DR. BABASAHEB AMBEDKAR MARATHWADA UNIVERSITY, AURANGABAD.

Syllabus

B.Sc. IIIrd Year (Microbiology)

[Semester-Vth & VIth]

[Effective from – June, 2011 & onwards]

B. Sc. [Microbiology] III Year
[Semester-V]
Paper- XVII Microbial Genetics

Unit -1:- **(15)**

- DNA/RNA as genetic material,
- Molecular properties of DNA – DNA melting, breathing, bending, flexibility, Novel structure of DNA, Linking number, role of major & minor groves in protein binding..
- History of molecular biology and genetic engineering
- DNA as genetic material- experimental proof- Griffith's experiment
  Hershey_Chase experiment
- RNA as genetic material – experimental proof
- DNA replication- models of replication, experimental evidence for semiconservative replication, enzymology of replication, mechanism of DNA replication
- Post replication modifications (methylation); role of restriction endonuclease, hsd, dam,dem system of methylation

Unit :2. **(15)**

- Salient features of Genetic code
- Transcription-structure of RNA polymerase, mechanism of transcription, post transcriptional modification.
- Translation- activation of amino acids, charging of t- RNA, Ribosomes, m-RNA, t-RNA
  a) initation
  b) elongation
  c) termination
- Regulation of gene expression at the level of transcription
  a) lac Operon

Bacterial Recombinations:

- Transformation-discovery, nature of transforming principle, competence factors, mechanism of transformation
- Conjugation-discovery, structure and properties of plasmids (F, R, Col.,) Plasmid incompatibility, process of conjugation-(F, HFr, and F mediated transfers)
- Transduction –mechanism of generalized and specialized transduction, Abortive transduction, lysogenic conversion.

Unit- 3 : Mutations (15)

- Spontaneous mutations (replica plating , fluctutation test)

  Base pair substitutions and frame shift mutations.

  Mutagenesis by Physical and chemical agents. -

  UVrays, Xrays , Base analogues, Agents modifying purines and pyrimidines, Nitrous acid, DMS, EMS, EES, Agents producing distortions in DNA -- Proflavin, Acridine orange , Intercalating agents (Ethidium bromide) .
- Suppressions – true reversion versus genetic suppression (intragenic and intergenic suppressions)
- Site directed mutagenesis

Paper –XVIII : Microbial metabolism

Unit :1 (18)

- **En**zymes : Definition, properties, specificity, active site, activation of enzymes, mechanism of action of enzymes (lock and key , induced fit, ping-pong)
- Nomenclature and classification of enzymes
- Factors affecting catalytic activity of enzymes (pH, temp.,enzyme conc., substrate conc., metal ions, time)
- Michaelis_Menten equation : derivation and significance
- Types of enzymes :extracellular, intracellular, constitutive and inducible.
- Enzyme inhibition : Irreversible ,reversible (competitive, uncompetitive , incompetitive) and metabolic antagonism, feedback inhibition.
- Elementary knowledge and uses of isoenzymes.
- Immobilized enzymes and allosteric enzymes.
- Commercial uses of enzymes (any five) – (food, leather, textile, environment, pharmaceuticals and clinical)
- Types of co-enzymes (NAD, FAD, Lipoic acid, VitB_{12}, Thiamine pyrophosphate) and reactions catalysed (atleast two)

Unit :2. (15)

Definitions: Metabolism, anabolism , catabolism, free energy.

- Bioenergetics: chemical links between catabolism and biosynthesis,energy coupling through ATP and through pyridine nucleotides, Central role of ATP-ADP system.
- Modes of energy yielding metabolism : Definition and features of fermentation, respiration and photosynthesis.
- Fermentation of carbohydrates:

  EMP, HMP, ED, Phospoketolase pathway (pentose ,hexose).

  Alcoholic, homolactic, mixed acid, butanediol, butyric, acetone-butanol fermentations.

- Aerobic respirations:
  RETC : location functions, components, redox carriers, oxidative phosphorylation artificial electron acceptors, bacterial cytochrome systems
- TCA cycle, glyoxylate cycle, anaplerotic sequences, regulation of TCA.

Unit : 3 (15)

- Catabolism of saturated (16 carbon) and unsaturated fatty acids (16 carbon) by β oxidation
- Degradation of proteins and amino acids : proteolysis, putrefaction.
- Transformation of aminoacids : oxidation, reduction, decarboxylation, deamination . (one example of each).
- Nucleic acid catabolism: DNA, RNA depolymerization, degradation of nitrogenous bases (mention end products without pathway)
- Biosynthesis of nucleotides: Purine and pyrimidine nucleotides, conversion of ribonucleotides to deoxyribonucleotides.
- Carbohydrate synthesis : peptidoglycan.

Practical paper XIX

1) Determination of one step growth curve of bacteriophage.
2) Replica plating for isolation of streptomycin resistance spontaneous mutant of *E. coli.*
3) Isolation of *lac* mutants of *E.coli*. (Lac) by UV induced mutagenesis and chemical mutagens.
4) UV damage and photoreactivation.
5) Study of transformation in *E. Coli*.,
   i) Preparation of competent *E. Coli*.
   ii) Enumeration of transformed cells.
   iii) Determination of plasmid transfer efficiency.
6) Study of conjugation in *E.Coli.* (plate method.)
7) Demonstration : Polymerase chain Reaction (PCR)

Practical Paper XX

1) Preparation of buffers and reagents.

2) Study of enzymes :- α-amylase, caseinase, catalase, deaminase, desulfurase, gelatinase, lecithinase, oxidase.

3) Effect of pH , temp, substrate concentration on α- amylase activity.

4) Demonstration of nitrate reduction

5) Demonstration of decarboxylation of amino acid.

6) Isolation of photosynthetic bacteria by column method

7) Primary screening for :

   i) Starch hydrolyzers.

   ii) Organic acid producers.

   iii) Antibiotic producers.

B.Sc. III Year [Semester-VI]

Paper- XXI Recombinant DNA Technology

Unit:1 (15)

- Recombinant DNA technology :definition, tools used for cloning, restriction endonucleases (types, nomenclature, recognition sequences, with examples).
- Modification of blunt ended DNA (T4 ligase, homopolymer tailing, linkers and adapters)
- Vectors : properties of good vector, cloning and expression vectors. (pBR322, pUC8, pSC101,) ,Bacteriophage vectors (λ phage,M 13 phage vectors), phagemid, cosmids, YAC /MAC.
- Genetic engineering – principles, cloning organisms, uptake of DNA (Calcium chloride treatment, electroporation, protoplast fusion, liposome), selection of recombinant clones.
- Genomic library (construction and identification of desired clone)

Unit :2. (15)

- Nucleic acid & protein blotting techniques : Southern blotting, western blotting, northern blotting.
- Colony hybridization
- DNA sequencing (Maxam &Gilbert)
- Probes (preparation &labeling) , its uses
- PCR

Unit :3 (15)

- Gene therapy
- **A**pplications of genetic engineering
  a) Agriculture
  b) Human and animal health
  c) Industries
  d) Environment
- Ethical issues of genetic engineering
- Transposition- Discovery, structure and types of bacterial transposons, mechanism of transposition, spread of antibiotic resistance, mutation due to transposition

Paper – XXII Industrial Microbiology

Unit :1. (15)

- Design of typical fermenter, types of fermenters (Single, multiple, recycle, airlift)
- Screening methods: primary, secondary.
- Strain improvement methods , increasing product yield.
- Preservation methods (lyophilization, freezing, mineral oil, soil stocks)
- Inoculum development
- Fermentation media : raw materials , media formulation, pretreatment, sterilization , contamination and its control, inoculum media, buffers, antifoam agents and precursors.
- Scale up of fermentation
- Phage contamination and control
- Down stream processing

Unit : 2 (15)

- Antibiotic fermentations : Penicillin.
- Vitamin fermentation : Vit. B-12
- Amino acid fermentation : L-lysine (direct and indirect)
- Organic Solvent : Ethyl alcohol fermentation
- Organic acid fermentation : Citric acid

Unit :3 (15)

- Enzymes- α – amylase (bacterial &fungal)
- Bakers yeast production
- Vaccines _ Genetic recombinant vaccines
- Biofertilizers – (Azo, Rhizo, and PSB)
- CH_4 fermentation
- Biopesticide production

B.Sc III year practical
Practical paper XXIII

1) Isolation of genomic DNA from *E. coli.*
   i) Purification of DNA by phenol extraction method.
   ii) Concentration of DNA by ethanol precipitation.
   iii) Separation of DNA using agarose gel electrophoresis.
2) Restriction analysis of *E. coli*.
3) Isolation of *E. coli* plasmid DNA
4) Separation of plasmid DNA by agarose gel electrophoresis.
5) Western blotting
6) SDS PAGE
7) Measurement of B-galactosidase activity using ONPG.

B.Sc III year practical
Practical paper XXIV

1) Production, detection and estimation of :

---------- Ethanol using *S cerevisiae* var, ellipsoideus.

---------- Glutamic acid by *Micrococcus glutamicus*

---------- Citric acid by *Aspergillus* spp.

---------- α-amylase using *Aspergillus sp/Bacillus sp.*

---------- Penicillin by *Pencillium* spp.

----------Biosurfectants using Pseudomonas spp.

2) Strain improvement (Physical/chemical agents) for α-amylase production using *Aspergillus sp/ Bacillus sp.*

3) Paper / TLC : Chromatographic separation and identification of fermented products. (amino acids, citric acid).

4) Separation of proteins using agarose gel electrophoresis.

5) Bioassay of Penicillin/ Vit B12

6) Study tour and report presentation.

Reference books for B.Sc.III year

1.**A.H.Rose:** Chemical Microbiology-An introduction to Microbial Physiology,Butterworth World student,LONDON.
2.Campbell Peter N. &Smith Anthony D.: Biochemistry illustrated,Churcill Livingstone ,NEW YORK.
3.Deb A.C.: Fundamentals of Biochemistry,New central Book Agency, CALCUTTA.
4.Lehninger Albert L.: Principals of Biochemistry,CBS Publishers,DELHI.
5. Lehninger Albert L.: Biochemistry,Kalyani Publishers,NEW DELHI.
6.Moat Albert G.&Foster John W.: Microbial Physiology,John Wiley & Sons,Inc.
7.Moat A.G.: Microbial Biochemistry.
8.Steinner R.F.: Life chemistry-An introduction to Biochemistry, D van Nostrand co.Inc. LONDON.
9.Stryer Lubert: Biochemistry,W.H.Freeman and Co.,SAN FRANCISCO.
10. T.Palmer: Understanding enzymes.
11. A.H.Patel: Industrial Microbiology,McMillan(India)Ltd.,BOMBAY.
12. Casida L.E.: Industrial Microbiology,Willey Estern Ltd.,NEW DELHI.
13. Prescott & Dumn: Industrial Microbiology,MacGrow HillCo.Ltd.
14. Bis Swanger Hans: Practical Enzymology,Wiley-VCH Verlag Gmbh & Co.
15. Chatwal Anand: Instrumental Methods-Chemical Analysis, Himalaya Publishing House.
16. Plummer David: An Introduction to Practical Biochemistry,Tata MacGrow HillBooks, Co. **17. Jayaraman J.:**Laboratory Manual in Biochemistry**,**New age ,International Publishers**.**
18. Tikekar P.G.: Practical Biochemistry for Medical Students,Purvi Pustak Kendra,BOMBAY.
19. Avinash & Kakoli Upadhay:MOLBIO,Himalaya Publications
20.Barry J.M.&Barry F.M.: Molicular Biology.
21.Freifelder David: Microbial Genetics, Jones & Bartlett,Publications.
22.Gardner Eldon,Simmon Michael&Snustad Oeter: Principals of genitics,John Wiley & sons,NEW YORK.
23.James D.Watson: Molicular Biology of the gene,W.A.Benjamin,Inc.
24.Joshi P.: Genetic engineering & its applications,Agrobious,JODHPUR(India).
25.Nilima Rajvaidya & D.Markendey: Genitical and Biochemical applications of Microbiology,APH publishing Co.NEW DELHI.
26.Singh B.D.: Biotechnology-Kalyani Publishers,DELHI.
27.Strickberger M.: Gemetics,prentice Hall of India Pvt.Ltd.,NEW DELHI.
28.Walker J.M.& Gingold A.D.: Molecular Biology & Biotechnology,Panima Publications,NEW DELHI.

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