



**BHARATHIDASAN UNIVERSITY**  
**TIRUCHIRAPPALLI – 620 024**

**M. Phil. MICROBIOLOGY (FT / PT) PROGRAMME**

(for the candidates admitted from the academic year 2007 - 2008 onwards)

**Semester I**

|             | Title of the Course     | Marks |    |       | Credits |
|-------------|-------------------------|-------|----|-------|---------|
|             |                         | IA    | UE | Total |         |
| Course -I   | Research Methodology    | 25    | 75 | 100   | 4       |
| Course - II | Microbial Genomics      | 25    | 75 | 100   | 4       |
| Course- III | Microbial Biotechnology | 25    | 75 | 100   | 4       |

**Semester II**

|             |                                                  |             |    |     |   |
|-------------|--------------------------------------------------|-------------|----|-----|---|
| Course – IV | Elective (Any one)                               | 25          | 75 | 100 | 4 |
|             | 1. Microbial Biodiversity and Molecular Taxonomy |             |    |     |   |
|             | 2. Rhizoremediation Technology                   |             |    |     |   |
|             | Dissertation and Viva-Voce                       | 200(150+50) |    |     | 8 |
|             | Viva Voce 50 marks                               |             |    |     |   |
|             | Dissertation 150 marks                           |             |    |     |   |

**QUESTION PAPER PATTERN ( Course I – IV )**

Part - A: Two questions from each unit ( without choice). Each question carries 2 marks. (10 x 2 = 20)

Part – B: One “EITHER OR” questions from each unit Each question carries 5 marks ( 5 x5 = 25 ).

Part – C: One question from each unit. Each question carries 10 marks. The candidate has to answer three questions out of the five questions (3 x 10 = 30 )

## **M.Phil. Microbiology**

### **Semester - I**

#### **Course I – Research Methodology**

##### **Unit – I: Microscopy**

Light microscopy – Bright field, Dark field, Phase Contrast, Fluorescent and Polarization microscopes, Inverted microscope. Electron microscopy – TEM & SEM – principle structure and applications. Confocal microscope – Atomic force microscope– Micrometry – Microtomes - Microphotography.

##### **Unit – II: Analytical Instruments**

Basic principles, Beer lambert's law, Colorimetric methods, & instrumentation. Principles of spectroscopy, Single & double beam spectrophotometers, UV- visible, flame photometer, Atomic absorption spectrophotometer, NMR, Mass spectrometry. Application of Radioisotopes in biological sciences. Measurement of radioactivity – X –ray film, GM counter & scintillation counting methods.

##### **Unit – III: Separation techniques**

Centrifugation- principle, types, simple & differential, Ultra centrifugation – preparative and analytical. Chromatography – Principle and applications using paper, thin layer, column (gel filtration, ion exchange and affinity), GC & HPLC. Electrophoresis – Principle, types and applications - PAGE (proteins), Agarose (Nucleic acids), Pulse field Gel Electrophoresis, Two dimensional electrophoresis (IEF)

##### **Unit – IV: Research techniques in molecular biology.**

Isolation and quantification of genomic DNA, plasmids & proteins. Blotting & hybridization. Polymerase Chain Reaction – principles, types and applications, PCR based DNA finger printing, RAPD & STRR analysis. Restriction mapping. Cloning strategies, DNA sequencing – manual and automated methods.

##### **Unit – V: Biostatistics & Bioinformatics**

Presentation of Data - Measures of central tendency - Mean, Median Mode, Correlation coefficient, Standard deviation, student 't' chi-square test. Analysis of variance (ANNOVA) and its uses. Internet basics- world wide web (www) genbank sequence data bases – NCBI, EMBL, DDBJ – retrieving database entries. Sequence alignment and database searching – FASTA, BLAST. Phylogenetic analysis. Secondary and 3D structure prediction using DNA and protein sequences. Data processing and publishing. Guidelines in preparation of manuscripts & thesis.

## References:

1. Arora, P.N. & Malhon, P.K (1996). Biostatistics. Imalaya Publishing House, Mumbai.
2. Sokal & Rohif (1973). Introduction to biostatistics. Toppan & Co., Japan.
3. Baxevanis, A.D. & Ouellette, B.F.F. (2001). Bioinformatics: A practical guide to the analysis of genes and proteins – Wiley Inter science – New York.
4. Zar, J.H. (1996). Biostatistical Analysis. Prentice Hall, Upper Saddle River, New Jersey, USA.
5. John G Webster (2004). Bioinstrumentation .Student edition, John Wiley &sons, Ltd.
6. Keith Wilson& John Walker (2003) Practical Biochemistry Principles & techniques.5<sup>th</sup> edition, Cambridge university press.
7. Grumani N (2006) Research methodology for biological sciences.1st Edition , MJP Publishers, A unit of Tamilnadu Book House .
8. Palanivelu P (2001) Analytical biochemistry and separation Techniques A Laboratory maual. 2<sup>nd</sup> edition, Published by Tulsi Book Centre, Madurai, Tamilnadu.

## Semester - I

### Course II - Microbial Genomics

#### UNIT - I: Genome Mapping

Genome – size-complexity- structure and function of prokaryotic and eukaryotic genome. Physical mapping of genome-Sequencing whole genome- Restriction mapping – FISH – STS mapping - Hybridization assays - Physical mapping without cloning- Mapping by genetic techniques – DNA markers - RFLPs, SSLPs, SNPs – Linkage analysis – Cross breeding and pedigree analysis.

#### UNIT- II: Sequencing methods and Strategies

Basic DNA sequencing,-Modifications of chain-terminator sequences- Automated DNA sequencing- DNA sequencing by capillary array electrophoresis- shotgun sequencing – Overlapping clone contigs - High throughput sequencing- sequencing strategies- Alternative DNA sequencing – EST sequencing and sequence skimming.

#### UNIT - III: Genome Analysis

Overview of sequence analysis- Gene prediction- Tools for genome analysis. Detecting open-reading frames-using homology to find genes- software programs for finding genes- Identifying the function of a new gene- Analyses not based on homology-Genome annotation- Molecular phylogenetics.

#### UNIT- IV: Comparative Genomics

Comparative genomics of prokaryotes, organelles, Eukaryotes and other aspects. Representational difference Analysis of cDNA and Genome Comparisons-Gene Expression during Host-pathogen interactions- genomics of Mycobacterium tuberculosis- Helicobacter pylori-Approaches to bacterial mRNA extraction and labeling for microarray Analysis.

#### UNIT-V: Functional Genomics

DNA micro array – Construction and Design- Application of DNA micro array for comparative and Evolutionary Genomics. Gene silencing, RNAi, SiRNA, SHRNA-Proteome

analysis – Protein-protein Interactions. Application of Microbial Genomics – Reverse Vaccinology: from genome to vaccine- Microbial genomics for Antibiotic Target Discovery.

### References:

1. C. M. Fraser, T. D. Read and K. E. Nelson (Eds) Microbial Genomes, Humana Press, USA
2. Principles of Genome Analysis: A Guide to Mapping and Sequencing DNA from Different Organisms by S. B. Primrose (Paperback - Jan 1998)
3. Genome Mapping: A Practical Approach (Practical Approach Series) by Paul H. Dear , Medical Research Council Laboratory of Molecular Biology, Hills Road, Cambridge CB2.
4. Principles of Gene Manipulation and Genomics - Page xviii by Richard M. Twyman, Sandy Blackadder Primrose - Science - 2006 - 644 pages
5. Microbial Genome Methods by Kenneth W. Adolph (Hardcover - Oct 28, 1996)
6. Genome Mapping and Sequencing by Ian Dunham (Hardcover - Sep 1, 2003).
7. Brendan Wren (Editor), Nick Dorrell (2002) Functional Microbial Genomics (Volume 33) (Methods in Microbiology), Academic Press, UK.
8. Sandy B. Primrose Richard M. Twyman (2005) Principles of Genome Analysis and Genomics, Blackwell Publishing, USA.

## Semester - I

### Course III – Microbial Biotechnology

#### Unit – I: Historical development of Microbial technology

Introduction – Contribution of Louis Pasteur, Robert Koch, Alexander Fleming, S.A. Wakesman and others in the development of microbiology and the early discoveries. Industrially important microorganisms. Products obtained from microorganism. Isolation, purification and preservation of microbes. Cell culture techniques – aseptic transfer.

#### Unit – II: Microbes in Medicine

Clinically important microorganisms and their effects on infection and immunity. Production of toxins by microorganism. Disease caused by pathogens and their control. Production of medicinally important substances by microbes. Production of useful non-microbial products produced through recombinant microbes – insulin, vaccines, and antibiotics. Production of antibodies in *E. coli*.

#### Unit – III: Microbial Products and their bioprocesses

Single cell protein – *Chlorella*, *Spirulina*, Yeasts, Mushrooms – SCP from wastes. Economic implications of SCP. Microbial production of enzymes – cellulase, lipase, *Taq* polymerase, and restriction endonuclease. Production of wine, vinegar and alcohol. Biofertilizers – cyanobacteria, *Azospirillum*, VAM and *Azolla*. Strategies applied for drug discoveries.

#### Unit – IV: Biodegradation and Bioremediation

Microbes involved in biodegradation of organic wastes and xenobiotic compounds – heavy metals, pesticides, insecticides. Bioinsecticides – BT toxin. Microbial leaching – Extraction of metals from ores. Biofuels, Microbial hydrogen production. biodegradation of oils and petroleum products.

#### Unit – V: IPR, Biosafety and bioethics

World Trade Organization (WTO) with reference to biotechnology affairs – Basic requirement of patentability, process of patenting, patenting biological materials. National & International patent laws. Biosafety regulations and assessment of biotechnology products – drugs/vaccines & GMO. Biosafety protocols – Biological weapons. Principles of bioethics – ethical conflicts in biotechnology.

## References:

1. Raledge C and Kristiansen B Eds (2001) Basic Biotechnology, 2<sup>nd</sup> edition, Cambridge University Press.
2. Balasubramanian D, Bryce CFA, Dharmalingam K, Green J, Jayaraman K. (1996). Concepts in Biotechnology, University Press, India.
3. Baxevanis AD and BFF Ouellette, Wiley O. (ed) (2001) Bioinformatics – A practical guide to the analysis of genes and proteins. Interscience, New York,
4. Borowitzka MA, Borowitzka LJ (1989) Microalgal Biotechnology, Cambridge University Press.
5. Alan T. Bull. Microbial Diversity and Bioprospecting. ASM press. Washington, D.C
6. Brenden Wren and Nick Dorrell, Functional Microbial Genomics (Volume 33) (Methods in Microbiology), Academic Press
7. Alexander Hillisch and Rolf Hilgenfeld. Modern Methods of Drug Discovery, Birkhauser, Switzerland
8. Doolittle RF. (1990). Molecular evolution. Computer Analysis of Protein and Nucleic acid Sequences Methods in Enzymology. Academic Press, New York.
9. Gerbardt P, Murray RG, Wood WA , Kreig NR. (1994) Methods for General and Molecular Bacteriology – American Society for Microbiology Washington D.C.
10. Glick BR, Pasternak JJ (1998) Molecular Biotechnology - Principles and Applications of Recombinant DNA, ASM Press, Washington DC.
11. Higgins D, Taylor W. (2000). Bioinformatics, sequence, structure and databanks A practical approach. Oxford University Press.
12. Glazer AN, Nikaido H. (1994) Microbial Biotechnology – Fundamentals of Applied Microbiology WH Freeman and Company, New York.
13. Glick BR, Pasternak JJ. (1994) Molecular Biotechnology, ASM Press, Washington DC.
14. Miyamoto MM, Cracraft JL. Phylogenetic Analysis of DNA sequences. Oxford University Press. Oxford.
15. Pnolella P (1998) Introduction to Molecular Biology, WCB Mc Graw Hill, Boston, Massachusetts.

## Semester - II

### Course IV -1- Microbial Biodiversity and Molecular Taxonomy

#### Unit - I: Biodiversity

Prokaryotic and eukaryotic microbial diversity - bacteria, cyanobacteria, prochlorales, cyanelles, microalgae, microfungi, zooplankton & protozoans. Habitats, nutrition, ultrastructure and mode of reproduction. Isolation, cultivation and preservation of microorganisms.

#### Unit - II: Symbiosis

Microbial symbiosis, - bacterial - Rhizobium & Frankia. Cyanobacterial symbiosis with Bryophytes (*Anthoceros*), Pteridophytes (*Azolla*), Gymnosperms (*Cycas*), Angiosperms (*Gunnera*). Lichens, VAM. Structure, nutrition and mode of reproduction of symbiotic microorganisms.

#### Unit - III: Classification

Introduction, Haeckel's three kingdom concept. Whittaker's five kingdom concept. Three domain concept of Carl Woese. Classification of bacteria according to Bergey's manual of determinative bacteriology. Criteria for classification and identification of microorganisms - morphological, physiological & biochemical. Numerical taxonomy. Phage typing. Nomenclature - bacteriological code.

#### Unit - IV: Molecular Taxonomy

Introduction - need for molecular taxonomy. DNA finger printing - RFLP, Plasmid profiles, G+C content. Importance of 16S rRNA in taxonomy & phylogeny. PCR based finger printing - RT PCR, 16S rDNA amplification, cloning, transformation, DNA sequencing. RAPD, STRR & LTRR, Blotting and hybridization. DNA Microarrays/Chips.

#### Unit - V: Bioinformatics for genomics

Genome sequence comparison, alignment and data base searching. GenBank - NCBI, EMBL & DDBJ - retrieving sequences. Tools used for phylogenetic analysis - Ribosomal Database Project, FASTA, BLAST, Phylip. RNA structure prediction, Restriction enzyme patterns. Designing primers & probes. DNA barcoding. Submission of rDNA sequences for getting accession numbers - Bankit & Sequin guidelines.



## References:

1. Groombridge, B (Ed.) 1992. Global Biodiversity - Status of the Earth's Living Resources. Chapman & Hall, London.
2. UNEP, 1995, Global Biodiversity Assessment , Cambridge Univ. Press, Cambridge.
3. Virchow, D. 1998. Conservation & Genetic Resources , Springer - Verlag, Berlin.
4. Gary K.Meffe & .Ronald Carroll ,C.1994. Principles of Conservation Biology, Sinauer Associates, Inc., Massachusetts.
5. Danial Lim ,1998, Microbiology, McGrawHill Companies , New York.
6. Edward A. Birge ,1992, Modern Microbiology - Principles and application. Wm.C. Brown Publishers , Inc. U.S.A.
7. HH Rashidi & LK Buehler (2002). Bioinformatics Basics: Applications in Biological Science and Medicine, CRC Press, London
8. Gibas, C and P. Jambeck (2000). Developing bioinformatics Computer skills. Shroff Publishers and Distributors Pvt. Ltd., Calcutta
9. Brige EA (1992) Modern Microbiology, WmC, Brown Publishers, Dubugue, USA.
10. Bryant DA (1994) The Molecular Biology of Cyanobacteria, Kluwer Academic Publishers, London.
11. Gerherdt P, Murray RG, Wood WH. Kreig NR (1994) Methods for General and Molecular Bacteriology, American Society for Microbiology, Washington DC.
12. Landecker EM (1996) Fundamentals of Fungi -Prentice Hall International Inc.
13. Pelczar Jr. MJ, Chan ECS, Krieg NR (1993). Microbiology - Mc Graw Hill. Inc, New York.
14. Des Higgins & Willie Taylor (2002). Bioinformatics: Sequence, structure and databanks, Oxford University Press
15. Baxevanis AD & Ouellette BEF (2001) Bioinformatics: A practical guide to the analysis of genes and proteins, Wiley Interscience - New York

**Course IV -2- Rhizoremediation Technology**

**Unit - I: Rhizosphere**

Definition and characteristics features of the rhizosphere: Physical, chemical and biological processes. Methods of rhizosphere research related with sustainable in modern agriculture. Applications to environmental technologies, soil protection and remediation.

**Unit - II: Rhizosphere's microorganisms**

Distinguishing features; Soil Monera (Prokaryotic), Soil protista, Soil fungi and Viruses. Distinct attributes of procaryotes. Individual groups of soil microorganisms. Types of interactio; plant-microbe interactions, microbe-microbe interaction.

**Unit - III: Xenobiotic nature of rhizosphere**

Source and types of xenobiotic compounds, organisms involved in degradation of chlorinated hydrocarbons, substituted simple aromatic compounds, polyaromatic hydrocarbons, agrochemicals, surfactants and microbial treatment of soil pollution.

**Unit - IV: Bioremediation**

Definition and concepts bioremediation, biogeochemical cycles, Isolation and screening bioremediation's microbes, organic compound contaminants bioremediation, heavy metal and xenobiotic compounds bioremediation. Engineering and molecular biological techniques used in bioremediation.

**Unit-V: Bioinoculants**

Microbial association: Symbiosis, asymbiosis, associate symbiosis – bacteria; actinomycetes; BGA; mycorrhizae: Nitrogen fixers, phosphate solubilizers and mobilizers – application of biofertilizers in agriculture. Out line of biopesticide, bioinsecticides, bioherbicides and its application to the agriculture. Enhancement of novel microbes.

**Reference:**

1. Environmental Biotechnology by Alan Scragg; Longman.
2. An Introduction to Environmental Biotechnology by Milton Wainwright: Kluwer, Academic Press.
3. Environmental Biotechnology by C. F. Forster and D. A. J. Wase.
4. Principles & Applications of Soil Microbiology by M. Salvia David.
5. Bio-remediation Technologies, Technomic Publishing Co., USA. S.K. Sikdur & R.L. Irvine.
6. Environmental Bio-monitoring: The Biotechnology Ecotoxicology Interface, Cambridge University Press, James M., Lynch & Alan Wiseman.
7. Phytoremediation & Rhizoremediation: Springer; Mackova, Martina; Dowling, David; Macek, Tomas (eds).
8. Subbha Rao, N.S. Soil Microbiology. IV edition. 2000. Oxford and IBH Publishing Co. Pvt. Ltd.
9. Motsara, Bhattacharya, P Beena Srivastava. Biofertiliser Technology, Marketing and Usage. FDCO, New Delhi.
10. Gillings, M and Holmes, A. Plant Microbiology. 2004. BIOS Scientific Publishers London and New York.