MICROB-DSC102

BASIC BACTERIOLOGY

Marks: 100 (Theory = 75 marks
Practicals = 25 marks)

Duration: Theory = 45 hours (3 credits)
Practicals = 30 hours (1 credit)

Course Objectives:

The main objective of this course is for students to acquire in-depth knowledge of bacterial cell structure and organization, cultivation methods and growth patterns, and reproduction. Further, the student gains insights into the vastness of bacterial diversity and its significance.

Pre-requisite: Student should have studied Biology/ Biotechnology/ Biochemistry in 12th standard

Course learning Outcomes:

Upon successful completion of the course the students will be able to:

CO1: Evaluate the morphological features and cellular organization of bacteria and archaea, and distinguish between cell wall and cell membrane compositions of gram positive bacteria, gram negative bacteria, and archaea. Will gain insights into the roles of enzymes and antibiotics affecting cell wall structure as well as the formation of spheroplasts, protoplasts, and L forms.

CO2: Isolate pure bacterial cultures and enumerate bacteria using serial dilution and plating techniques. Will learn about various culture media and methods employed to maintain bacterial cultures and preserve bacteria.

CO3: Discourse on the different phases of bacterial growth, and will understand the consequences of binary fission as a means of reproduction. Will learn about various nutritional and physical factors affecting bacterial growth.

CO4: Prepare various types of media; understand the use of membrane filtration to sterilize heat sensitive media components; have hands-on experience of isolating bacteria and fungi from air.

CO5: Streak bacterial cultures on nutrient medium, prepare bacterial slants and stabs, and enumerate bacteria by different plating methods.

Contents:

Theory: **45 hours**

Unit 1: Structure and organization of the bacterial cell wall and appendages: Shapes, sizes and arrangements of bacterial cells. Cell wall and cell membrane organization: Structure of cell wall in Eubacteria and Archaea, difference between cell wall structure and composition of Gram positive versus Gram-negative bacterial, structure of outer membrane, difference between eubacterial and archaeal cell membranes. Bacteria lacking cell walls, action of antibiotics and enzymes on bacterial cell wall, formation of protoplasts, spheroplasts and L forms. Cell envelope layers outside the cell wall: capsule, slime layer, glycocalyx, S-layers. External appendages**:** flagella, fimbriae and pili. **15**

Unit 2: Cytoplasmic organelles: ribosomes, mesosomes, nucleoid, chromosome and plasmids, intracytoplasmic membranes, inclusions (storage inclusions: PHB, polyphosphate granules, sulfur globules, cyanophycin granules; micro-compartments: Carboxysome; other inclusions: magnetosome, gas vacuole). **10**

Unit 3: Bacteriological techniques: Culture media: Chemical types (synthetic and complex), Functional types (supportive and enriched, selective and differential). Cultivation of aerobes and anaerobes, concept of viable but non culturable bacteria (VBNC). Culturing and Preservation methods: Streaking of bacterial culture, spread-plating, serial dilution plating, counting viable cells. Enrichment culture technique. Preservation of bacteria and maintenance of stock cultures. Microbial culture collection centers (ATCC and MTCC). **8**

Unit 4: Bacterial growth and reproduction: Different phases of bacterial growth in a batch culture**,** determination of generation time, analysis of growth rate. Factors affecting bacterial growth: Nutritional and physical factors. Endospore: Structure, formation, stages of sporulation and germination of endospore. Methods of asexual reproduction: budding, fission and fragmentation. **12**

Practicals: **30 hours**

Unit 1: Introduction to bacterial growth and analysis: Principle, working and applications of instruments used in cultivation and morphological analysis of microorganisms: bacteriological and BOD incubators, light microscope (using simple staining of bacteria). Concept of laminar flow: biological safety cabinets of levels 1 to 4.

Preparation of media and capture of aeroflora: Preparation of Synthetic medium (minimal medium) and Complex media (nutrient agar, potato dextrose agar, MacConkey agar). Capture of aero-microflora on nutrient agar and potato dextrose agar plates.

15

Unit 2: Isolation, preservation and quantitation of bacteria: Isolation of pure cultures of bacteria by Quadrant streaking method on nutrient agar plates. Preparation of bacterial culture slants and stabs on nutrient agar. Preservation of bacterial cultures by preparation of glycerol stocks. **15**

Suggested Reading:

Theory:

1. Brock Biology of Microorganisms by M.T. Madigan,J. Aiyer,D. Buckley,W.Sattley and D. Stahl.16th edition. Pearson, USA. 2021.

2. Prescott's Microbiology by J. M. Willey, K. Sandman and D. Wood. 11th edition. McGrawHill Higher Education, USA. 2019.

3. Microbiology: Principles and Explorations by J.G. Black and L.J. Black. 10th edition. Wiley, USA. 2019.

4. Microbiology: An Introduction by G.J. Tortora, B.R. Funke,and C.L. Case. 13th edition. Pearson, USA. 2018.

5. Principles of Microbiology by R. M. Atlas. 2nd edition. W.M.T. Brown Publishers, USA.

1997.

6. Microbiology by M. J. Pelczar, E. C. S. Chan and N. R. Krieg. 5th edition. McGraw Hill, USA. 1993.

Practicals:

1. Microbiology: A Laboratory Manual by J. Cappuccino and C.T. Welsh. 12th edition. Pearson Education, USA. 2020.

2. Basic Lab Manual of Microbiology, Biochemistry and Molecular Biology by A. Ray and R. Mukherjee. Taurean Publisher, India. 2019.

3. Benson's Microbiological applications: Laboratory manual in general microbiology by A.E. Brown and H. Smith H. 15th edition. McGraw-Hill Education, USA. 2022.

4. Manual of Microbiology: Tools & Techniques by A.K. Sharma. 1st edition. Ane Books, India. 2007.

Facilitating the achievement of Course Learning Outcomes

S. no.	Course Learning Outcomes	Teaching and Learning activity	Assessment Tasks
1.	Evaluate the morphological features and cellular organization of bacteria and archaea, and distinguish between cell wall and cell membrane compositions of gram positive bacteria, gram negative bacteria, and archaea. Will gain insights into the roles of enzymes and antibiotics affecting cell wall structure as well as the formation of spheroplasts, protoplasts, and L forms	PowerPoint presentations/ videos, pictures showing bacterial cells and their components. Explaining differences between Gram+ve and Gram-ve bacteria; eubacterial and archaebacterial structures with the help of diagrams and discussion of the action of antibiotics and enzymes on cell wall.	Test based on diagrams ofvarious cell components and their differences.
2.	Isolate pure bacterial cultures and enumerate bacteria using serial dilution and plating techniques. Will learn about various culture media and methods employed to maintain bacterial cultures and preserve bacteria.	Demonstration of various techniques for isolation and culturing of bacteria. Discussion for comparing of methods of preservation of bacteria.	Evaluation of streaking/spread plate / serial dilution plating techniques.

3.	Discourse on the different phases of bacterial growth, and will understand the consequences of binary fission as a means of reproduction. Will learn about various nutritional and physical factors affecting bacterial growth.	Class lectures on mathematical and graphical expression of changes in bacterial populations by asexual reproduction. Calculation of generation time and growth rate to be explained.	MCQ /Quiz based on examples of asexual reproduction and growth curve.
4.	Prepare various types of media; understand the use of membrane filtration to sterilize heat sensitive media components; have hands-on experience of isolating bacteria and fungi from air.	Weighing media components, dissolving them, setting pH and sterilization of media using autoclave along with learning about the abundance of microbes in air	Testing for sterile media preparation and membrane filtration technique
5.	Streak bacterial cultures on nutrient medium, prepare bacterial slants and stabs, and enumerate bacteria by different plating methods.	Preparation of serial dilution, plating methods will enable students get good practice in inoculating/subculturing bacteria	Testing efficacy of working under aseptic conditions to minimize contaminations of culture plates, observing purity of cultures and learning to purify mixed cultures

***Assessment tasks are indicative and may vary**