UNIVERSITY OF CALCUTTA

SYLLABUS

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THREE-YEAR HONOURS
DEGREE COURSE OF STUDIES



MICROBIOLOGY

2010

Syllabus for Microbiology Honours

Part I

Paper I

Group A: Biomolecules

Unit I 1. Stereochemistry

2. Carbohydrates

3. Amino acids

Unit II 1. Protein

2. Nucleic Acids

3. Lipids

Group B: Biophysical Chemistry and Biometry

Unit I 1. Physicochemical properties of water

2. Thermodynamics

3. Microscopy

4. Radioactivity

Unit II 1. Spectroscopy

2. Biometry

Paper II

Group A: General Microbiology

Unit I 1. Development of microbiology

2. position of microbes

3. Staining

4. Morphology and subcellular structures

5. Bacterial cell wall biosynthesis and structure

Unit II

- 1. Eukaryotic microbes
- 2. Microbial nutrition
- 3. Microbial growth
- 4. Control of growth of microbes

Group B: Practical

Unit I

- 1. Operation of Light-Microscope; oil-immersion
 - objective
- 2. Preparation of culture,media,Cultivation of Microorganisms, Pure-culture, Staining techniques
- 3. Micrometry

Unit II

- 1. Qualitative tests of carbohydrates
- 2. Separation of aminoacids
- 3. Estimation of amino acid
- 4. Biometry

Detailed syllabus

Paper I

Group A: Biomolecules (50 marks)

UNIT I

1. Stereochemistry: (15)

General concepts on: Plane of symmetry, centre and axis of symmetry; Concepts of chirality; optical isomerism; geometrical isomerism; DL, RS nomenclature; Projection formula (Fischer & Howarth); Isomers: anomers, epimers.

Stereochemistry of cyclohexane: idea of axial & equatorial bonds (related to chair form conformation), Important chemical reactions relating to configurations, Mutarotation and its mechanism.

2. Carbohydrates: (15)

Definition, classification and structural concept of:

Monosaccharides: Hexoses (only Glucose), Pentoses (Ribose, Ribulose, Xylose)

Disaccharides: Sucrose, Lactose, Maltose Amino Sugars: Glucosamine, Muramic Acid

Inversion (hydrolysis) of cane sugar.

Chemical reactions of monosaccharides (glucose & fructose i.e., aldose and ketose) with HNO_3 , Br_2 - water, HIO_4 , Phenylhydrazine. Principle of chemical estimation of sugar. Anomeric effect (Methylation effect). Polysaccharides: Chemical structure of Starch (α -amylose, amylopectin), glycogen & cellulose. Smith degradation and enzymic hydrolosis of α -amylose & amylopectin.

3. Amino Acids: (10)

Definition, classification, structure, stereochemistry of amino acids; Physico-chemical properties (Ionization & Biuret reaction) of amino acids. Amphoteric molecule, Zwitterion, pK values; Isoelectric point, Electrophoresis, Formol titration of Glycine (only reaction & principle). Reaction with Ninhydrin, FDNB, Dansyl & Dabsyl chloride, Fluorescamine, Van-slykes reaction. Reactions of carboxyl & amino groups. Synthesis of Glycine. Separation of amino acids by-lon-exchange, Gel filtration, Paper Chromatography and Thin layer Chromatography. Peptides: peptide bond, biologically important peptides (glutathione, oxytocin-important functions).

UNIT II

1.Proteins (15): Classification (Primary, Secondary, Tertiary, Quaternary- definition, examples) Forces that stabilize structure of proteins: H-bonds, hydrophobic interaction, electrostatic attraction, Van der Waal's interaction, dipole-dipole interaction.

Types of proteins:

- i) Fibrous (α -helix, β sheet, e.g. collagen): definition and structure.
- ii) Globular (Haemoglobin, Myoglobin): definition & examples.
- iii) Simple proteins and conjugated protein: definition & examples—physical denaturation and renaturation

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2. Nucleic acid: (15)

Purine, pyrimidine - definition and structure. Nucleoside, nucleotide: definition and structure. DNA & RNA: Double helical structure. A-DNA, B-DNA & Z-DNA (structure and differences). Chemical Properties: Hydrolysis (acid, alkali), enzymatic hydrolysis of DNA. General structure and types of RNA (tRNA, mRNA, rRNA). Viscosity, Buoyant density. Hyperchromic effect. Cot curve, T_m.

3.Lipids (10)

Definition, nomenclature, classification - (simple, complex, derived lipids - structure & example) phospholipids, glycolipids, - (structure, composition); hydrolysis, saponification, saponification number, I_2 number, acetylation, acetyl number, volatile fatty acid number - definition and related problems, Isomerism - cis-trans isomerism.

Fatty acids: Saturated (palmitic acid, stearic acid), unsaturated (oleic acid): Structure of free fatty acids (example only). General chemical reaction of fatty acids - esterification. Hydrogenation and halogenations

Suggested textbooks:

1. Finar, IL. Organic Chemistry, Part I and Part II.

Group B

Biophysical Chemistry and Biometry (50 marks)

UNIT I

1. Physico-chemical properties of water: (10)

lonic product of water; pH - definition, effect of pH in enzyme catalyzed reaction. Acids, bases and buffers in biological system; Arrhenius, Bronsted-Lowry theories of acid and bases. Polyprotic acids, ampholytes, dissociation of polyprotic acid; titrable and true acidity. Surface tension, viscosity: application to biomolecules.

2. Thermodynamics: (15)

Zero-th law, 1st law & 2nd law of thermodynamics: application in biological systems, Concept of free energy, standard free energy change. Equilibrium constant; enthalpy; entropy. Transport across membrane - passive diffusion, facilitated diffusion & active transport - (definition and examples); gradient of chemical potential as driving force in transport, equilibria and transport across membranes; diffusion, osmosis, sedimentation, osmotic pressure. Donnan equilibrium, diffusion potential, membrane potential. Electrophoresis.

3. Microscopy: (5)

General principles of optics in relation to microscopy; different components of light wave (UV, IR, visible); principles and applications of Compound Microscope; Light Microscope; Dark field Microscope; Bright field Microscope; Phase Contrast Microscope; Fluorescent Microscope; Electron Microscope; Resolving power; Numerical aperture:Chromatic Aberration.

4. Fundamentals of radioactivity: (10)

Law of Radioactivity, Decay constant, half life, average life. Properties of α , β , γ radiations, unit of radioactivity, radioactive carbon dating. Applications of radioactive isotopes (14 C, 3 H, 32 P) in biological systems, (in Radioimmunoassay, glucose metabolism, DNA synthesis). Principle of liquid scintillation counter. Radiation absorption- biological effectiveness- Linear energy transfer- radiation protection and safety aspects.

UNIT II

1. Spectrophotometry: (15)

Concept of electromagnetic radiations - UV, visible, IR.

Orbital theory: Bonding and antibonding; simple association of 1t orbital to form TT orbitals. Concept of chromophore - Wit's chromophore theory, auxochorome, red shift, blue shift, Lambert-Beer's law - derivation & deviation; absorptivity, line diagram & working principle of spectophotometer. Extinction co-officient. Instrumentation and application of UV and visible spectrophotometry, Light Scattering, fluorescence spectroscopy, fluorescence energy transfer, infrared spectroscopy.

2. Biometry: (15)

Introduction: Types of Biological Data, Population and samples.

Descriptions of Samples and Populations: Frequency Distributions, Descriptive statistics (measures of Central tendency and measures of Dispersion, Boxplot)

Probability: Introductory concepts, Binomial distribution, Random sampling

Distribution Theory: Normal distribution and sampling distributions.

Statistical Inference: Statistical estimation, standard error of the mean, confidence interval and hypothesis testing of the population mean -t test. Brief discussions on the comparison of two independent population means. The Chi square test and its applications.

Analysis of Variance: Multi sample Hypotheses

Linear Regression and Correlation.

Suggested text books:

- 1. Tinoco, Sauer and Wang, *Physical Chemistry, Principles and Applications in Biological Sciences*, Prentice Hall, 4th Edition (2001).
- 2. Zar, JH., Biostatistical analysis, Pearson Education.
- 3. Atkins, *Physical Chemistry for the Life Sciences*, W.H. Freeman (2005).
- 4. Atkins, *The Elements of Physical Chemistry*, W.H. Freeman, 3rd Edition (2000)
- 5. Cantor & Schimmel, *Biophysical Chemistry*, Part I, II, III, Freeman Press (1980-1981).

- 6. Eisenberg & Crothers, *Physical Chemistry with Applications to the Life Sciences*, Benjamin/Cummings Publishing Co. (1979).
- 7. K. E. van Holde, W. C. Johnson, and P.S. Ho, *Principles of Physical Biochemistry* (1998).
- 8. P. Atkins and J Paula, *Physical Chemistry for the Life Sciences* (2006).
- 9. C.R. Cantor and P.R.Schimmel, Biophysical Chemistry (1980), Part I: The Conformation of Biological Macromolecules, Part II: Techniques for the Study of Biological Structure and Function.
- 10. Forthofer, RN., Lee, ES. (1995) Introduction to Biostatistics: A guide to design, analysis and discovery, Academic Press.

Paper II

Group A: General Microbiology (50 marks)

UNIT I

- 1. Notable contributions in the development of Microbiology: (3)
- Spontaneous generation (abiogenesis).
- ii) Biogenesis.
- iii) Germ Theory of Disease.
- iv) Koch's Postulates.
- v) Scope of Microbiology.

2. Position of microorganisms in biological world: (4)

Whittaker's Five-kingdom and three-kingdom concept of living organisms (General characteristics of those groups); General features of Eubacteria and Archaebacteria (major difference within Eubacteria).

3. Stains & Staining techniques: (10)

Definition of auxochrome; Chromophores; Acidic and Basic dyes; Classification of stains; Simple and differential staining: theories of staining, mordant and its function; Gram staining; acid fast staining; endospore staining; negative staining; capsule staining; flagella staining; mechanism of gram staining.

4. Bacterial Morphology and subcellular structures: (18)

Morphology of bacteria, Slime layer, Mycelial morphology: Actinomycetes, Capsule, Cell wall, Ribosome, Cytoplasmic membrane (Fluid mosaic model of Singer - Nicholson); Cytoplasmic inclusion bodies - (inorganic, organic); Exospores & Cysts: types & structure; Endospore, Flagella, Pilus, Fimbriae (structure, composition and functions). Plasmids and episomes. Nuclear material, Bacterial Chromosome (Fundamental differences with eukaryotic chromosome).

5. Bacterial cell wall biosynthesis and structure(5)

UNIT II

1. Eukaryotic microbes: (10)

General characteristics, vegetative & reproductive structure of the following groups of microorganisms:

Algae: Cyanophyta, Chlorophyta, Bacillariophyta, Phacophyta, Rhodophyta, Fungi: Phycomycetes, Asocomycetes, Basidiomycetes, Deuteromycetes; Protozoa: Giardia, Plasmodium & Entamoeba.

2. Microbial Nutrition: (10)

Nutritional types (definition and example) - Photoautotrophs, Photoorganotrophs, Chemolithotrophs (ammonia, nitrite, sulfur, hydrogen, iron oxidizing bacteria); Chemoorganotrophs, Effect of oxygen on growth - classification on the basis of oxygen requirement and tolerance.

3. Bacterial Growth: (5)

Growth phases - Generation time. Kinetics of growth, Batch culture. Continuous culture. Synchronous culture (definition and brief description). Physical factors influencing growth - Temperature. pH, osmotic pressure, salt concentration.

4. Control of growth of Microbes: (15)

Sterilization, disinfection, antiseptic, sanitizer, germicide, antimicrobial agent (definition, application & examples); physical method of disinfection and sterilization - dry heat, moist heat, filtration, radiation (mode of action, applications); Chemical control – dye solutions, alcohol, acid, alkali, halogen, heavy metal, phenol, phenol derivatives,

formaldehyde, ethylene oxide, detergents (mode of action, applications). Assessment of chemical disinfectant; phenol coefficient-definition and method of determination.

Chemotherapeutic agents - sulphonamides, antibiotics, (definition types); mechanism of action and antimicrobial spectrum of penicillin, streptomycin, tetracycline, chloramphenicol, Nalidixic acid and metronidazole; drug resistance - phenomena and mechanism.

Suggested textbooks:

- 1. Stanier, RY., et al., General Microbiology, 5th ed. Macmillan Press.
- 2. Pelczar. M., et al., Microbiology, 5th ed.,2000, Tata-McGraw Hill
- 3. Atlas. RM., Principles of Microbiology, 2nd ed., 1997, McGraw-Hill
- 4. Salle, AJ., Fundamental Principles of Bacteriology, 7th ed.,1999, Tata- McGraw Hill
- 5. Prescott, LM., Microbiology, 6th ed. 2005, McGraw-Hill.

Group B : Practical (50 marks)

Unit I

- **1. Operation of Light-Microscope**; use of oil-immersion objective. (5)
- 2. Preparation of culture media: (10)

Complex media (Nutrient Broth; NA slant; Lactose broth); Chemically defined, Synthetic media (Czapekdox broth / agar). YPD / select media which will be used for the experiments specified.

- a) **Cultivation of Microorganisms**: on agar-slant/agar-plate streak culture:Bacteria (*Bacillus subtilis, Staphylococcus aureus, Escherichia coli*); Yeast (*Saccharomyces cerivisiae*) Moulds (*Penicillium notatum, Aspergillus niger*).
- b) **Pure-culture**: by streak-plate/pour plate methods (12)
- c) **Staining techniques** for examination of microorganisms :(15)
- i) Bacteria-preparation of heat-fixed smear and (a) Simple straining (*E.coli, Bacillus subtilis, Staphylococcus aureus*) (b) Gram-staining-Gram positive (*B. subtilis, S. aureus, M.lutea*); Gram-negative (*E. coli, K. aerogenes*) (c) Capsule staining (*K. aerogenes, K. pneumoniae*) (d) Endospore st.aining (*B. subtilis*).
- ii) Fungi-Lactophenol Cotton blue staining of Yeast (Saccharomyces cerevisiae) :

Molds (Penicillium notatum, Aspergillus niger).

3. Micrometry: (5)

Microscopic measurement of bacterial cell (*B.subtilis*) yeast (*Saccharomyces cerevisiae*), fungal spores (*P. notatum, A. niger*).

4. Enumeration of microbes: Yeast by Haemocytometer. (5)

Unit II

1. Qualitative tests of carbohydrates: (6)

Glucose, fructose (Benedict's Test); sucrose (Acid hydrolysis & Benedict's Test); proteins (Biuret method); lipids (TLC & detection by Iodine vapor).

- **2. Separation of aminoacids** (Lysine, glycine, tryptophan, proline) by Thin Layer Chromatography. (6)
- 3. Estimation of amino acid (glycine) by formol titration. (4)

4. Biometry: (10)

Hypothesis Test: *N*: testing Goodness of Fit and Contingency: t- Test for analysis of experimental samples. Study of Poisson Distribution of Microbes in a given sample (e.g. bacteria in a sample of water collected from a reservoir) - using Hacmocytometer.

PART II

Paper III

Group A: Cellular and Molecular Biology

Unit I 1.DNA Replication

2.Transcription in prokaryotes

3. Mechanism of translation in prokaryotes:

Unit II 1. Eukaryotic cell biology

Group B: Metabolism and Bioenergetics

Unit I 1.Enzymes

2. Amino acid metabolism

Unit II 1.Carbohydrate metabolism:

2. Purine and Pyrimidine metabolism (10)

3. Lipid metabolism

Paper IV

Group A: Environmental and Food Microbiology

Unit I 1. Air Microbiology

2. water Microbiology 3.Food Microbiology

4. Preservation of food

5. Microbiologically Fermented food

Unit II 1. Soil Microbiology

2. Biogeochemical cycles

Group B: Practical

Unit I 1.Enumeration of microbes

2.Growth curve

3.Assay of antibiotics

4.MIC

Unit II 1.Water microbiology

2. Milk microbiology

3.Phenol coefficient

DETAILED SYLLABUS

Paper III

Group A: Cellular and Molecular Biology

Unit I

1. DNA Replication: (10)

DNA-Replication-Meselson-Stahl experiment as evidence for semiconservative replication; Mechanism of replication-Rolling-circle model & Theta (8) structure (bidirectional)

2. Transcription in prokaryotes: (15)

Mechanisms (Initiation, elongation, termination); promoter structures, subunits of bacterial polymerases, functions and domains responsible for activity, elongation process, mechanism of termination, □-dependent and independent termination; *lac, trp, ara* operons.

3. Mechanism of translation in prokaryotes: (15)

Description of ribosomal cycle including phenomena of initiation, elongation, termination; description of factors involved in these processes; genetic code; tRNA: clover-leaf structure & function; rRNA: structure and function; role of aminoacyl tRNA synthetases. Non-ribosomal peptide synthesis: cyclic peptide antibiotics e.g. Gramicidin etc.

Unit II

1.Eukaryotic cell biology: (35)

Eukaryotic Cell Membrane, Difference in membrane constituents between eukaryotes and prokaryotes: target of antimicrobial drugs; Elementary idea of intracellular organelles, Transport across membrane - (Active, Passive, Facilitated), Comparison between eukaryotic and prokaryotic flagella and cilia; Microfibrils in protozoan physiology; Mitochondria vs kinetoplasts (found in unicellular protozoan parasite)

Cell Biology of Yeast: Budding and fission yeasts, mating types and its determination (only elementary ideas) Mitosis & Meiosis, Cell cycle; Secretory pathway in yeasts (preliminary ideas about Sec mutants, transport of materials to the bud. Secretion in bacteria (3 types of secretion, Sec proteins, secretory metabolites)

Protein degradation: prokaryotic and eukaryotic-only elementary concepts (GroE, GroEL etc vs proteosomal system)

Suggested textbooks:

Stryer. L. et al. Biochemistry, 5th edition, WH Freeman, 2006.

Voet, D., and Voet JG. Biochemistry, Wiley, 1995.

Alberts, B., et al. Molecular Biology of the Cell, Garland, 4th ed., 2002

Lodish, H., et al. Molecular Cell Biology, WH Freeman, 2003.

Group B: Metabolism and Bioenergetics

Unit I

1.Enzymes: General properties, Nomenclature and classification; Co-factors definition and function with special reference to the representative substances - a) Co-enzymes (NAD+, NADP+, Co-enzyme-A, TPP, Pyridoxal phosphate); b) Prosthetic groups (FAD+ - Succinic dehydrogenase); c) Metal ions: Zn²⁺, Mg²⁺, Fe²⁺, Fe³⁺, Mn²⁺ - required for enzyme action

Enzyme Kinetics - Michaelis-Menten equation; Enzyme Inhibition - Competitive-cite succinate on Malonate dehydrogenase as example, Non-competitive - Cite Iodoacetamide on triose phospate dehydrogenase and EDTA as example; Suicide inactivation-action of penicillin on bacterial cell wall biosynthesis as an example; Regulatory enzymes-Allosteric - Cite CTP on aspartate transcarbamylase as example; Feedback inhibition - Cite Threonine to Isoleucine as example; Ribozyme (catalytic RNA) and Abzyme (use of antibody as enzyme) - definition only

2.Amino acid metabolism (10)

Transamination, deamination, transmethylation and decarboxylation. Glucogenic and ketogenic amino acids, Outline of Urea Cycle; Microbial metabolism of glycine, phenylalanine and lysine

Unit II

1.Carbohydrate metabolism: (25)

Aerobic respiration-Glycolysis (EMP-pathway) with energy production: entry of galactose & fructose in EMP-path; TCA-cycle with energy production: pentose-phosphate pathway: Electron Transport Chain (in brief) & ATP generation sites; ATP & ADP cycle (oxidation-reduction potential and electromotive force). Photophosphorylation, oxidative

phosphorylation (chemiosmotic theory); Anaerobic respiration - Utilizing NO₂, Sulfur (SO₄), CO₂ as electron acceptors; Stickland-reaction; Entner-Doudoroff pathway Fermentation - Glucose metabolism in anaerobic condition general concept only Bacterial photosynthesis (Cyanobacteria and Green-sulphur bacteria); Difference with eukaryotic photosynthesis

2. Purine and Pyrimidine metabolism (10)

Synthesis of purines: elementary concept, source of the precursors of purines, ribose 5-phosphate; synthesis of AMP and GMP from IMP-only preliminary idea; Importance of folic acid and target of sulfonamides; Microbial reduction of purines to deoxy-purines: thioredoxine; Biosynthesis of pyrimidines: Aspartate transcarbamoylase (ATCase); Origin of Thymine: importance of folic acid (conceptual); Degradation of nucleotides: xanthines, uric acid; catabolites of pyrimidines: NAD and Coenzyme A (only elementary ideas)

3.Lipid metabolism (5)

Detailed account for oxidation of even-and odd-carbon numbered, saturated and unsaturated fatty acids; Brief idea of fatty acid biosynthesis; Metabolism of Triglycerides and phospholipids

Suggested textbooks:

Biochemistry—D.Voet and JG Voet

Biochemistry—L.Stryer

Principles of Enzymology—T.Palmer

Paper IV

Group A: Environmental and Food Microbiology

Unit I

1.Air Microbiology: (5)

Different types of microorganisms in the air, aerosols, sampling techniques, airborne pathogens, techniques of room sterilization.

2. Microbiology of water: (10)

Microbiological analysis of water (total count, indicative organism), B.O.D. & C.O.D. - determination and implication. Coliform test - detection of faecal and non-faecal coliform); IMViC test; determination of MPN microbiological treatment of sewage and industrial waste water. Anaerobic Treatment (safety tank).

3. Food Microbiology: (15)

Milk as a growth medium of bacteria, Normal microflora in milk, undesirable microbes in milk and normal microflora of meat, poultry, eggs, fruits and vegetable; Fresh food, Fresh milk, canned food and stored grains; Microscopic examination and Culture, phosphatase test of pasteurized milk.

4.Preservation of food (5): High temperature (Boiling, Pasterurization. Appertization) Low temperature (Freezing): Dehydration. Osmotic Pressure.

Chemical Preservations. Radiation.

5.Microbiologically Fermented food (5): Curd. Cheese. Idli. Yogurt. Acidophilic Milk Microorganisms as food-SCP: Food borne diseases - Salmonellosis. shigellosis.

Unit II

1. Soil Microbiology: (25)

Physical and chemical characteristics of various soil types-different microbial groups in soil, method of study,. Rhizosphere, Phyllosphere. Brief account of microbial interactions - (symbiosis, neutralism, commensalism, competition, ammensalism, synergism, parasitism, and predation); Biological nitrogen fixation - symbiotic and asymbiotic; Root - nodule formation in legumes; Compost and Biofertilizers, Biological Pest control. Plant disease (brown spot of rice, black stem rust of wheat, stem rot of jute, rice disease by Tungo virus, grey blight of tea,_red rot of sugarcane, TMV, Blast of rice, leaf blight of potato. Powdery mildew of cucurbit) dissemination and control.;

2.Biogeochemical cycles (15)- Carbon, Nitrogen, Phosphorus, and Sulphur Cycles – role of micro organisms in the process;: Microbiology of methane production

Suggested textbooks:

Salle, AJ. Fundamental Principles of Bacteriology, 7th Ed., Tata-McGraw Hill Publishing Co.

Subba Rao, NS. Soil Microbiology, 4th Ed., Oxford & IBH Publishing Co. Pvt. Ltd.

Dubey, R.C., and Maheswari, D.K. Textbook of Microbiology, S. Chand & Co.

Group B: Practical

Unit I

- 1.Enumeration of microbes- Isolation of pure culture from natural sources
- (a) Bacteria from soil-by serial dilution and pour-plate/spread plate method. (b) Yeast from rotten banana or apple-by the method same as (a), (c) Molds from infected citrus fruits-by streak-plate method, (d) Microbes from air-by agar-plate exposure method.

2.Growth curve

- **3.Assay of antibiotics-**. Microbiological assay of antibiotics: Antibiotic sensitivity test by paper disc and by Cup-Plate method
- **4. MIC-** ii) Determination of Minimal Inhibitory Concentration (MIC) by serial dilution method for assaying commonly used antibiotics (using appropriate test bacteria). (36)

Unit II

- **1.Water microbiology-** i) Microbiological examination of water: (Drinking water, Supply water, Pond water)
 - a) Presumptive test
 - b) Confirmatory test
 - c) Completed test: for coliform
- ii) IMViC reactions.

2. Milk microbiology

Microbiological examination of milk: By Methylene-blue dye reduction test;

3.Phenol coefficient- Determination of Phenol-coefficient (Dettol), Test organism to be used: E. coli.

PART III

Paper V

Group A: Microbial Genetics

Unit I 1.Structure: DNA, gene and chromosome:

2. Genetic exchange

Unit II 1. Mutation and Repair

2.Recombination

Group B: Industrial Microbiology and Recombinant DNA Technology

Unit I 1.Industrial microbiology

2.General method of preservation of industrially important

culture strains

Unit II 1.Recombinant DNA Technology

Paper VI

Group A: Medical Microbiology and Virology

Unit I 1.Normal Microbial Flora (normal) of human body

2. Mechanism of Bacterial Pathogenicity

3.Antimicrobial Therapy

4.Common Microbial Diseases

Unit II 1. Virology

Group B: Immunology

- Unit I. 1.Introduction: overview of the Immune system.
 - 2.Cells and organs of Immune system
 - 3. Types of Immunity
 - 4.Antigens
- Unit II
- 1. Immunoglobulins
- 2. Antigen Antibody interactions
- 3. Complement
- 4. Hypersensitivity: definition, types, examples.
- 5. Vaccines

Paper VII

Practical

Unit I

- 1. **Isolation and characterization** of one industrially important enzyme, immobilization of cells..
- 2. **Determination of Km, V_{max} and pH optima**, effect of activator, inhibitor of alkaline phosphatase

Unit II

- 1. **Protein estimation** by Lowry method
- 2. Absorption spectra of DNA and protein, hyperchromic shift f DNA
- 3. Phage titration

Paper VIII

Practical

Unit I

- 1. Antigen-Antibody reaction -
- 2. Restriction digestion of plasmid DNA.

Unit II

- 1. **Isolation of plasmid-DNA (**E.coli DH 5α) by using a standard method of any authentic teaching kit: Gel-electrophoresis (Agarose-gel), quantification of DNA and purity.
- 2. Transformation of *E. coli* by using plasmid DNA by CaCl₂ method.
- 3. Conjugation experiments using any standard teaching kit.

Detailed Course

Paper V

Group A: Microbial Genetics (50 marks)

UNIT I

1. Structure: DNA, gene and chromosome: (20)

Experimental evidence for DNA as genetic material (Experiments of Griffith, Avery and MacLeod; Hershey and Chase); Experimental evidence for RNA as genetic material (TMV).

Nucleic Acid structure: DNA double helix: crystallographic proof, alternative forms of DNA, intercalating agents, secondary and tertiary structure of RNA.

Structure prokaryotics gene; genomic organization in prokaryotes (nucleoid, DNA supercoiling, topoisomerases), Extrachromosomal inheritance: Plasmids (genes found, copy number, compatibility). Episomes.

Structure of eucakryotic genes (description and experimental proofs), multigene family. Genome organization (ARS, centromere, telomere, chromatin structure), various forms of repetitive DNA (satellite, LINEs and SINEs), psedogenes. Extrachromosomal inheritance (mitochondria and plastids)

2. Genetic exchange: (15)

Transformation, Conjugation, Hfr bacteria and chromosome mapping. Transduction-generalized (P1) and specialized (lambda-phage).

Transposable elements: Bacterial Transposons.

UNIT II

1. Mutation and Repair (20)

Spontaenous (Spontaneous mutation Luria - Delbruck's Fluctuation Test) and induced mutations, Mutagenic agents - Physical, Chemical and Biological (Phage-mu). Genetic Techniques to detect mutations in bacteria and fungi (isolation and characterization of nutritional auxotrophic mutation); Different forms of mutations and how they arise (tautomeric shift, base analog, alkylating agent, apurinic lesions, UV radiation and

thymine dimers, replicational error); Ames test is used the assess the mutagenecity of compounds.

Repair: reversal of UV damage in prokaryotes: photoreactivation, base excision and nucleotide excision repair, post replicational repair, mismatch repair, SOS repair, error prone repair.

2. Recombination (10)

Homologous recombination (Holiday structure : RecBCD system); gene conversion; site specific recombination (lambda)

Suggested textbooks:

- 1. Molecular Biology of the Gene (5th edition): By James D Watson et al
- 2. Concept of Genetics (6th edition): By Klug and Cummings
- 3. Genetics: Principles and Analysis (4th edition): By Hartl and Jones
- 4. Genetics: Analysis and Principles: By Robert J Brooker

Group B: Industrial Microbiology and Recombinant DNA Technology (50 marks)

UNIT I

1.Industrial microbiology: (25)

Microbial culture selection by screening method with reference to the Antibiotic and Enzyme production. Strain improvement, equipment and instrumentation (fermenters-General description of different types - Stirred Tank, Bubble column, Air Lift, Packed-bed Bioreactor)

Fermentation - static, submerged, agitated, solid phase, batch, feed-batch, continuous. Use of Immobilized cells and enzymes (Ca-alginate beads: polyacrylamide, micro-film)-definition and general characteristics. Industrial production of (using most common and low-cost raw materials) Ethyl Alcohol, Acetic Acid, Penicillin, Vitamin B12' Lysine, a. -amylase (inoculum building, fermentation, separation, assay and purification of products-general discussion). Concept of Primary and Secondary metabolites in Microorganisms.

2.General method of preservation of industrially important culture strains: (5)

UNIT II

1.Recombinant DNA Technology: (35)

Isolation & purification of nucleic acids & protein, RFLP, RAPD, Finger printing, Southern blotting, Dot blotting. Northern blotting. Western blotting - techniques.

Cloning vectors (pBR. 322, pUC8I9. YACs). Cloning. pCR techniques. Ti plasmid as transformation vector.

Construction of DNA libraries (Basic ideas and outlines of methods). Restriction and Modification enzymes: Enzymes used in Recombinant DNA techniques: DNA ligase. Polynucleotide Kinase. DNA Polymerase. etc.

Over Expression of Recombinant Proteins in Bacteria: Insulin, Human Growth Hormone, FSH.

Suggested textbooks

Industrial Microbiology—Presscott & Dunn
Industrial Microbiology—H. Patel
Indutrial Microbiology—L.E.J.R.Casida
Cell and Molecular Biology---J E..Darnell, H. Lodish, D.Baltimore,
The Cell --- De.Robertics
Principle of Gene Manipulation—Bob Old and S B Primrose

Paper VI

Group A: Medical Microbiology and Virology (50 marks)

UNIT I

1. Normal Microbial Flora (normal) of human body: (5)

Thoracic, Abdominal, Urogenital & Skin.

2.Mechanism of Bacterial Pathogenicity: (15)

Entry, colonization, growth, mechanism of damage of host cell. Production of endo-and exo-toxins - definition and general properties. (a) Neurotoxin: exotoxin & toxoid,

botulinum toxin, tetanus toxin; (b) Enterotoxin: Cholera toxin, Salmonella toxin, Klebsiella toxin. (c) Cytotoxin: Shigella toxin, Diphtheria toxin.

3.Antimicrobial Therapy: (15)

General properties of antibacterial agents (inhibitors of cell wall synthesis, disruptors of cell membranes, inhibitors of protein synthesis, inhibitors of nucleic acid synthesis and antimetabolites), antifungal agents, antiviral agents, antiprotozoan and antihelminthic agents: selective toxicity, spectrum of activity, modes of action, side effects, resistance of microorganisms. Gene Therapy: Definition and outlines of different methods.

4.Common Microbial Diseases: (15)

(Names of pathogens, disease symptoms, preventive measures and vector control where applicable).

- i) Bacterial Tuberculosis, Leprosy, Tetanus, Cholera, Gonorrhea, Anthrax, Typhoid
- ii) Viral Flu, Polio, AIDS.
- iii) Fungal Candidiasis.
- iv) Protozoan -Malaria, Amoebiasis and leishmaniasis

UNIT II

1.Virology: (30)

General characteristics of viruses: What are viruses? Difference between bacteria and viruses, Components of viruses, sizes and shapes of different viruses (describe with at least one example), host range and specificity

Classification of viruses based on the nucleic acid content: DNA (dsDNA, ssDNA) and RNA (ssRNA, dsRNA) viruses with examples

Human cancer viruses (SV40, HTLV - 1 & 2, Epstein-Barr virus only)

Virus like agents: viroids; prions;

Viral replication: General characteristics of replication, Replication of T4 phage, Phage growth and the estimation of phage numbers, Lytic and lysogenic life cycle of bacteriophage lambda; mechanism(s) that determines lytic and lysogenic life cycle, SOS response of *E coli* host;.Replication of an animal virus (ds DNA); Bacteriophage isolation

Suggested textbooks

Medical Microbiology---David Greenwood

Molecular Biology----Padmanabhan and Shastri

A Genetic Switch---Mark Ptashne

Group B: Immunology (50 marks)

UNIT I

1.Introduction: overview of the Immune system. (2)

2.Cells and organs of Immune system: (10) Hematopoietic stem cells, stromal cells, hematopoietic growth factors, Lymphoid organs (primary and secondary) and cells, Mononuclear cells, Granulocytic cells, Mast cells, Dendritic cells- characteristics and functions.

3.Types of Immunity: (14) (i) Innate immunity - mechanism of immune response (anatomic, physiological, phagocytic and inflammatory barriers).

(ii) Adaptive immunity: Humoral and Cell-mediated immunity - mechanism of immune response---antigen processing and presentation, types and structures of Major histocompatibility complex molecules (MHC) and their role in antigen presentation, clonal selection of lymphocytes, definition of cytokine, generation of humoral and cell mediated response by cellular interactions (general concept only).

4.Antigens: .(6) chemical nature, antigenicity, immunogenicity, hapten, epitopes, mitogens (definition, properties, examples); Adjuvant (definition, examples, function)

UNIT II

1. Immunoglobulins: (9) Isotypes- definition, basic and fine structures, general characteristics and functions. Monoclonal and polyclonal antibody (definition and characteristics).

- **2. Antigen Antibody interactions**: (5) Precipitation reactions-Radial immunodiffusion, double immunodiffusion, immunoelectrophoresis; Agglutination reactions-Hemagglutination, passive agglutination, bacterial agglutination, agglutination inhibition.
- **3. Complement**: (6) The complement components, function, complement activation-(i) Classical, (ii) Alternate and (iii) lectin pathways (characteristics & functions).
- **4. Hypersensitivity**: definition, types, examples. (3)
- **5. Vaccines : (7)** Active and passive immunization (definition, characteristics, examples and functions). Attenuated and inactivated viral or bacterial vaccines (definition, characteristic, functions, examples).

Suggested textbooks

Immunology—Jenis Kuby

Basic Immunology—Abbas

Paper VII (100 marks)

Practical

Unit I

- 1. Isolation and characterization of one industrially important enzyme, immobilization of cells.
- 2. Determination of Km, V_{max} and pH optima, effect of activator, inhibitor of alkaline phosphatase

Unit II

- 1. Protein estimation by Lowry method
- 2. Absorption spectra of DNA and protein, hyperchromic shift of DNA
- 3. Phage titration

Paper VIII (100 marks)

Practical

UNIT I

- 1. Antigen-Antibody reaction
 - a) Agglutination (blood typing etc.),
 - b) Ouchterlony's agar diffusion method,
 - c) Single radial immunodiffusion (Mancini's method),
 - d) Immunoelectrophoresis (by standard teaching kits).

2. Restriction digestion of plasmid DNA

UNIT II

- **1.** Isolation of plasmid-DNA (E.coli DH 5α) by using a standard method of any authentic teaching kit: Gel-electrophoresis (Agarose-gel), quantification of DNA and purity.
- **2. Transformation** of *E. coli* by using plasmid DNA by CaCl₂ method.
- 3. Conjugation experiments using any standard teaching kit.

Existing, Syllabus of B.Sc. General Course in Microbiology effective from the academic session 2003-04 (vide University Notification No. CSR/!86/2002, dt. 8th October, 2002), will remain unchanged.

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