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Syllabus for

Ph. D. Entrance Test

Subject:- MICROBIOLOGY

SECTION – A : Research Methodology (50 % weightage)

Unit-I Introduction

What is Research?

Objectives of Research

Scientific Research

Research types and its methods,

Research process and steps in it.

Importance of research methodology in scientific research

Unit – II Review of Literature

Essential constituents of Literature Review in Microbiology

Need for Reviewing Literature

What to Review and for what purpose

Literature Search Procedure

Sources of Literature in Microbiology

Planning of Review work

Note Taking, Library and documentation

Unit –III Planning of Research and sampling

The planning process

Selection of a Problem for Research

Hypothesis formation

Measurement, Research design and plan

Sampling Techniques or Methods

Choice of sampling Techniques Sample size

Sampling and Non-Sampling errors, Estimation Mean, Estimation of Standard Error and Confidence Interval

Unit –IV Data collection and analysis

Types of Data
Collection of data
Processing of data
Tabulation
Graphical representation
Statistical softwares used.
Analysis of data by statistical methods.

Unit- V Report/Project writing

Types of Reports
Planning of Report Writing
Documentation
Data and Data Analysis Reporting in a Thesis
Writing of project,
Funding agencies.

SECTION – B : MICROBIOLOGY (50 % weightage)

Unit I

A. Biostatics and Computer Applications

- Introduction to Biostatics. -

-Data collection and presentation

-Graphical representation of data.

-Measures of variability.

-Probability theory and distribution

-Introduction to computer.

-Software and Hardware

-Medline and Pubmed

B. Biochemistry

- Enzyme classification

-**Enzyme kinetics** - Derivation of Michaelis - Menton equation and its significance in enzyme kinetic studies. Lineweaver-Burke plot, Haldane-Briggs relationship, sigmoidal kinetics, steady state kinetics and transient phases of enzyme reaction.

-Extremozymes, types of enzymes-Theories of enzyme action.

-**Carbohydrate catabolic pathways**- EMP, HMP, ED, Phosphoketolase pathway, TCA cycle, Glyoxylate bypass.

Microbial growth on C1 Compounds (Cyanide, Methane, Methanol, methylated amines and carbon monoxide)

--**Carbohydrate fermentations** - Alcohol, lactate, mixed acid, butyric acid, acetone-butanol, propionic acid, succinate, methane, and acetate, butanediol, acettoin fermentations.

-**Degradation of aliphatic and aromatic compounds**.- microorganisms involved, mon-terminal, biterminal oxidation of propane, decane, etc. and aromatic hydrocarbons and aromatic compounds via catechol, protocatechuate, meta-cleavage of catechol and protocatechuate, dissimilation of catechol and protocatechuate, homogentisate and other related pathways

- **Endogenous metabolism**- enzymatic synthesis, degradation and regulation of reserve materials - glycogen, polyphosphates and polyhydroxybutyrate (PHB), PHB production

-Biosynthesis of amino acid- formation of glutamic acids, conversion of glutamic acid to glutamine, proline and arginine, formation of alanin, serinine, glycine and cysteine), Biosynthesis of nicotinic acid and pantothenic acid, biosynthesis of Purines and Pyrimidines.

C. Enzyme technology

-Extraction and purification of microbial enzyme- Importance of enzyme purification, different sources of enzymes. Extra cellular and intracellular enzymes. Physical and chemical methods used for cell disintegration. Concentration of the enzyme extract, Enzyme fractionation by precipitation using salts and solvents, liquid – liquid extraction, ion exchange chromatography, gel filtration, affinity chromatography and other special purification methods. Analysis of purity, Enzyme crystallization techniques, Criteria for purity of enzymes, tests of homogeneity of purified enzymes.

-Enzyme Inhibition- Irreversible and reversible enzyme inhibitions. Competitive, uncompetitive and non competitive enzyme inhibitions with suitable examples and their kinetic studies.

Allosteric inhibition- Positive and negative cooperativity, sigmoidal kinetics and allosteric enzymes. Models accounting cooperativity – Hill, Adair, MWC and KNF models. Cooperative binding of oxygen to haemoglobin – significance of sigmoidal behaviour. Types of allosteric inhibition and their kinetic studies and significance in metabolic regulation. Aspartate transcarbamoylase as allosteric enzyme.

Regulation of enzyme activity- Allosteric regulation, feedback regulation and cascade system (Genetic regulation), covalent modification.

-Principles and techniques for enzyme immobilization- Principles, parameters, carriers/matrices used for immobilization, techniques of enzyme immobilization viz. adsorption, covalent bonding, entrapment and membrane confinement.

-Protein engineering, Enzyme engineering.- Objectives of Protein Engineering, basic strategy of enzyme engineering. Protein engineering versus enzyme engineering as a biocatalyst. Techniques of Protein Engineering, Chemical modification and Site directed mutagenesis to study the structure- function relationship of industrially important enzymes. Hybrid enzymes. Examples of Protein Engineering applications- Improvement in stability, catalytic efficiency, selectivity and substrate specificity, purification and biopharmaceutical applications etc.

-Enzyme therapy - Treatment of genetic deficiency diseases, Enzymes in cancer therapy, Enzyme inhibitors and drug design, therapeutic importance of ribozymes and abzymes

-Enzyme sensors

Unit II

A. Bioinstrumentation

- Principle and Applications of Basic Instruments – PCR, pH meter, Gel Documentation, LAF, Biosafety cabinets, - Centrifugation and its type

-Microscopy , its type and applications

-Spectroscopy techniques, theories and it applications - UV-Visible, IR, NMR, Fluorescence, Atomic Absorption, CD, ORD, Mass, Raman Spectroscopy.

-Theories, principles and application of different chromatography techniques –paper, TLC, GC, HPLC, HPTLC, gel filtration, ion-exchange, affinity, hydrophobic.

- Theories, principles and application of different electrophoresis techniques. -Starch, agarose, IEF, 2D GEL PAGE

-Radioisotopic techniques and its application, Tracer techniques, autoradiography. GM counter, dosimetry

B. Food Microbiology

-Dairy fermentation – cheese, acid product, milk, kefir,

- Wine fermentation,

-Probiotics, bio surfactants

-Microbial enzyme in food and dairy industry-

-Production of Beaker's yeast, Tea, vinegar

-Food spoilage/diseases its types - Clostridium, Salmonella, Shigella, Staphylococcus, Campylobacter, Listeria. -Microbial intoxication, Mycotoxins.

-Food preservation methods

-Quality assurance - FDA, EPA, HACCP, ISI.

C. Bioprocess engineering and Technology.

-**Types and designs of Bioreactor** - Design of basic bioreactor, bioreactor configuration, design features, individual parts, baffles, impellers, foam separators, spargers, culture vessel, cooling and heating devices, probes for on-line monitoring computer control of fermentation process, measurement and control of process. Ideal batch reactor, ideal continuous flow stirred tank reactor, packed bed reactor bubble column reactor, fluidized bed bioreactor, Trickle bed reactor (Their basic construction, working, and distribution of gases)

-**Upstream process** - Inoculum development, formulation of production media, sterilization of media, maintenance of stock culture, scale up of the process from shake flask to industrial level.

Growth of culture in fermenter , choosing cultivation methods , Modifying batch and continuous reactors, immobilization cell systems, active and passive immobilization , solid state fermentation process.

Down -stream processes -.

Down stream processes : Introduction , Recovery of particulates filtration , centrifugation , sedimentation , emerging technologies for cell recovery , product isolation , extraction , solvent extraction , aqueous two phase system , sorption , precipitation , reverse osmosis, ultra filtration. Product recovery traits: Commercial enzymes, Intracellular foreign proteins from recombinant E. coli, polysaccharide and biogum recovery, antibiotic, organic acids, ethanol, single cell protein

- **Mass Transfer and Sterilization** - Gas liquid mass transfer in cellular systems, basic mass transfer concept, Rate of metabolic oxygen utilization, Determination on oxygen transfer rates, determination of Kla, Heat transfer, aeration / agitation and its importance.

Sterilization of bioreactors, nutrients, air supply, product and effluents, process variable and control, scale up of bioreactor.

Unit III

A. Microbial Genetics

-**Difference in organization of prokaryotic and eukaryotic genome**.- Primary structure - linear polyncleotide, Secondary structure- double stranded helical structure (Watson and Crick model), Tertiary Structure: negative and positive superhelices, geometry of superhelical DNA, enzymatic activity altering DNA supercoiling (Role of Top I & II),

-Different forms of DNA and RNA

-DNA replication and modification

-**Types of mutation** - Transitions, Transversions, Misscence Mutations, Suppressions & Reversions

Mutagens modifying replicating DNA – 5- Bromouracil, 2 aminopurine.

Mutagens altering resting DNA - Nitrous acid, Hydroxyl amine, Alkylating & Intercalating agent.

U. V. Mutagenesis.Advance applications of mutation: Site directed mutagenesis

Repair Mechanisms: - Photoreactivation, Exicision, Retrieval systems, Mismatch repair & SOS Repair

-Recombination and its model.

-**Phage genetics and different phages** - T4, Lambda, M13, phi x 174

-**Transposons and molecular mapping** - Discovery, types of transposons- Insertion sequences and composite transposons, Transposons in Prokaryotes(Bacteria and Phages, animal viruses) , transposons in maize, Mechanism of transposition- duplication of target sequences, replicative, non-replicative and conservative transposition, role of integrase and resolvase in Cointegrate formation, Role of transposons in acquisition of multiple drug resistance, Detection of transposition, Mutations i.e., Deletions, Inversions and Frame-shift due to transposition.

-Prokaryotic transcription and translation

-**Regulation of gene expression** -Operon concept and its regulation- Regulation at the level of transcription- Operon concept, Constitutive genes and Regulating genes, types and properties of promoters for constitutive and Regulated expression, Co-ordinated control of structural genes, stringent response, catabolite repression, instability of bacterial RNA,

Positive regulation in E. coli [Arabinose operon] and

Negative regulation in E. coli [lac operon],- Basic operon model, Molecular structure and organization of inducers and repressors, Inducible operon, role of CAP-CAMP ,Repression, fate of mutation in Cis and

Trans acting genes of lac operon, Regulation by attenuation by trp operon., Regulation of cI repressor (Lambda operon) , Insertion of Tn element in switch off of expression.,Regulation of DNA Repair genes. Regulation at the level of translation

Antisense t RNA technology, regulation of synthesis Tn m RNA

B. Environmental Microbiology

-Eutrophication, its effect, types, control

- Ecosystem, biotic and abiotic environment

-Ecosystem characteristics, structure and function

-**Bioremediation of xenobiotics** - Definition of recalcitrant/ xenobiotic compounds, their presence in the natural ecosystem, Concept and consequences of biomagnification, Microbiology of degradation of xenobiotics in the environment, ecological considerations, decay behavior, biomagnification and degredative plasmids, hydrocarbons, substituted hydrocarbons, oil pollution, surfactants and pesticides. Genetically Modified Organisms released and its environmental impact assessment and ethical issues.

-**Global environmental problems**. - Ozone depletion, UV-B, green house effect and acid rain, their impact and biotechnological approaches for management. .

-**Effluent treatment techniques** –aerobic, anaerobic and different types

C. Virology and Immunology

-Classification and morphology of viruses.

-Cultivation and multiplication of viruses.

-Emerging viruses and its control.

-Pathogenicity of viruses.-animal viruses Adenovirus, Herpes virus, Picorna virus, Poxvirus and Orthomyxovirus, pathogenesis of plant [TMV], NPV

-Innate and adaptive immunity

-Cells and organs of immune system

-Antigens, Antibody and their type

-Antigen Antibody reaction,

-Immuno-assays: SRID, ELISA-PCR, RIA, Western Blotting, Immunofluorescence and their application. Immune deficiencies and autoimmunity.

-vaccines and its types

Unit IV

A. Microbial Physiology

-Bacterial photosynthesis

-Aerobic and anaerobic respiration

-Structure and organization of bacterial membrane.

-Bacterial Sporulation

-Bacterial Chemolithotrphy - Oxidation of molecular hydrogen by Hydrogenomonas species. Ferrous and sulfur/sulfide oxidation by Thiobacillus species.

Nitrogen metabolism - Biochemistry of biological nitrogen fixation, properties of nitrogenase and its regulation, ammonia assimilation with respect to glutamine synthetase, glutamate dehydrogenase, glutamate synthetase, their properties and regulation.

B. Biodiversity

-Microbial Diversity- distribution, abundance, ecological niche. Types- Bacterial, Archael and Eucaryal. Whitaker's five kingdom concept, General characters of actinomycetes, fungi, algae, protozoa and yeasts

-Community ecology- community structure, benevolent interactions (control within the microbial communities of rhizosphere), antagonistic interactions, (competition, antibiosis, predation etc.). Rhizosphere, rhizoplane, siderophore, flavonide from plants, lectines, octapine, nipotine, indole acetic acid. Marine ecosystem

-Alkalophiles, barophiles, Halophiles and acidophiles

C. Recombinant DNA Technology

-**Tools and technique involved in genetic engineering**- Electrophoretic techniques in DNA analysis-Agarose gel electrophoresis, PAGE, Pulse field gel electrophoresis, DISC gel electrophoresis, electroelution, autoradiography, Restriction mapping, DNA sequencing-Maxam-Gilbert, Sanger's dideoxy and automated methods of DNA sequencing. Gene silencing, Principle, technique and applications of chromosome walking, chromosome jumping, RFLP, RAPD, AFLP, DNA fingerprinting

Chromosome microdissection and microcloning, Microarray- principle, methodology, advantages and applications.

-**Vector used in gene cloning**- Strategies of -Cloning vectors and expression vectors

Vectors of E.coli_ : Plasmid vectors: Properties of plasmids, PBR 322-genetic evolution, map and function, pUC vectors ,Phage vectors: Lambda phage vectors: gt phages , Charon vectors, EMBL vectors, M13 mp vectors,Higher capacity vectors: Cosmids, Phagmid bluescript vectors

High level expression/production vectors: PET vectors, PINPOINT vectors, BAC

Vectors for yeast : 2 µ plasmid vector, ARS vectors, mini chromosome vectors and YAC .

Shuttle vectors: SV 40 plasmid vectors, retrovirus vectors.

Vectors of plant: Ti plasmid vector

-Enzymes in gene manipulation -

-**Techniques of gene cloning** - Isolation of gene of desired interest, Construction of genomic and cDNA libraries, Selection and Identification of clones containing recombinant vectors

-Application of genetic engineering and PCR - Areas of applications of rDNA technology, Production of recombinant Insulin, Hepatitis B surface antigen, Production of monoclonal antibodies, rDNA in gene therapy (ADA Deficiency)Construction of BT cotton plant and transgenic tomatos/potatos.

PCR alternative to gene cloning- advantages, principle and Procedure, optimization of PCR, Designing of primers, Identification of PCR products, Variations in basic PCR- Inverse, asymmetrical , multiplex, Hot start, ligation mediated, RT , Real-time quantitative PCR, DD PCR and Immuno PCR.

Applications- DNA cloning for sequencing, DNA-based phylogeny, or functional analysis of genes; the diagnosis of hereditary diseases; the identification of genetic fingerprints (used in forensic sciences and paternity testing); the detection and diagnosis of infectious diseases. PCR based Site directed mutagenesis, Identification of pathogens.

Unit V

A. Microbial fermentation

-Microbial Production of therapeutic compounds.

- Penicillin, Streptomycin, Rifamycin and Tetracycline. Biotransformation of steroids, antibiotics..

Vitamin B12 and riboflavin fermentation.

-Biofuels and plant tissue culture

-**Modern trends in microbial production** –Bioplastics - PHB, PHA, biopolymers- dextran, alginate, xanthan, pullulan, biosurfactants, SCP, Biofertilizers, Biopesticides.

-IPR and Patents

B. Bioinformatics and its applications

- **Database and its types** –Nucleic acid, Protein.

-Bioinformatics tools for protein and DNA analysis

-Whole genome analysis

-**Types of array** -DNA and protein microarray

-Sequence analysis using bioinformatics tools –BLAST, FASTA,-different software's and its applications

-Proteomics and its analysis tools- Mass spectrometry

C. Pharmaceutical microbiology

-**Antimicrobial chemotherapy** - Introduction and selection of antimicrobial agents

Concept of Bioassay, therapeutic index, MIC and LD50.

Penetrating defenses, as cellular permeability barriers, Cellular transport system and drug diffusion.

Definition and classification of antibiotics, with respect to their mechanism of action, Antibacterial spectrum, Structural activity and relationship (SAR), acquisition of drug resistance, pharmacokinetics and adverse drug effect β- Lactum(Penicillin, Amoxicillin, cefuroxime), aminoglycosides (Streptomycin, Gentamicin), Tetracyclines (Tetracyclin, doxicyclin) , Macrolides (Erythromycin, Azethreomycin), Peptide antibiotics (Bacitracin, polymixin,), Sulphonamides (sulfamethoxazole), co-trimoxazole and quinolones (ciprofloxacin) Chloramphenicol, trimethoprim.

Definition, classification, Mechanism of action and examples of chemical disinfectants, antiseptic and preservatives.

Definition, classification, Mechanism of action and examples of antiviral (Acyclovir, zidovudine), Antifungal (amphotericin B, Fluconazole) and Antitumor (Bleomycin, ductinomycin) antibiotics.

-**Drug delivery system in gene therapy**- Approaches and safety considerations associated with gene therapy. Immunological problems associated to gene therapy. Pre-requisites and candidate diseases for human gene therapy. Drug carrier, Macromolecular, cellular, and synthetic Viral and non viral mediated gene delivery.

Introduction, concept and types of drug targeting, cellular level events of drug targeting, targeting ligands, blood cell receptors for endogenous compounds/ ligands, carrier and vesicular system for targeting, specialized liposomes for cellular drug targeting.

-**Microbial production and spoilage of pharmaceutical products**- Manufacturing procedure and in-process control of Pharmaceutical products: Bacterial and Viral vaccine, sterile injectables, Solid dosage forms, liquid orals and Ointments

New Vaccine production: DNA vaccines, synthetic, peptide vaccines, multivalent subunit vaccines, edible vaccines and their trials.

Microbial production and applications of therapeutic / diagnostic enzymes: Asparaginase, Streptokinase, beta lactamases

Microbial production contamination and spoilage of Pharmaceutical products (sterile injectables, ophthalmic preparations and implements) and their sterilization

Applications of Biosensors in pharmaceutical industries.

-**Regulatory practices and policies in Pharma industry** - FDA, Govt. regulatory practices and polices.

Concept of R & D and Financing R and D, Quality control and market planning.

Significance of IP, BP and USP. Reimbursement of drugs, Biological and legislative aspects.

Rational drug design (Quantitative structure activity relation QSAR of drug) and computational aspect of drug design.Screening and utilization of bioactive phytochemicals.

Patenting of drugs and Biological products

-Quality assurance and validation –

- Regulatory aspects of QC, QA, and QM. GMP , GLP and CMP in Pharma Industry. ISO, WHO, USFDA certification. Microbial Limit test of Pharma products. Sterility testing , pyrogen testing and LAL test of Sterile Pharma products. Sterilization- heat, D- value, Z-value and survival curve, radioactive, gaseous and filtration. Chemical and biological indicators. Designing layout for microbiology laboratory.

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