrifuges vary from 4000 to 6000 rpm (round per minute) related with 3000 to 7000 gm.

(2) **High Speed Refrigerated Centrifuge :** Its maximum rotor speeds of 25000 rpm related with 60000 gm with a range of interchangeable fixed angle and swinging bucket rotors are available.

(3) **Ultracentrifuge :** The preparative ultracentrifuges have spinning rotors that can attain 80000 rpm with a relative centrifugal field of up to 600000 gm.

Sterilization

Q.1. What is Sterilization

Ans.: Sterilization is a process of making an article, surface or medium free from any type of microorganisms. Through sterilization, any kind of micro-organisms are killed.

Q.2. **Which method we use for Sterilization.**

Ans: Method of Sterilization:

(1) Physical Method of Sterilization :

(a) **Moist Heat:** It is done through autoclaving.

Dry Heat: Dry heat is produced by an hot air oven, glass syringe, forceps, scalpel, flasks, Petri dishes etc sterilized in an oven at 150^{o}C for 30 minutes.

(b) **Radiation:** Ultra Violet (UV) radiation is used in inoculation chamber or laminar air flow.

(c) **Sterilization by Membrane Filtration:** Certain substances like enzymes, antibiotics, amino acids, vitamins, etc. are heat sensitive; hence their sterilization is not possible by autoclaving. Such heat-sensitive substances are sterilized through various types of filters which may retain the bacteria. Millipore membrane filters are commonly used.

(2) Chemical Method of Sterilization :

(a) **Alcohol:** 70% ethanol or 70% isopropanol is used to sterilize the working table top, inoculation chamber etc.

(b) **Aldehyde :** Laboratory is fumigated with formaldehyde when the number of contaminants gets increased.

(c) **Inorganic Chemicals :** There are certain heavy metals which are toxic to any organisms such as salts of copper mercury etc. $HgCl_2$ solution (0.1%) is most commonly used as disinfectants for seeds, explants or any materials.

Culture

Q.1. What is Culture? Define the different types of Culture Media?

Ans.: The growth of an organism on a medium is called culture. The food base that supports the growth of an organism is called culture medium.

The culture media are devised in such a way that the organism should get the entire nutritional requirement. Basically, the culture media are of three types.

(i) **Natural Medium :** Ex diluted blood, urine, milk, vegetable, juices, peptone or animal cells/tissues/organs.

(ii) **Semi-synthetic Medium :** Ex – potato dextrose agar, czapek-dax agar, nutrient agar, beef extract agar, media etc.

(iii) **Synthetic Medium :** The synthetic medium will be of different types as

(a) General purpose medium Ex-for routine microbiological work.

(b) Differential medium Ex-differentiate the groups of microorganisms by opting such media that contain dyes and colour indicators to give biochemical response Ex-mac-monkey and eosin ethylene blue agar.

(c) **Selective Medium :** Contains compounds found in differential media and certain agents that further inhibit growth of most of the micro-organism and promote growth of the required ones.

(d) **One Purpose Medium :** Highly selective medium used to isolate specific micro-organizers ex-brilliant green agar for isolation of salmonella from faces.

(e) **Assay Medium :** Assay antibiotics, amino acid, vitamins etc.

Q.2 What is the preparation of liquid media or Broth?

Ans.: Components –

Nutrient Broth Medium

HCL (1 N)

NaOH (1 N) } Controlled the pH

NaCl – 5.0 gm.

Beef Extract - 3.0 gm.

Distilled Water - 1 ltr.

pH - 7.0

Procedure : Weigh the chemical ingredients of the nutrient broth and transfer them into a beaker containing 500 ml distilled water neat on hot plate and dissolve the ingredients. Add more distilled water to make the volume to 1 litre. Measure the pH by pH meter. Adjust the pH to 7.0 by adding drops of either HCl or NaOH solution. Prepare cotton plugs and apply them into the mouth of flask. Tightly cover the mouth of cotton plugs with aluminum foil and tie with a rubber band. Place the flask in side the autoclave sterilises at 121°C for 30 minutes. Use the broth when required or store room temperature for further use.

Q.3. Write the composition of media.

(i) PDA (ii) Nutrient Agar Media

Ans.: (i) **PDA (Potato Dextrose Agar)**

- Potato Tubers - 200 gms.
- Dextrose - 20 gms.
- Agar - 15 gms.
- Distilled Water - 1 litre
- pH - 5.6

(ii) **Nutrient Agar Media :**

Peptone – 5.0 gms.

Beef Extract – 3.0 gms.

Agar – 15 gms.

pH - 7.0

Distilled Water - 1 ltr.

NaCl - 5.0 gms.

Q.4. Explain the quality control of media?

Ans.: (i) The media are sterile ex–free from microbes.

(ii) It has stable and shelf life.

(iii) It has effectiveness.

(iv) If required, it also has selectiveness.

Q.5. What is the technique for pure culture of Microorganisms?

Ans.: Bacteria exist in mixed population. It is very rare to get a single and pure form for studying the cultural, morphological, and physiological characters of individual species. It is essential to separate them from the others to get in the form called pure culture. There

are many important methods for isolating pure culture from mixed culture.

(iii) Streak plate method from culture to pure culture.

(iv) Pour plate method for pure culture.

(v) Spread plate method.

(vi) Serial dilution agar plate method (or viable plate count method).

Q.6. Write the methods of culture preservation and maintenance.

Ans.: The methods of preservation are :

(i) Freeze drying or Lyophilization – Ex-bacteria, yeasts, fungi and some viruses.

(ii) Storage in liquid nitrogen.

(iii) Storage of strains at 70^0C.

(iv) Storage on glass beads at 60 to 70^0C.

(v) Storage in gelatin discs.

(vi) Storage in mineral oils.

(vii) Storage in soil ex fungi aspergillums, rhizopur, alternara, penicillin.

(viii) Maintenance by sub culturing.

Q.7. What is Agar?

Ans.: It is that component of the medium that helps in the solidification of the liquid medium (Broth). It is known as agar. It is a complex polysaccharide consists of 3,6–unhydro-L-Galactose & D-Galactopyranose.

Q.8. What is Sub-culturing?

Ans.: Sub-culturing is recommended for small collection centers. It has a draw back that it is time consuming and labour intensive process.

Stain's

Q.1. Explain the Gram's Staining and difference between Gram's Positive & Gram's Negative?

Ans.: Gram's staining is most important differential technique used in bacteriology. There are two groups - grams positive bacteria and gram negative bacteria.

S.No.	Characteristics	Negative Bacteria	Positive Bacteria
1-	Grams reaction	Dark-violator purple	Red or brown
2-	Cell wall composition	Low in liquids 1-4%	High in lipids (11-22%)
3-	Susceptibility	More	Less

	to penicillin	susceptible	susceptible
4-	Effect of basic dyes	Marked inhibition	Less inhibition
5-	Resistance to physical disruption	More resistant	Less resistant
Ex.:		Streptococci Mycobacterium leprae	Salmonella typhus Neisseria meningitides

Q. 2 Write components of following Stains :

Ans.: (i) Gram's Stain

(ii) Fungal Stain

(iii) Acid Fast Stain

(iv) Haemapoxylin

(v) Negative stain

Ans.: (i) Gram's Stain

(a) Crystal Violet (Hucker's)

Solution A

Crystal Violet (90% dye) – 3 gm.

Ethanol (95%) – 20 ml.

Solution B

Apmoi, Pxaate – 0.8 gms.

Distilled Water - 80 ml.

Prepare solution A & B separately, then mix & keep in a brown colour bottle.

(b) Gram's Iodine

Iodine – 1.0 gm.

Potassium Iodide – 2.0 gm.

Distilled Water – 300 ml.

(c) Ethanol (95%)

Ethand (100%) – 95 ml.

Distilled Water – 5 ml.

(d) Safrarin

Safranine (2.5% solution prepared in 95% ethanol) – 10ml.

Distilled Water – 100 ml.

(ii) Fungal Stain

(a) Latophenol + Cotton blue

Lactic Acid – 20 ml.

Phenol Cry Stables – 20 gm.

Glycerol (Glycerin) – 40 ml.

Distilled Water - 20 ml.

Cotton Blue – 2 ml.

(iii) Acid Fast Stain

Carbol functisin (Ziehl's)

Solution A

Basic Fuchsia (90% dye content) - 3 gm.

Ethylalcohd (95%) - 10.0 ml.

Solution B

Phenol Crystals (CP) - 5.0 gm.

Distilled Water - 95.0 ml.

Acid Alcohol -

Ethyl Alcohol 95% - 97.0 ml.

HCl 37% - 3.0 ml.

Ethylene Blue

Methylene Blue

Methylene Blue (90% dye content) - 0.3 gm.

Distilled Water -100.0 ml.

(iv) Haematoxyline

Haematoxyline - 2.0 gm.

Glacial Acetic Acid - 10.0 ml.

Absolute Alcohol 100.0 ml.

Glycerol - 100.0 ml.

Potassium Alum - In excess

Distilled Water - 100.0 ml.

(vii) Negative Stain (Nigrosim)

Nigrosin (water solude) - 10.0 gm.

Distilled Water - 100 ml.

Formation - 0.5 ml.