# Syllabus for Change of Discipline Test (CDT) for seeking admission in M.Sc. Marine Microbiology (Approved in BoS held on 22.12.2021)

#### History of Development and Scope of Microbiology

Types and distribution of microorganisms, classification schemes - Binomial nomenclature, Whittaker's five kingdom and Carl Woese's three Domain classification systems and their utility. General characteristics, reproduction, life cycles and economic importance of different groups: Acellular microorganisms (viruses, concept of viroids, virusoids, satellite viruses, bacteriophages; viral taxonomy), cellular microorganisms (Prokarya: Archaea and Eubacteria; Eukarya: Algae, fungi and protozoa) and prions, theories of evolution and origin of life, Germ theory of diseases, aseptic surgery. Role of microorganisms and their applications, types of associations: mutualism, commensalism, synergism, syntrophism, competition, antagonism, amensalism, parasitism, predation; symbiosis. Diversity in microbial cytology. Structure of eukaryotes and prokaryotes (Archaebacteria and Eubacteria) and differences. Cell organization and ultrastructure of prokaryotic cell (Bacterial cell). Cell wall: structure and composition in Gram's positive and Gram's negative bacteria, spheroplast, protoplast, L-forms; Flagella and pili; Cell membrane: architecture, structure & function; Slime and capsule: composition, function; Cytoplasmic organelles; Nuclear material: nature and function; Endospore: structure, sporulation and germination. Eukaryotic cell organelles: nucleus, endoplasmic reticulum, golgi apparatus, mitochondria, chloroplast, lysosomes, peroxisomes, protein sorting and transport, cytoskeleton and cell movement, the plasma membrane. Signal transductionreceptors involved in signal transduction, extracellular matrix and cell interactions. Introduction to cell signalling: Components of various signalling pathways, downstream effects of signalling on cell adhesion, cellular differentiation, cell cycle and apoptosis. Stem cells and their applications.

#### **Microbial Biochemistry**

Biomolecules and metabolic pathways - trioses, tetroses, pentoses, hexoses, optical isomerism, pyranose and furanose forms, alpha/beta forms, reducing sugars, disaccharides, glycosidic bonds, polysaccharides, storage (glycogen, starch), structural - cellulose. Principles of carbohydrate determination, qualitative and quantitative tests for carbohydrates, reducing sugar, non-reducing sugars. Concept of aerobic and anaerobic respiration, fermentation, major pathways in heterotrophs & regulation: EMP, HMP, ED pathway, TCA pathway, ETC, alcohol fermentation, Pasteur effect, mixed acid fermentations, linear and branched fermentative pathway. L & D forms, zwitterion, amphoteric nature, R - groups, naturally occurring amino acids and their detection by Ninhydrin method. Bonds and structures of proteins. Qualitative and quantitative protein determination, UV absorption, Biuret & Folin Lowry method. Fatty acids & triglycerides. Quantitative and qualitative tests of lipid determination, Purines & pyrimidines, nucleosides & nucleotides; principle of determination of DNA by diphenylamine and RNA by Orcinol methods. Enzymes, definition, protein nature, active site, specificity, holo-enzyme, apo-enzyme, coenzyme, cofactors, prosthetic group, monomeric, oligomeric and allosteric enzymes. Classification and nomenclature of enzymes, structures and specific activity of enzymes, factors affecting enzyme action. Mechanism of action of enzymes, activation energy, transition state, multi-enzyme complex,- pyruvate dehydrogenase, isozyme - lactate dehydrogenase. Types of mechanisms of solute transport: passive diffusion, facilitated diffusion, active transport, group translocation.

#### **Microbial growth and Bio-energetics**

Definitions of growth, measurement, batch and continuous culture, generation time and specific growth rate, growth in response to environmental factors - temperature (psychrophiles, psychrotrophs,

mesophiles, thermophiles, thermodurics), pH (acidophiles, alkaliphiles), solute and water activity (halophiles, xerophiles, osmophiles), oxygen (aerobes, anaerobes, microaerophilic, facultative aerobes, facultative anaerobes), hydrostatic pressure (barophiles). Microbial growth in response to nutrition and energy- autotroph/phototroph, heterotrophs; photo-organo-heterotroph, chemo-lithotroph: chemo-litho-autotroph, chemo-litho-heterotroph, chemo-heterotroph, photo-litho-autotroph. *Bioenergetics:* Gibb's Free Energy, standard free energy change and equilibrium constant, Coupled reactions and additive nature of standard free energy change, energy rich compounds: Phosphoenolpyruvate, 1,3- Bisphosphoglycerate, Thioesters, ATP. ATP as a high energy system, ATP hydrolysis and other high energy phosphate compounds, utilization of ATP in chemical work, ETC and oxidative phosphorylation, substrate level phosphorylation.

# **Cell Biology**

Cell to cell interactions: eukaryotic cell membrane, extra cellular matrix and cell matrix interactions, cell surface protrusions, types of cellular junctions - adhesion, tight, gap and plasma-desmata. Protein sorting and transport: Ribosomes, Endoplasmic Reticulum – targeting and insertion of proteins in the ER, protein folding, processing and quality control in ER, smooth ER, export of proteins and lipids. Golgi apparatus - organization, protein glycosylation, protein sorting and export. Cell Signalling: cyclic GMP and MAP kinase pathway, chemotaxis and phototaxis, quorum sensing: CFTR, calmodulin. Cell Cycle: regulation of eukaryotic cell cycle, mitosis and meiosis, cell death and apoptosis, development of cancer, causes and types: Oncogenes, tumor suppressor genes, properties and development of cancer cells (activation of cell division), symptoms, causes, risk factors, classification (benign and malignant), types (Carcinoma, Sarcoma, Leukemia, Lymphoma and Myeloma), stages of cancer (histological classification).

### **Microbial Genetics**

Genetic material: DNA, Watson - Crick model of DNA; Prokaryotic DNA (Circular DNA, Supercoiled, Palindromic), Plasmids; Eukaryotic DNA (Repetitive sequences, split genes, nucleosomes), mitochondrial and chloroplast DNA; Guanine quadruplex (G4) DNA. RNA: mRNA, rRNA, tRNA, non-coding RNA, micro-RNA and Si RNA. Replication of DNA: Modes of replication - Conservative, semi conservative (Meselson - Stahl experiment) and dispersive; Processes and enzymes involved in replication; Inhibitors of replication; Models of replication in prokaryotes and eukaryotes - Rolling circle model/sigma, theta and linear. Differences between prokaryotic and eukaryotic replication process. Transcription: Initiation, Elongation, Termination; post transcriptional modification - RNA splicing (Ribozyme), Reverse transcriptase and its implication, Inhibitors of transcription. Concept of operon. Differences between prokaryotic and eukaryotic transcription process. Translation: Concept of genetic code, Properties: codon / anticodon, Wobble hypothesis, start and stop codons; Ribosomes as sites of protein biosynthesis; amino acid activation and specificity; Initiation, Elongation, Termination; post translational processing and modification; Inhibitors of protein synthesis. Differences between prokaryotic and eukaryotic translation process. Gene expression and regulation: General Structure of Operon. Structural and regulatory genes. Induction and repression; catabolite repression. Positive and negative regulation of lac operon. Structure and regulation: Trp operon. Gene transfer mechanisms: Transformation: Griffith's experiment; Avery, MacLeod and McCarty's experiment, Factors affecting transformation, Competence factor, Steps in transformation. Transduction: Davis' U-Tube experiment. Lytic and lysogenic cycles. Generalized, Specialized, Complete and Abortive Transduction. Conjugation: Gene transfer by F+ strains, Hfr donor, F-prime state. Chromosome mapping. Mutations: Spontaneous Mutations: Concept of spontaneous mutations and mechanisms. Principle, methodology and significance of replica plating and fluctuation test. Auxotrophs, Complementation Test. Types of mutations: Point mutations: base pair substitution, tautomerism (transitions, transversions). Frame shift (slippage). Missense, nonsense, silent, conditional, suppressor (intragenic, extragenic). Large deletions, site directed mutagenesis. Induced mutations: Physical /chemical mutagens. Teratogenicity testing – Ames test. DNA damage and repair mechanisms (light/dark repair). *Molecular recombination and molecular taxonomy:* General features of recombination, types of recombination. Models for reciprocal and non-reciprocal recombination – Fox and Holliday's model, evidence for Fox and Holliday's model. Rec A and Rec BCD complex. rRNA and molecular taxonomy.

### **Genetic Engineering**

*Tools and strategies:* Restriction endonucleases: Type I, II, III. Mode of action, nomenclature and applications of Type II restriction enzymes in genetic engineering. Restriction and modification. Milestones in genetic engineering. *DNA modifying enzymes and their applications:* DNA polymerases, Klenow fragment, kinases and phosphatases, terminal deoxynucleotidyl transferase, DNA ligases, S1 nuclease, and RNAase H. Use of linkers and adapters. Synthesis of cDNA, sticky end and blunt end cloning. *Cloning and Expression vectors:* Plasmids, pBR and pUC series, Ti plasmid-based vector; Bacteriophage, lambda and M13 based vectors; cosmids; phagemids, Bacterial Artificial Chromosomes (BACs); Yeast Artificial Chromosomes (YACs). Shuttle vectors, Expression vectors (Fusion and Pure proteins), *E. coli* lac promoter-based vector, Yeast Episomal Plasmids (YEPs), Mammalian vector (SV40).

# **Bacteriological Techniques**

Pure culture isolation: Streaking, serial dilution and plating methods; cultivation, maintenance and preservation of pure cultures; cultivation of anaerobic bacteria. Sterilization: Autoclave (moist heat), hot air oven (dry heat), Tyndallization, membrane filtration. Culture media: components of media, natural and synthetic media, chemically defined media, complex media, selective, differential. Physical methods of microbial control: heat, low temperature, filtration, desiccation, osmotic pressure. Chemical methods of microbial control: types of disinfectants. Staining principles: mordants, fixatives and decolorisers, definition of dyes, chromogen, chromophore and auxochrome group, types of staining – Gram staining, monochrome staining and negative staining.

# **Techniques in Microbiology**

Microscopy: Bright Field Microscope, Phase contrast, Fluorescence, Confocal and Electron (Scanning and Transmission) Microscopy; Micrometry. Spectroscopy: Principle of UV-Vis and IR spectroscopy and their application in analysis of biomolecules. pH and Buffers: pH Meter: Principle, calibration and application. Buffers and buffering capacity. Chromatography: Principles and applications: Paper, Thin layer, Si gel Column, HPLC, Reverse phase, Gel filtration, Ion exchange and Affinity Chromatography.

Electrophoresis: Principle and applications: Native polyacrylamide gel electrophoresis, SDSpolyacrylamide gel electrophoresis, and Agarose gel electrophoresis. Centrifugation: Preparative and analytical centrifugation, fixed angle and swinging bucket rotors. RCF and sedimentation coefficient, differential centrifugation, density gradient centrifugation and ultra-centrifugation. DNA amplification and sequencing: PCR, Sanger's method, Maxam and Gilbert's method, Shot gun sequencing. Omics: Metabolomics, metagenomics, transcriptomics, proteomics.

# **Applied Microbiology**

*Microbial products of commercial interest:* Nutraceuticals: Probiotics, Prebiotics, Synbiotics, PUFA, Antioxidants, Vitamins, Polyphenols, SCP, Applications. Biosensors: Definition, Components, Basic Characteristics, Elements, Principles, Applications. Detailed study of glucose and BOD sensor.

Bioplastics: Definition, Properties, types and composition, synthesis, microorganisms involved in biodegradation, uses, Environmental impact. Applications of Microbes in Biotransformation and Bioremediation: Definition, types of microbial transformations/bioconversions (oxidation, reduction, hydrolysis, condensation, isomerisation, formation of C=C double bonds). Microbial remediation of common pesticides, organic (hydrocarbons, oil spills) and inorganic matter (metals). Screening and enrichment of organisms, biocatalysts and techniques, biotransformation of hydrocarbons and heavy metals. Immobilisation methods and Applications: Introduction, preparation of immobilised enzymes, support matrix. Methods of immobilisation (adsorption, covalent bonds, entrapment, copolymerisation, encapsulation), advantages and disadvantages, applications. RNAi: Definition, RNA silencing, mechanism, applications (therapeutics and agriculture). Intellectual Property Rights (IPR): Introduction, types of IPR, copyrights, trademark, patents, types of patents, process of patent application. Nanotechnology: Definition of nanoparticles, types, characterization and properties. Applications - drug delivery systems, bioremediation, antifouling, degradation of xenobiotics and fiber retting. Waste Management; Solid Waste management: Sources and types of solid waste, methods of solid waste disposal (composting and sanitary landfill). Liquid waste management: Composition and strength of sewage (BOD and COD), primary, secondary (oxidation ponds, trickling filter, activated sludge process and septic tank) and tertiary sewage treatment.

# **Microbes in Human Health**

*Water potability:* Treatment and safety of drinking water, methods to detect potability of water samples: (a) Standard qualitative procedures: presumptive test/MPN test, confirmed and completed test for faecal coliforms, (b) Membrane filter technique. *Medical microbiology and immunology:* List of important human diseases and their causative agents. Definitions of immunity (active/passive), primary and secondary immune response, antigen, antibody and their types, vaccines. *Bacterial diseases:* The causative agents, mode of transmission, pathogenesis, symptoms, chemotherapy and prophylaxis of the following. Respiratory Diseases: Pneumonia (*Streptococcus pneumoniae*), Influenza (*Haemophilus influenzae*), Tuberculosis (*Mycobacterium tuberculosis*). Gastrointestinal Diseases: Bacterial diarrhea (*Escherichia coli*), typhoid (*Salmonella typhi*), Cholera (*Vibrio cholerae*), bacterial dysentery (*Shigella dysenteriae*). Skin infections: *Staphylococcus aureus, Vibrio parahaemolyticus*. Genito-Urinary Tract Infections: Syphilis (*Treponema pallidum*), UTI (*E. coli* and *Proteus vulgaris*). *Protozoan diseases:* Amoebic dysentery. *Fungal diseases:* Cutaneous mycoses: Athlete's foot (*Tinea pedis*). Opportunistic mycoses: Candidiasis (*Candida albicans*).

### **Microbial Ecology and the Environment**

*Concept of an ecosystem:* producers, consumers and decomposers, energy flow in the ecosystem, ecological pyramids (pyramid of numbers, pyramid of energy, pyramid of biomass), food chains, food webs and ecological pyramids. Community structure: ecological succession, trophic structurezonation and stratification. Introduction, types, features, structure and functions of the following ecosystems: terrestrial ecosystem (forests, grasslands, deserts); aquatic ecosystems (ponds, streams, lakes, rivers, oceans, coastal zone, estuaries). *Microorganisms and their habitats:* Aquatic Environment: Microflora of fresh water and marine habitats; Air: Aeromicroflora and dispersal of microbes; Animal Environment: Microbes in/on human body (Microbiomics) and animal (ruminants) body; Extreme Habitats: Microbes thriving at high and low temperatures, pH, high hydrostatic and osmotic pressures, salinity, and low nutrient levels. *Microbial Interactions:* Definitions and examples of important microbial interactions –mutualism, commensalism, synergism, syntrophism, competition, antagonism, amensalism, parasitism, predation; symbiosis. Examples of each type of interaction - mycorrhizal, root nodules, ruminant symbiosis, nematophagus fungi, symbiotic, bioluminescent bacteria, lichens. *Soil as microenvironment:* Soil profile and soil microflora; organic matter decomposition – humus formation. Rhizosphere and endophytic microflora and their role. R:S ratio, Microbivory, Microbial associations in phytosphere: rhizosphere - phyllosphere spermosphere. Degradation of cellulose, hemicelluloses, lignin and pectin in soils. Microbial succession in decomposition of plant organic matter. Biogeochemical cycles: C, N and role of microorganisms. Plant-Microbe Interactions: Plant diseases: Mode of entry of pathogens, disease symptoms. Beneficial associations: Rhizobium (Nitrogenase, Nodulation, Hydrogenase), Azolla, Frankia (infection process, nodulation), Mycorrhiza (Types- ecto/endo, mechanism of symbiosis). Phytostimulation and Bioinsecticides: Plant Growth Promoting Bacteria (PGPB). Microorganisms in soil, Root exudation, Effect of PGPB on plants, Root microbiome, PGPB: Direct (Nitrogen fixation, P solubilisation, IAA producers, ammonia producers, ethylene (ACC deaminase) and indirect (Siderophores, HCN). Biopesticides -Introduction, types (bacterial- Bacillus thuringiensis, viral -NPV, fungal -Trichoderma, Metarhizium), mode of action, genes involved, factors influencing their action and target pests. *Biofertilizers:* definition, importance. Types (i) Nitrogen fixing – Azotobacter, Rhizobium, Azolla, Frankia, Cyanobacteria and Azospirillum. (ii) Phosphate solubilizing Microorganisms, (iii) Vesicular Arbuscular Mycorrhiza (VAM). Biochemistry of symbiotic and nonsymbiotic nitrogen fixation, Phosphate solubilisation and Potassium mobilization. Application methods: Steps in mass production of bacterial biofertilizers, quality guidelines for biofertilizers. Methods of preparation and application – liquid and carrier based, Mass production of blue green algae, Azolla and mycorrhiza. Plant response to biofertilizers.

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