

## MICROBIOLOGY PRACTICALS – (I, II, III)

Min. pass marks: 18	Distribution of Marks	
	Duration: 3 hours REGULAR	Max. Marks: 50 EX-STUDENT
1. Major Exercise	12	12
2. Minor Exercise	10	10
3. Preparation	8	8
4. Spots (5)	10	10
5. Record	5	-
6. Viva-voce	5	10
<b>TOTAL</b>	<b>50</b>	<b>50</b>

### B.Sc. Pt –III Microbiology- 2013

#### MB-19-Microbial culture, growth and food Microbiology

Duration : 3 hrs

Max .Marks 50

Note - The paper is divided into five units. Two questions will be set from each unit. The candidates are required to attempt one question from each unit. All questions carry equal marks.

#### Unit-I

Microbial culture: Disinfection, Sterilization, Isolation, Screening and maintenance of micro organism, bait technique, trophic grouping

Microbial culture media (artificial natural), physical chemistry of culture medium, pH, buffer. Methods of anaerobic cultivation. Inoculation, Incubation and measurement of growth of micro organism.

Physical factors of controlling growth of microorganism (temperature gaseous)

#### Unit-II

**Microbial growth kinetics:** Definition, brief description of different type of microbial culture (batch, fed batch and synchronous), growth phases, growth kinetics, growth field, method of growth determination.

Environmental factors affecting microbial growth temperature, pH, Osmotic pressure and nutrient concentration per cell.

#### Unit-III

**Food product:** Microbial mass as food -single cell protein (*Algae-Chlorella, Spirulina, Scenedesmus*)

**Mushroom cultivation:** *Agaricus bisporus, Pleurotus spp.*

**Fungi:** Filamentous fungi, yeast, *Candida*, members of *Saccharomyces* and *Torulopsis*  
Fermented food- Bread, Cheese, Vinegar, dairy products, Kinema, Fermented Beverages (Beer, wine)

**Bio Conversions:** Alcohol production, steroid conversion, production of enzyme-amylases, proteinases, cellulases.

Amino acid production-glutamic acid and lysine

#### Unit-IV

Microbial contamination and spoilage-contamination of Sugar products, Vegetable, Fruit, meat, meat product, dairy products, Fish, Sea food and Poultry.

Spoilage and canned foods, Detection of spoilage and characterization.

**Food Preservations:** Pasteurization, appertization, aseptic packaging, use of high temperature, freezing, dehydration, Osmotic pressure, use of chemicals, organic acid, esters, sulphur di-oxide, salt and high sugar concentration.

#### **Unit-V**

**Food borne infections and intoxications:** Bacterial and non bacterial infective and toxic types – *Vibrio*, *Bacillus*, *Clostridium*, *Escherichia*, *Salmonella*, *Shigella*, Nematodes, Protozoa, Algae, Fungi Virus.

**Infections and toxicosis:** Botulism, Cholera, Mycotoxicosis, Salmonellosis *E coli*-poisoning, preventive measures. Food sanitation in manufacture and retail trade, food control agencies, role of fruit product certifying agencies and related regulations, waste treatment, disposal and quality control.

### **MB-20-Fermentation Technology and Government rules**

Duration : 3 hrs

Max .Marks 50

Note - The paper is divided into five units. Two questions will be set from each unit. The candidates are required to attempt one question from each unit. All questions carry equal marks.

#### **Unit-I**

Bioreactors/Fementers: Design, components and operation of fermenters, stirred tank, bubble columns airlift, bioreactors, static submerged and agitated fermentation.

Physicochemical standards used in fermentors /Bioreactor, Limitations of Bioreactors.

#### **Unit-II**

**Fermentation process:** Media formulations, sterilization, solid substrate fermentation, steps of fermentation process.

Advantage and disadvantages of solid substrate and liquid substrate fermentation.

In-situ recovery of fermentation products, fermentation monitoring, Bioprocess calculations based on material and energy balance.

#### **Unit-III**

Fermentation in batch culture: Microbial growth kinetics, measurement of growth (cell number direct and indirect method)

Nutrients product formation, heat evolution, effect of environment (temperature, pH, high nutrient concentration)

Continuous culture system, Aeration, Agitation, Oxygen transfer kinetics.

Concept of Newtonian and non Newtonian fluids plastic fluids apparent viscosity foam and antifoam.

#### **Unit-IV**

Downstream processing: Filtration, Precipitation ultrafiltration, Ultra ceutrifugation, cell disintegration solvent extraction, chromatographic separation, membrane filter.

Biotransformation: Development of inoculum, Incubation production of ethyl alcohol, acetic acid, antibiotics and vitamin B<sub>12</sub> with reference to easily available raw materials.

Scale up, instrumentation control, physical, chemical, environmental sensors.

### **Unit-V**

**Biosafety:** Objective, rules and regulation, procedure of biosafety, containment (Physical and biological)

Risk assessment of genetically modified food.

Public opinion and ethical issues against the microbial technologies.

Patent practice and problem-patentability of micro-organisms patenting laws in India and its limits.

Rules and procedure for use of genetically modified organisms and environmental protection act 1986.

## **MB-21-Soil & Agriculture Microbiology**

Duration : 3 hrs

Max .Marks 50

Note - The paper is divided into five units. Two questions will be set from each unit. The candidates are required to attempt one question from each unit. All questions carry equal marks.

### **Unit-I**

Soil-definition, types, physical and chemical characters, soil profile, soil micro organisms Bacteria, fungi, Actinomycetes, algal, protozoa and viruses.

### **Unit-II**

Interactions between plants and microorganisms-types of interactions (Symbiosis, mutualism, commensalisms, competition, amensalism, synergism, parasitism, predation, microorganisms of rhizosphere, rhizoplane and phylloplane. Mycorrhiza- Types and its application.

### **Unit-III**

Microorganisms and biogeochemical cycles- Nitrogen, phosphorous, sulphur and carbon. Bioleaching and Biodegradation. (cellulose, pectin, plastic and pesticides)

### **Unit-IV**

Biofertilizers :-definition, types, kind of association, mode of application and merits. Biochemistry, genetics and physiology of nitrogen fixation-symbiotic, non symbiotic and associative symbiotic. Nitrogen fixation by blue green algae.

### **Unit-V**

Biopesticides-introduction, types, mode of action and factors influencing, target pests. A brief account of the symptoms, etiology, life cycle and management of bacterial (citrus canker, blight of paddy), mycoplasmal (little leaf of brinjal), Viral (TMV, tomato leaf curl) and fungal (white rust of crucifers and stem rust of wheat) diseases.

## MB-22-Medical Microbiology

Duration : 3 hrs

Max .Marks 50

Note - The paper is divided into five units. Two questions will be set from each unit. The candidates are required to attempt one question from each unit. All questions carry equal marks.

### Unit-I

**Introduction:** Definition, brief history, types of medically important microbes, host-parasite relationship.

**Normal microflora of human body:** normal microflora of skin, conjunctive (eye), nose, mouth and upper respiratory tract, intestinal tract, urinogenital tract, blood & tissues.

### Unit-II

Pathogenicity, reservoirs of infections, infections, virulence, communicability of disease/pathogenic microbes.

Microbial ecology of pathogens of animals and birds. Effects of pathogenic microbes.

### Unit-III

Diagnosis, symptoms, Toxic components, etiology and epidemiology and disease development in animals and fowls.

Fowls pox, fowl spirochetosis, infections bronchitis.

Diseases of silkworm, livestock and pets (dogs & cats)

### Unit-IV

**Diseases of human beings-I:** Diagnosis, symptoms, toxic components, etiology and disease development in human body-bacterial diseases (Diphtheria, Tuberculosis, Leprosy, Cholera, Typhoid, Gonorrhoea, Syphilis, Tetanus, Streptococcal infection of the skin, Dysentery, Salmonellosis, Botulism and Scarlet fever). Control, treatment and management of bacterial diseases.

### Unit-V

**Diseases of human beings-II:** Diagnosis, symptoms, toxic components, etiology and disease development in human body-viral and fungal disease.

Viral-Aids, Poliomyelites, Mumps, Influenza, Viral Hepatitis, Small pox, Chicken pox, Common cold and Rabbits.

**Fungal:** Superficial mycoses, cutaneous mycoses, sub-cutaneous mycoses and systemic mycoses.

Control, treatment and management of viral & fungal diseases.

## MB-23-Genetic Engineering and rDNA Technology

Duration : 3 hrs

Max .Marks 50

Note - The paper is divided into five units. Two questions will be set from each unit. The candidates are required to attempt one question from each unit. All questions carry equal marks.

#### **Unit-I**

Introduction and historical background of genetic engineering. Isolation and purification of DNA from bacterial, plant and animal cells. Vectors: Plasmids, cosmids and phages. Restriction enzymes, ligases, S1 nucleases, DNA polymerases. Reverse transcriptase.

#### **Unit-II**

C-DNA synthesis and cloning: mRNA enrichment, reverse transcription, DNA primer Linkers, adapters, blunt end ligation, homopolymer tailing. C-DNA library construction and screening. Genomic library construction and screening. PCR and DNA sequencing.

#### **Unit-III**

Techniques used in identification of recombinant DNA clones. Cloning and expression of foreign genes in prokaryotes (E.coli). Cloning and expression of foreign genes in eukaryotes (eg. yeast). Solid phase automated synthesis of DNA Applications of transposons in gene tagging

#### **Unit-IV**

Production of transgenic mice. Gene targeting. Using yeast to study eukaryotic gene function Therapeutic products produced by genetic engineering-blood proteins, human hormones, vaccines. Transgenic animals, production of proteins of pharmaceutical value.

#### **Unit-V**

Gene transfer-microinjection, electroporation, microprojectile, shot gun method ultra -sonication, Liposome fusion, microlasers. Use of *Agrobacterium tumefaciens* and *A.rhizogenes*, Ti plasmids, gene targeting in plants. Transgenic plants.

## **MB-24-Tools and Techniques**

Duration : 3 hrs

Max .Marks 50

Note - The paper is divided into five units. Two questions will be set from each unit. The candidates are required to attempt one question from each unit. All questions carry equal marks.

#### **Unit-I**

**Microscopy:** General, magnification, microscopes types, use of techniques of preparing specimens, resolving power, optical microscope-Basic idea of light microscopy, Types- bright field, dark field, ultra-violet, fluorescence and phase-contrast microscopes.

Electron microscope-Basic idea of structure and functioning of E.M., Preparation of material for electron microscopy, Types-TEM, SEM, Scanning probe microscope, scanning tunnel microscope atomic force microscope.

Techniques in light microscopy-wet mount, hanging drop preparations.

### Unit-II

**Microbial stains:** Stains V/s Dyes, various types of stains, nature of stains, uses of stains.

**Staining techniques:** Simple, differential negative, gram-stain, mechanism of gram stain, Ziehl Nelson (acid fast) stain, special stains.

Sterilization techniques: sterilization of glassware and culture media.

### Unit-III

**Micro analytical Techniques:** Disintegration of microorganisms- Mechanical & non-mechanical methods.

Separation of sub cellular structures and organelles- Differential, gradient, zonal or band and equilibrium or isopycnic density gradient centrifugation.

### Unit-IV

**Cyto chemical Analysis:** Cytochemistry of microbes, cytochemical staining methods, radio autography (autoradiography) principles and procedure of radio autography.

**Chromatography:** principle and procedure of absorption, column, thin layer (TLC), partition, and gas-liquid ion-exchange chromatography.

**Electrophoresis:** Principle, equipment and procedure of various types (vertical & horizontal) electrophoresis, SDS-PAGE electrophoresis.

### Unit-V

**Culture of microbes:** Preparation of culture media, aseptic transfer of bacteria, pure culture, serial dilution technique, preservation and maintenance of cultures, concept of stock culture. Measurements of microbes- Ocular & stage micrometer

## B.Sc- Pt-III (Microbiology) Practicals

Practical No.	Paper No.	Max. Marks	Min. Marks	Paper Titles
Practical -01	MB-25	50	18	Microbial Culture, Growth and food Microbiology Fermentation Technology and Government role
Practical -02	MB-26	50	18	Soil and Agricultural Microbiology + Medical Microbiology
Practical -03	MB-27	50	18	Genetic Engineering and R-DNA Technology +Tools and Techniques

### Practical- Paper- I Microbial Culture, Growth & Food Microbiology

1. Principles and application of light and phase contrast microscope, Incubator, Colorimeter, Centrifuge, Spectrophotometer (Visible and UV).
2. Preparation of liquid and solid nutrient media for growth of micro-organism, cleaning, sterilization, culturing methods, dilution technique in microbiology.
3. Isolation and maintenance of organisms by plating, streaking and serial dilution methods, slant and stab cultures, storage of micro organisms.
4. Staining techniques in microbiology-
  - a. Simple staining
  - b. Negative staining
  - c. Differential staining
  - d. Spore staining
5. Microscopic examination of bacteria, yeast and moulds. Study of micro organisms by Gram stain, Acid fast stain.
6. Identification of micro organisms by biochemical tests.
7. Growth, Bacterial growth curve, measurement of bacterial population by turbidometry and serial dilution method.  
Effect of physiological factors (physical and chemical) on growth of micro-organisms e.g. temperature, pH, Carbon and nitrogen sources.
8. Microbial examination of food.
9. Effect of antibiotics on microbes.
10. Microbial production of citric acid using *Aspergillus niger*.
11. Detection of number of bacteria in milk by standard plate count.
12. Isolation of microbes from natural sources using specific media.
  - a. Nutrient agar
  - b. Potato dextrose agar
  - c. BG-11
13. Microbial analysis of water, milk, butter, fruit and vegetables using selective standard method.
14. Isolation of Microbes from rhizosphere and rhizoplane.
15. To perform microbial activity in composting.

## **Practical- Paper- II**

### **Fermentation Technology and Government Role**

1. Isolation of industrially imported micro-organism for microbial processes.
2. Determination of thermal death point (TDP) and thermal death time (TDT) of microorganism for design of a sterilizer.
3.
  - a. Determination of growth curve of a supplied microorganism and also determine substrate
  - b. Compute specific growth rate ( $\mu$ ), growth yield ( $y$ ) from the above experiment.
4. Comparative studies of ethanol production using different substrates.
5. Production and estimation of Alkaline Protease.
6. Saurkraut fermentation.
7. Use of alginate for cell immobilization.
8. Determination of mixing time in bioreactors.
9. Visit to dairy, Bakery, distillery and sugar factory and submission of visit report.

## **Practical- Paper- III**

## Soil and Agricultural Microbiology

1. Analysis of soil: Texture, pH, moisture content, water holding capacity, percolation and capillary action.
2. Isolation and study of microbes (bacteria and fungi) from Rhizosphere and Rhizoplane
3. Isolation of Rhizobium from root nodules of legumes (Trigonella / Cicer / Soybean)
4. Isolation of free nitrogen fixers (Azotobacter, Azospirillum) from soil
5. Study of plant pathogens based on theory

## Practical- Paper- IV Medical Microbiology

1. Isolation of Pathogenic bacteria from natural sources: Cough, skin-wash, blood, tissue, stool etc.
2. Culture and identification of pathogenic bacteria using specific media
3. Isolation and identification of fungi causing diseases in animals and human beings.
4. Study of causative viruses of pathogenetic importance with the help of slide, **model, election micrograph or computer simulation** :
  - a. HIV
  - b. Hepatitis B Virus
  - c. T phage
  - d. Polioviruses
  - e. Rabies
5. Field trip or visit to a microbiological laboratory of any advanced medical college or institute of medical science to study various activities and exposure to the research being done there.
6. Study of diseases (bacterial & viral) of pets and their causative agents. (Help of veterinary hospital / college may be taken).

## Practical- Paper- V Genetic Engineering and rDNA Technology

1. Isolation of DNA from : a) Bacteria (genomic) b. Plants
2. Digestion of DNA with restriction enzymes.
3. Quantitation of nucleic acids.
4. Bacterial culture and antibiotic selection media
5. Protoplast fusion, Anthers culture and haploid production
6. Charts on genetic engineering –
  - a. pBR 322
  - b. pUC 180 and 19
  - c. SV 40
  - d. Bacteriophage
  - e. Gene Cloning
  - f. Selection of recombinants by replica plate technique

## Practical- Paper- VI

## Tools and Techniques

1. Study of organization and working of microscopes :
  - a. Optical Microscopes : dissecting and compound
  - b. Exposure to organization and working of phase contrast microscope and electron microscopes.
2. Preparation of various microbial stains and their use.
3. Use of staining techniques –
  - a. Single and differential, negative
  - b. Gram's stain
4. Sterilization of glassware and media (use of autoclave). Aseptic transfer.
5. Study and use of micro analytical techniques.
6. Separation of sub- cellular organelles (use of centrifuge and other techniques)
7. Separation of cell organelles by sucrose gradient.
8. Electrophoresis : SDS – PAGE
9. Thin layer chromatography
10. Paper chromatography : circular and vertical
11. Culture of microbes using specific media and techniques.
12. Measurement of microbes using micrometer (Ocular and stage)
13. Visit to microbiological laboratory for exposure of various advanced tools and techniques.