

**UNIT-1****5 h**

**Enzymes and Vectors** - DNA Polymerase, Polynucleotide kinase, Alkaline phosphatase, Terminal deoxynucleotidyl transferase, Reverse transcriptase, Restriction endonucleases, RNase, Klenow fragment, DNA ligases. **Vectors for cloning**- Plasmids, Bacteriophage lambda, Filamentous coliphages, Cosmids, YACs and BACs, Eukaryotic cloning vectors for yeast (YIp, YEp, YCp.), higher plants (Ti based vectors, binary and cointegrate, chloroplast-based vectors) and for animal cells (Adenovirus, Adeno-associated virus, Baculovirus, Herpesvirus, Retrovirus). Shuttle vectors, Expression vectors.

**UNIT-2****8 h**

**Basic techniques** - Isolation and purification of genomic and plasmid DNA, Pulse field gel electrophoresis. Nucleic acid blotting-Southern blotting, Northern blotting, Western blotting, PCR, RT-PCR, Real-time quantitative PCR, RNase protection assay, *in situ* hybridization. **DNA sequencing methods**- Maxam and Gilbert method, Sangers dideoxy method, Automated DNA sequencing, Pyrosequencing. **Site-directed mutagenesis**-. Analysis of genetic variation-Single nucleotide polymorphism, RFLP, RAPD, Restriction mapping. DNA chips and microarray.

**UNIT-3****10 h**

**Cloning strategies** - Natural gene transfer methods- transformation, transduction, conjugation. Calcium chloride mediated transformation, Transfection with phage vectors. Packaging phage- $\lambda$ DNA *in vitro*. DNA-mediated transformation-DNA/calcium phosphate coprecipitate method, Phospholipids as gene-delivery vehicles, Electroporation, Genomic DNA libraries- Development of  $\lambda$  replacement vectors for genomic library construction, cDNA cloning- cDNA libraries, Rapid amplification of cDNA ends. Shot gun cloning. Cloning in bacteria, plants and animals

**UNIT-4****8 h**

**Recombinant clones** - Screening libraries with gene probes, Sequence-dependent screening -colony hybridization, plaque hybridization, Screening by 'gain of function', Immunological screening, Chromosome walking,. Studying gene function through protein interactions-Two hybrid screening, phage display libraries. Analysis of gene expression-Gene expression based on T7 RNA polymerase, visible marker genes, Gene silencing.

**UNIT-5****8 h**

**Applications and legal issues** – Applications of genetic engineering in Agriculture, Veterinary, Industry, Forensic Science and Medicine. Transgenic crops, animals, recombinant enzymes, pharmaceuticals- humulin, somatropin and somatostatin. Ethical, Legal, Social, and Environmental Issues.

**Text Books:**

1. Sandy Primrose (2006). *Principles of Gene Manipulation and Genomics*. 7<sup>th</sup> Ed., Black Well Publishers.
2. Brown T.A. (2004). *Gene Cloning and DNA analysis*. 2<sup>nd</sup> Edition, ASM press.

**Suggested Reading:**

1. David Frifielder, Stanely R. Maloy. (1994). *Molecular biology and Microbial genetics*. 2<sup>nd</sup> Edition, Jones and Barlett Publishers.
2. Peter J Russell (1997). *Genetics*. 5<sup>th</sup> Ed., Benjamin-Cummings Publishing Co.
3. Glick BR and Pasternak JJ (2003). *Molecular Biotechnology*, 2<sup>nd</sup> Ed., ASM press.

**UNIT - I****4 h**

History of Microorganisms in food. Importance and significance of microorganisms in food. Factors – Intrinsic and Extrinsic parameters - affecting the growth of microorganisms in food.

**UNIT - II****6 h**

Determination of micro organisms and their products in food: Sampling, Microscopic and culture dependent methods- Direct microscopic observation, culture, enumeration and isolation methods; Animal and Cell Culture Models for Foodborne Bacterial Pathogens, Physical, Chemical and Biological methods. Culture independent techniques – Metagenomics, Biosensor based detection of food pathogens, PCR Based, DGGE. Molecular Typing and Differentiation of Foodborne Bacterial Pathogens. Analytical methods for microbial metabolites- microbial toxins and metabolites.

**UNIT -III****10 h**

Food preservation: High temperature, Low temperature, Drying, Chemical, Modified atmosphere, Radiation, other food protection methods and Microbial Resistance.

Food spoilage: Organisms involved, characteristic features, dynamics and significance of spoilage of different groups of foods - Cereal and cereal products, vegetables and fruits, meat, poultry and sea foods, milk and milk products, packed and canned foods.

**UNIT - IV****8 h**

Food fermentations: Manufacture of fermented foods: Meat and fishery products, plant products and mixed products, breads, beverages. Spoilage and defects of fermented foods. Microbial cells as food: SCP, mushroom cultivation. Dairy Microbiology: Milk, Fermentation, Fermented and Non-fermented Dairy Products.

Source and applications of microbial enzymes, microbial antioxidants, biosurfactants, microbial polysaccharides, flavors and microbial colors. Probiotics and their advantages. Genetically modified foods.

**UNIT - V****8 h**

Indicators of water and food safety and quality: Microbiological criteria of foods and their Significance. The HACCP and ISO systems for food safety. Food borne diseases: Bacterial food borne diseases ( Staphylococcal intoxicification, Botulism, Salmonellosis, Shigellosis, EPEC Diarrhoea, *Clostridium Perfringens* gastroenteritis, *Bacillus cereus* Gastroenteritis) Food Borne Viral Pathogens (Norwalk virus, Reovirus, Rotavirus, Adenovirus, Parvovirus, Hepatitis A Virus) Food Borne Animal Parasites Protozoa – Giardiasis, Amebiasis, Toxoplasmosis, Cryptosporidiosis. Cysticercosis/Taeniasis. Roundworm – Trichinosis, Anisakiasis. Mycotoxins: Aflatoxicosis, Deoxynivalenol, Mycotoxicosis, Ergotism.

**Text Books**

1. Adams, M.R. and M.G. Moss. (2007): *Food Microbiology*, 3<sup>rd</sup> Edition, Royal Society of Chemistry (RSC) Publishing.
2. James M Jay, Martin J Loessner, and David A Golden. (2005) *Modern Food Microbiology*, 7<sup>th</sup> Edition. CBS Publisher.

**Suggested Reading:**

1. Pina M. Fratamico, Arun K. Bhunia, and James L. Smith. (2005). *Food-borne Pathogens: Microbiology and Molecular Biology*. 1<sup>st</sup> Edition, Caister Academic Press.
2. Sperber, William H.; Doyle, Michael P. (2010). *Compendium of the Microbiological Spoilage of Foods and Beverages*. 1<sup>st</sup> Edition, Springer.
3. Frazier, W.C. (1988). *Food Microbiology*, Mc Graw Hill Inc. 4<sup>th</sup> Edition.

**MB 503**

**FERMENTATION TECHNOLOGY**

**3 Credits**

**UNIT- I**

**8 h**

Fermentation Pathways for Industrial Products: Biochemical pathways of metabolic reactions for utilization of carbon sources and formation of different metabolites by micro organisms; possibility of control of the reactions for the increased formation of useful metabolites. Strain Development - Various techniques of modifying the strains for increased production of industrial products. Use of chemicals, UV rays, genetic engineering to produce newer strains.

**UNIT- II**

**8 h**

Media for Fermentation: Importance of media components for production of industrial products by fermentation; use of different sources of carbon, nitrogen, minerals and activators for commercial fermentation; importance of pH, temperature and aeration in fermentation; optimization of fermentation media.

**UNIT- III**

**6 h**

Different Types of Fermenters: Laboratory and plant fermenters; shake flasks and advantages; laboratory fermentation systems with various controls and sampling and data collection provisions; aeration and agitation; production fermenters; sterilization of media; cooling systems; inoculation, temperature and pH control systems; scale-up of fermentation process.

**UNIT- IV**

**8 h**

Downstream Processing: Introduction, removal of microbial cells and other solid matters, Filtration: Theory, Batch filters (plate and frame filters, pressure leaf filters), Continuous filters (rotary vacuum, Cross flow filtration); Centrifugation, Cell aggregation and flocculation, Types of centrifuges (basket centrifuges, tubular bowl centrifuge etc); Cell disruption: Physical mechanical methods and chemical methods.

**UNIT- V**

**6 h**

Various equipments for product recovery; micro-filters and Ultra-filtration systems for separation of cells and fermentation medium and for concentration of medium containing product; solvent extraction of product; evaporation and crystallization; drying techniques; chromatographic systems for separation; instrumentation and controls.

**Text Books:**

1. Vogel, H.C. and C.L. Todaro, (2005). *Fermentation and Biochemical Engineering Handbook : Principles, Process Design and Equipment*. 2<sup>nd</sup> Edition, Standard Publishers.
2. El-Mansi, E.M.T, (2007). *Fermentation Microbiology and Biotechnology*. 2<sup>nd</sup> Edition, CRC / Taylor & Francis.

**Suggested Readings:**

1. Chisti, Y. *Fermentation, Biocatalysis and bioseparation, Encyclopedia of Bioprocess Technology*, Vol. 5, John Wiley and Sons, New York.
2. Michael L. Shuler and Fikret Kargi (2001). *Bioprocess Engineering: Basic Concepts*. 2<sup>nd</sup> Edition, Prentice Hall.
3. Pepler, H.J. and Perlman, D. (2005). *Microbial Technology: Fermentation Technology*. 2<sup>nd</sup> Edition Vol. 2. Elsevier India Private Limited.
4. Pepler, H.J. and Perlman, D. (2005). *Microbial Technology: Fermentation Technology*. 2<sup>nd</sup> Edition Volume 2. Elsevier India Private Limited.

**Unit I:****6 h**

Historical developments in medical microbiology: Normal flora of human body, opportunistic infections; Host-parasite interactions. Mechanisms of microbial resistance to host cellular and humoral defenses. Molecular basis of microbial pathogenicity. Brief account of major air, water and soil borne diseases of microbial origin.

**Unit II:****6 h**

Types of infections – Bacterial, Fungal and Viral; modes of transmission, portal of entry. Nosocomial infection, common types of hospital infections and their diagnosis and control. General concepts for clinical specimen collection and handling of clinical specimen, specimen processing and biosafety. Various methods of drug susceptibility testing, antibiotic assay in body fluids. Brief account on available vaccines and schedules; passive prophylactic measures.

**Unit III:****10 h**

**Bacterial infections:** Pathogenesis, Laboratory diagnosis, prophylaxis and treatment of the diseases caused by Staphylococcus; Streptococcus; Neisseria; Bacillus; Corynebacterium; Clostridium; Enterobacteriaceae family; Non-fermenters including Pseudomonas; Yersinia; Haemophilus; Brucella, Bordetella, Mycobacterium, Non sporing anaerobes, Rickettsia, Mycoplasma and Chlamydia.

**Unit IV:****7 h**

**Viral infections:** Pathogenesis and laboratory diagnosis of important viral diseases; Chicken pox, Rabies virus, common cold, hepatitis, meningitis, encephalitis, AIDS, SARS, Herpes simplex infections, Mumps Measles, Influenza, Arboviruses Slow viruses and Tumor viruses.

**Unit V:****8 h**

**Fungal infections:** Classification of medically relevant fungi; Collection of clinical samples and laboratory diagnosis of important human fungal diseases: Cutaneous and subcutaneous mycoses; Systemic mycoses; Opportunistic mycoses; Antifungal agents and their susceptibility test.

**Parasitology:** Important diseases caused by: Entamoeba; Giardia; Trichomonas; Plasmodium; Trypanosoma; Leishmania; Taenia; Schistosoma; Paragonimus; Ascaris; Ancylostoma; Necator; their laboratory diagnosis, treatment and prevention.

**Text Books:**

1. Greenwood D. 2007. *Medical Microbiology* 4th Ed., I.K. International.
2. Murray P.R., Tenover F.C., and Tenover F.C., and Tenover F.C. 2007. *Clinical Microbiology*, ASM Press.

**Suggested Readings:**

1. Nester E. W., Anderson D. G. and Nester M. T. 2006. *Microbiology: A Human Perspective*, McGraw Hill.
2. Harvey, R.A., Champe, P.C. and Fisher, B.D. 2007. *Lippincott's Illustrated Reviews : Microbiology. Lippincott Williams and Wilkins*, New Delhi/New York.
3. David Wilks, Mark Farrington and David Rubenstein 2010. *Infectious Diseases Manual: Blackwell Science*
4. George F. Brooks, Karen C.Carroll, Janet S.Butel, Stephen A. Morse. 2007. *Jawetz, Melnick & Adelberg's Medical microbiology*. 24<sup>th</sup> Ed. McGraw-Hill Professional.

**Unit I:****6 h**

Historical account of microbes in industrial microbiology; sources and characters of industrially important microbes; their isolation, purification and maintenance; Screening of useful strains; primary screening and secondary screening; Strain improvement through random mutation and genetic engineering; Microbial growth kinetics in batch, continuous and fed-batch fermentation process.

**Unit II:****8 h**

Microbial production of Primary and secondary metabolites. Commercial production of antibiotics with special reference to penicillin, streptomycin and their derivatives. Microbial transformations: steroids and alkaloids production. Large scale production of recombinant molecules interferon, human proteins- insulin, vaccines, anticancer agents and siderophores,

**Unit III:****9 h**

Microbiology and production of alcoholic beverages; Malt beverages, distilled beverages, wine and champagne; Commercial production of organic acids like acetic, lactic, citric, and gluconic acids; Commercial production of important amino acids (glutamic acid, lysine and tryptophan), and vitamins (riboflavin and vitamin A).

**Unit IV:****7 h**

Immobilization of microbial enzymes and whole cells and their applications in industries; Industrial enzymes production; Cellulases, Xylanases, Pectinases, Amylases, Lipases and Proteases and their applications.

**Unit V:****6 h**

Biofuels (ethanol and methane) from organic residues; fuels from algae; Mushroom cultivation; other microbial products - Biopolymers and EPS, Bioplastics, Biosurfactants, Patent protection and IPR for biological inventions.

**Text Books:**

1. Nduka Okafor (2007). *Modern Industrial Microbiology and Biotechnology*. 1<sup>st</sup> Edition: Science Publishers.
2. Waites, M.J., Morgan, N.L., Rockey, J.S. and Higton, G. (2002). *Industrial Microbiology: An Introduction*. Blackwell Science Publishers.

**Suggested Readings:**

1. Richard H. Baltz, Julian E. Davies, and Arnold L. Demain (2010). *Manual of Industrial Microbiology and Biotechnology*. 3<sup>rd</sup> Edition, ASM Press.
2. Daniel Forciniti (2008). *Industrial Bioseparations: Principles and Practice*. 1<sup>st</sup> Edition, Wiley-Blackwell.
3. Reed. G. (1999). Prescott and Dunn's *Industrial Microbiology*. CBS Publishers.
4. Demain, A. L. (2001). *Industrial Microbiology and Biotechnology* IInd Edition. ASM Press, Washington.

**UNIT- I: 6 h**

**Introduction to Agricultural Microbiology:** Introduction, concepts and scope of agricultural microbiology, Agronomy and production of important crop plants. Interrelationships between plants and microorganisms, rhizosphere concept, quantitative and qualitative studies, R:S ratio rhizosphere; spermosphere; phyllosphere microorganisms; their importance in plant growth regulators. Microbial flora of healthy and diseased plants.

**UNIT- II: 8 h**

**Biological control of plant pathogens, Pests and weeds:** Factors predisposing plants to microbial infections- occurrence and conditions for spread of bacterial pathogens. Diagnosis of Plant Diseases –Infectious and Non-infectious diseases; Causative Agents of Plant Diseases – Viruses, Mollicutes, Bacteria, Fungi. Microbial control of crop pests and diseases; Bio-pesticides- Bacterial and fungal- genes involved, mode of action. Biocontrol agents for weed control.

**UNIT- III: 6 h**

**Soil Microbiology:** Soil microbes and soil fertility, microorganisms in soil processes carbon, nitrogen and sulphur cycle Nitrogen fixation: Biochemistry of Nitrogen fixation - mechanism of nitrogenase - hydrogenase - Assay of nitrogen fixation - physiology of legume root nodule, leghaemoglobin - Synthesis, Genes involved in photosynthesis and nitrogen fixation, Transformation- VAM.

**UNIT- IV: 8 h**

**Bio fertilizers:** Bacterial bio fertilizer: *Rhizobium*, *Azotobacter*- *Azospirillum*, *Acetobacter diazotrophicus* - phosphobacteria and Frankia. Algal fertilizer - Blue green algae, Azolla – Importance. Fungal fertilizers - Mycorrhizae – ecto and endomycorrhiza. Principles of mass production - Growth characteristics of different groups of organisms - Fermentation and other techniques. BSI standards and economics of biofertilizers.

**UNIT- V: 9 h**

**Plant tissue culture:** History and scope, totipotency and cell theory; growth and differentiation of plants; growth regulation of morphogenesis, somatic embryogenesis; application of tissue culture for plant improvement, micropropagation, embryo rescue, anther culture, germplasm conservation, production of secondary metabolites, **Biotechnology in agriculture:** the new green revolution, transgenic crops, gene protection technology, frost control technology, resistant varieties. Biotic and abiotic stresses. Reclamation of barren lands using microbial technology.

**Text Books:**

1. Iqbal Ahmad, Farah Ahmad, John Pichtel (2011). *Microbes and Microbial Technology: Agricultural and Environmental Applications*. 1<sup>st</sup> Edition, Springer.
2. Eldor A. Paul, (2007). *Soil Microbiology, Ecology and Biochemistry*. 3<sup>rd</sup> Edition: Academic Press.

**Suggested Reading:**

1. Eugene L. Madsen (2008). *Environmental Microbiology: From Genomes to Biogeochemistry*. 1<sup>st</sup> Edition, Wiley-Blackwell Publishing.
2. Agrios, G. N. (2000). *Plant pathology*. Harcourt Asia Pvt. Ltd.

3. Buchanan, B.B., Gruissem, W. and Jones, R.L (2000). *Biochemistry and Molecular Biology of Plants*. I.K. International Pvt. Ltd.
4. Bohra. J.S (2010). *Encyclopaedia of Agricultural Microbiology*. Anmol Publication.
6. Motsara, I.M.R., Bhattacharyya, P. and Srivastava, B. (1995). Bio fertilizer Technology, Marketing and usage-A source Book-cum- glossary- FDCO, New Delhi.

**MB 507 – ENVIRONMENTAL MICROBIOLOGY****3 CREDITS****UNIT-1** 5 h

**Microbial ecology:** Microorganisms in the tree of life: Description, taxonomy and diversity of major microbial taxa: Archaea, Algae, Fungi and protozoa. Interactions between microbes and organisms at other trophic levels: commensalism, mutualism, parasitism and predation with examples.

**UNIT-II** 8 h

**Microbial communities:** Communities in soil, water, air. Role of microbes in nutrient cycling: Nitrogen fixation, nitrogen cycling, ammonification, nitrification, denitrification. Extremophiles: Adaptation to extreme environments. Examples of microbial communities and sampling techniques.

**UNIT-III** 8 h

**Diverse roles of microbial communities in the environment:** Climate regulation: microbes and tooth decay, gut flora and food digestion in herbivores, biofilms and quorum sensing, fungal and bacterial endophytes in plants, role of protozoans to control eutrophication of water bodies.

**UNIT-IV** 10 h

**Microbes in waste and effluent treatment:** Bioremediation, sewage and waste treatment: solid and liquid industrial wastes, anaerobic digestion. Water: Water borne diseases. Water quality analysis. Waste water and water treatment, BOD, COD. Soil Composting. Microbes in metal extraction from soil, degradation of chemical pesticides and xenobiotics.

**UNIT-V** 5 h

**Microbes and energy:** Algal biofuels, artificial photosynthesis, benthic microbial fuel-cells. Examples of innovative techniques.

**TEXT BOOKS**

1. Raina M. Maier, Ian A. Pepper and Charles Gerba (2009) Environmental Microbiology (2<sup>nd</sup> edition). Academic Press. ISBN 978-0-12-370519-8.
2. Mohapatra P. K. Textbook of Environmental Microbiology.. IK International Pvt.Ltd. ISBN: 8190656672

**Suggested readings:**

1. Pepper IL, Gerba CP and Brusseau M (2006) Environmental and Pollution Science, Academic Press, USA
2. Atlas RM and Bartha R (1998) Microbial Ecology – Fundamentals and Applications, Addison, Wesley Longman Inc.

## Lab Courses for III Semester

**MB 551**

**GENETIC ENGINEERING LAB**

**1 Credit**

1. Plasmid DNA isolation and quantification
2. Preparation of competent cells
3. Transformation of plasmid DNA into *E.coli* cells (CaCl<sub>2</sub> mediated transformation)
4. Cloning of GFP
5. Analysis of cloned gene and agarose electrophoresis
5. PCR amplification of cloned gene
6. Restriction Fragment Length Polymorphism (RFLP) analysis
8. Bacterial Gene Expression (Using Lac promoter system)
9. RAPD Fingerprinting (Demonstration)

**MB 552**

**FOOD MICROBIOLOGY LAB**

**1 Credit**

1. Examination of microbial load in soft drinks, ice creams, packaged and canned foods.
2. Isolation and identification of food poisoning bacteria from contaminated foods, dairy products
3. Isolation and identification of important molds and yeast from food
4. Isolation of Aflatoxin producing organism, extraction and detection of afla-toxin from food or culture.
5. Production and estimation of lactic acid by *Lactobacillus* Sp. / *Streptococcus* Sp.
6. Rapid analytical techniques in food quality control using microbial Biosensors.
7. Role of microbes in fermented foods: Bread making, Sauerkraut.
8. Detection of number of bacteria in milk by standard plate count (SPC).
9. Determination of quality of milk sample by methylene blue reduction test.
10. Assessment of water quality by MPN technique.

- 1) Bioassay of nicotinic acid
- 2) Production of Ethanol by Yeast.
- 3) Isolation of enzyme producing microorganisms from soil
- 4) Production and extraction of thuricides.
- 5) Laboratory scale production of biofertilizers.
- 6) Production, quantification and extraction of Citric acid
- 7).Demonstration: Reactor Studies:** Batch, fed-batch, and continuous flow reactor analysis and residence time distribution.
  - i. Determination of Thermal Death Point (TDP) and Thermal Death Time (TDT) of microorganisms for design of a sterilizer.
  - ii. Monitoring of dissolved oxygen during aerobic fermentation.
  - iii. Preservation of industrially important bacteria by lyophilization.
  - iv. Product concentration by vacuum concentrator
  - v. Cell disruption for endoenzymes by sonication.
- 8) Demonstration: Down-stream Processing Lab** - Solid separation methods-filtration, sedimentation, centrifugation, product enrichment operations, precipitation, ultra filtration, two-phase aqueous extraction, high-resolution purification, preparative liquid chromatographic techniques, product crystallization and drying.

1. Collection, transport, storage and culturing of various clinical specimens for microbiological examinations.
2. Isolation and identification of respiratory tract bacterial pathogen – *Streptococci/ Klebsiella pneumoniae*.
4. Isolation and identification of gastrointestinal bacterial infection – *Salmonella / Shigella* spp.
5. Isolation and identification of UTI pathogens– *E. coli, Pseudomonas aeruginosa*
6. Laboratory examination of pus and skin specimens for *Staphylococcus aureus, Streptococcus pyogenes and Pseudomonas aeruginosa*.
7. Laboratory examination of sputum: Collection of sputum. Microbiological examination of sputum for pus cells and predominant bacteria. Ziehl-Neelsen staining to detect AFB culturing the specimen.
8. Fungal pathogens – *Candida albicans* and Dermatophytes (one)
9. Laboratory diagnosis of *E. histolytica, G. laniblia, Trypanosomas, Leishmania* and Plasmodium and common helminth infections. (Any two)