

## DETAILED SYLLABUS ( DAIRY MICROBIOLOGY COURSES)

(As per 5th Dean's Committee Report)

### 1. Fundamentals of Microbiology 3(2+1)

#### Theory

Overview of history and scope of microbiology: Discovery of Microorganisms and Microscopy (types, working principles and applications); Theories of Biogenesis and abiogenesis; Contributions of Leeuwenhoek, Pasteur, Tyndal, Joseph Lister, Robert Koch, Edward Jenner and Alexander Fleming; Scope and application of microbiology in fields like Dairy, Food, Pharmaceutical, Industrial, Medical and agriculture. Classification of Microbes: Microbial classification systems, numerical taxonomy, General properties and principles of microbial classification, Whittaker's five kingdom and Carl Woese's three domain classification system; Systematics of bacteria and Bergey's manual of systematic bacteriology, Phylogenetic tree. Prokaryotic and Eucaryotic microorganisms: Structure and functions of prokaryotic cells; Differences between prokaryotes and eukaryotes; Differences between cell wall of Gram positive and Gram negative bacteria; Structure of Archeal cell wall. Microbial growth and nutrition: Bacterial growth curve; factors affecting growth of bacteria, direct and indirect methods of measurement of bacterial growth; Bacteriostatic and bactericidal agents; Common nutrient requirements and nutritional types of microorganisms. Diversity of Microorganisms: Viruses: Structure and Classification; Bacteriophages; Differences between viruses and bacteria; Fungi: Classification of Fungi; Reproduction in Fungi; Protozoa and algae. Microbial Ecology and Environmental Microbiology: Microflora of air, soil and water and Microbes of Extreme environment like Archea. Basics of Microbial Genetics and Host-Microbe interactions: DNA as the genetic material, Structure of DNA/ RNA, DNA replication, transcription and translation; Basic concepts of immunology; Role of immune system in governing host-microbe interactions, Microbial Commensalism, Colonization, Infection, Disease and Vaccines

#### Practical

General instruction for microbiological laboratory. Microscope- simple and compound; Microbiological equipments; autoclave, hot air oven, incubator, centrifuge, colorimeter, laminar airflow, membrane filter. Simple staining- methylene blue; crystal violet; negative staining. Differential staining (Gram, spore, acid fast). Motility of microorganisms - hanging drop technique. Measurement of size of microorganisms by micrometry (ocular and stage). Preparation of commonly used growth media liquid and solid: simple and differential media. Isolation techniques for microorganisms – Streak, spread and pour plate. Enumeration of microorganisms in air and soil. Enumeration of microorganisms in water: total viable count, coliform (MPN). Visit to Microbiology Laboratory of Dairy/Food Industry.

### 2. Microbiology of Fluid Milk 2(1+1)

#### Theory

Microbes associated with raw milk: Significance of specific groups of microorganisms in milk i.e. psychrotrophic, mesophilic, thermophilic and thermophilic bacteria - their

morphological and biochemical characteristics and classification. Microbial contaminants in raw milk, their sources during various stages of production - milking, chilling, storage and transportation with special reference to psychrotrophic microorganisms; Microbiological changes in bulk refrigerated raw milk. Sources of contamination and microbial spoilage of raw milk: Microbial contaminants of raw milk supplies, their sources during various stages of production i.e. milking, chilling, storage and transportation with special reference to psychrotrophic microorganisms and preventive measures. Types of microbial spoilage - souring, curdling, bitter cream, proteolysis, lipolysis, abnormal flavors and discoloration. Mastitis milk - types of mastitis, causative micro-flora of mastitis, compositional and microbiological changes during mastitis infection, their processing and public health. Concept of clean milk production: Hygienic milk production system; Cleaning and sanitation of udder, animal, utensils, equipments and dairy farm environment; Microbiological quality of milk produced in organized and un-organized sector in India and comparative information in developed world; Microflora of aseptically drawn milk and its natural antimicrobial systems - immunoglobulins, lactoferrin, lysozyme and lactoperoxidase (LP) system. Microbiological aspects of fluid milk: Pasteurization, boiling, sterilization, ultra high temperature (UHT), non thermal (pulsed field) micro-filtration, bacterofugation, standardization and homogenization. Significance of heat resistant and post processing contaminants in fluid milk with special reference to proteases and lipase enzymes and their role in spoilage of processed milk. Bio-film formation during processing and their control measures. Public health aspects of fluid milk: Microbial zoonotic diseases transmitted through fluid milk; Milk borne diseases - food infection, intoxication and toxigenic infection caused E. coli, Salmonella typhi, Staphylococcus aureus, Bacillus cereus, Listeria monocytogenes, Shigella species, Campylobacter etc. Microbiological grading and legal standards of raw and processed milk.

### **Practical**

Morphological examination of common dairy microorganisms (size and shape, arrangement and sporulation). Estimation of microbial load in raw milk by standard plate count (SPC) and dye reduction tests (MBRT, RRT). Grading of processed/ market milk by total viable count, coliform and methylene blue reduction time. Enumeration of psychrotrophic, thermophilic, thermotolerant and spore forming bacteria in raw and market milk. Detection of sources of contamination: Air, water, utensils, equipment and personnel, line testing. Spoilage of milk caused by microorganisms: souring, sweet curdling, gassiness, lipolysis, ropiness, proteolysis and discoloration. Detection of mastitis milks: pH, SLST, somatic cell count, chloride content, Hotis test and CAMP test. Detection and estimation of coliforms: presumptive, rapid coliform and IMVIC Test.

## **3. Microbiology of Dairy Products 2(1+1)**

### **Theory**

Microbiology of Cream and Butter - Micro-environment and impact of critical process factors on entry of spoilage and pathogenic organisms in cream and butter; Microbiological aspects including defects in pasteurized (ripened/unripened cream), sterilized and UHT cream; Factors influencing the microbial growth during batch/continuous butter making process; Microbial Defects in butter - Bacterial/mold

discoloration, enzymatic deterioration and their control measures; Regulatory microbiological standards. Microbiology of Condensed, Evaporated and Dried products: Type of microorganisms associated with condensed, evaporated and dried products, their growth/ survival during manufacture and storage; Microbial defects - Bacterial thickening / Mold button formation in SCM; Gassiness/bloating, Bacterial coagulation (Sour and sweet), Bitterness, Fishy flavor in evaporated milk; pre-heating/DSI temperature and their impact on microflora of dried products; Effect of reconstitution on microbial quality of milk powder including baby foods and survivability of pathogens; Regulatory microbiological standards Microbiology of Ice Cream and Frozen desserts: Microenvironment in ice cream, microbiological quality of ingredients, critical process factors and their impact on entry of pathogens in ice cream and frozen desserts, their survival during storage, food poisoning outbreaks and legal standards. Microbiology of Indigenous Milk Products: Predominance of spoilage and pathogenic organisms in khoa and khoa based sweets – burfi, peda, gulabjamun, etc., paneer, Chhanna and Chhanna based sweets – rasogulla; kheer, shrikhand, dahi, kulfi etc.; Factors affecting the microbiological quality in reference to production, processing, storage and distribution; Microbial safety in relation to potential pathogens and their public health significance; Microbial defects, control measures and legal standards; Active packaging concepts and role in bio-preservation.

### **Practical**

Microbiological examination of raw, pasteurized, sterilized and UHT cream for Standard plate count (SPC) as well as lipolytic and coliform counts, direct microscopic count (DMC), dye reduction tests and sterility test. Microbiological examination of salted and unsalted butter for SPC, psychrotrophic, lipolytic, coliforms and yeast and mold count; K.Q test. Microbiological examination of concentrated milk for SPC, coliforms, spores, yeast and mold, thermophilic and thermophilic counts. Microbiological examination of dried milks for SPC, coliforms, Staph. aureus, B. cereus, E. coli, Salmonella, Sulphite reducing clostridia and Staphylococcal enterotoxins. Microbiological examination of ice-cream and other frozen desserts for SPC, coliforms and Staphylococcal counts; Detection of Salmonella spp./E. coli. Microbiological examination of khoa for SPC, coliforms and staphylococcal counts besides yeast and mold counts. Microbiological examination of paneer and shrikhand for SPC, Spores, coliforms, yeast and molds and Staphylococcal counts. Microbiological examination of packaging materials for SPC, Spores and Yeast and mold counts.

## **4. Starter Cultures and Fermented Milk Products 3(2+1)**

### **Theory**

Types, metabolism and propagation of starter cultures: History, classification and importance of starter Cultures in dairy industry; Single, multiple, defined and mixed strain starters; Probiotics and Special cultures like exopolysaccharide production; Propagation of starter cultures concentrates - direct bulk and direct vat starter cultures, factors affecting propagation; Metabolism of starter cultures (carbohydrate, protein, citrate) and production of metabolites and antibacterial substances; methods of starter distillates their merits/demerits. Activity, Purity, Preservation of Starters and Starter Failure: Quality and activity tests for dairy starters and their preservation- methods (liquid,

spray drying, vacuum drying, freeze-drying, frozen concentrate, concentrated dried cultures), merits and demerits; factors affecting the survival of cultures during preservation; Defects in starters and their control; Starter failure effect of antibiotic residues, sanitizers and bacteriophages. Phages-life cycle, sources, prevention, chemical and mechanically protected systems. Role of Starters in fermented milks: Role of starters in the preparation of various fermented milks; Types of fermented milks - dahi, yoghurt, acidophilus milk; different types of dahi and yoghurt; preparation; defects and their control. Kefir and koumiss : origin and characteristics; microbiology of kefir grains; Other fermented milks such as Bulgarian milk, cultured buttermilk, Leben, Villi and Yakult; Microbiology of fermented milk products; their nutritional and therapeutic significance. Cheese Starters: Classification, desirable properties, Artisanal and adjunct cheese cultures, primary and secondary flora of cheese; biochemical changes during ripening, bacterial and mold ripened cheeses: soft, semi-soft, semi-hard, hard, Brick and Brie cheese, Camembert and Roquefort cheese; Rennet: rennet substitutes, microbial rennet and recombinant chymosin

### **Practical**

Testing purity of starter cultures by gram's staining, catalase test; creatine test. Testing starter activity by dye reduction tests, Horrall-Elliker, White Head & Cox test. Preparation of single and mixed starter cultures. Evaluation of homo-fermentation and hetero-fermentation separately and in combination. Preservation of starter cultures by freeze-drying techniques. Preparation of concentrated starter (DVS). Effect of physical factors (temperature, pH, Salt and Sugar) on dairy starters. Testing milk for presence of inhibitory substances using *B. stearothermophilus* and *S. thermophilus* as indicator organisms. Effect of presence of antibiotic residues in milk on starter activity. Evaluation of associative growth of Starter cultures in milk. Detection of Bacteriophages in cheese whey by plaque assay method. Preparation and microbial examination of dahi, yoghurt, cultured butter milk, acidophilus milk and kefir. Analysis of cheese for total spore and anaerobic spore count. Microbiological analysis of cheddar cheese at different stages of manufacture of (storage and ripening).

## **5. Quality and Safety Monitoring in Dairy Industry 3(2+1)**

### **Theory**

Consumer Awareness about Microbiological Quality and Safety of Dairy Foods: Changing scenario; Concepts of quality control, quality assurance and food safety; Global quality and food safety standards, Integrated food law, its main features and functions. Introduction to Food Safety Management System: Concepts of Quality Management System (QMS)–ISO: 9000:2000; Principles of QMS; Standard requirements for QMS; HACCP concept and principle with special reference to biological hazards in dairy foods, TQM tools and techniques. Microbiological Risk Analysis Concepts: Risk assessment, risk management and risk communication; risk profiling of dairy products; Microbiological criteria and two and three class sampling plan / guidelines; Bio-safety concepts in handling of dairy pathogens and setting up of a microbiological/ pathogen lab in a dairy plant. Rapid Enumeration Techniques: Enumeration principles and procedure for rapid detection of predominant hygiene indicator organisms and pathogens like *E. coli* (*E. coli* 0157:H7), *Salmonella*, *Shigella*, *Staphylococcus aureus*,

*Bacillus cereus* and *Listeria monocytogenes*. Role of Biosensors for monitoring hygiene and safety of dairy foods: Detection of antibiotic residues in milk –Delvo SP, MDR test, penzyme test, charm assay, lateral flow assay (ROSA test) etc. Detection of aflatoxins, pesticides other inhibitors etc. and their public health importance in dairy foods. Plant and equipment hygiene: Concepts of hygiene and sanitation, microbial quality of water and environmental hygiene in dairy plant, chlorination of dairy water supply, quality of air, personnel hygiene, treatment and disposal of waste water and effluents.

### **Practical**

Rapid detection of total plate count, yeast and mold counts, Coliform, *E. coli*, Enterococci, Enterobacteriaceae count using D- count and 3M Petrifilm kits. Rapid detection of pathogenic bacteria based on antigen antibody principle: Staphylococcal enterotoxins, *E. coli* O157:H7, *Listeria monocytogenes* and *Salmonella* using VIDAS system. Rapid detection of antibiotic residues in milk using Delvo SP, MDR test, Charm assay, Lateral flow assay (ROSA test). Rapid detection of aflatoxin M1/ pesticides residues in milk using Charm Assay, Lateral Flow Assay (ROSA test) / Enzyme Inhibition Assay using Luminometer. Evaluation of common sanitizing agents used in dairy plants by a) suspension b) capacity test. Microbiological tests for assessing Environmental, equipment and personnel hygiene by swab and rinse methods. Determination of BOD in dairy waste water. Quality evaluation by HACCP in the preparation of dairy products.

## **6. Food and Industrial Microbiology 3(2+1)**

### **Theory**

Scope of food microbiology: Basic aspects, history and scope of food microbiology. Intrinsic and extrinsic factors that affect microbial growth in different foods. Microbial Spoilage of foods: Microbial spoilage of fruits, fruit juices, vegetables, cereals, meat, poultry, sea foods, carbonated soft drinks, canned foods; Sources of contamination; Control of spoilage. Food preservation: Principles of food preservation : physical methods viz. low temperature and high temperature preservation (D, Z and F Values); Drying Methods; Chemical preservatives, Natural antimicrobial compounds and bio-preservation; Mode of action of various preservation methods on microbes. Fermentation processes: Fermentation processes : Historical development, the range, components and types (i.e. submerged, surface and solid state fermentation); criteria for selection of industrially important microorganisms; preservation and improvement of industrially important micro-organisms using metabolic engineering/genetic engineering; media for industrial process; upstream and downstream processing. Types of fermenters: Fermenters: types (batch, fed batch and continuous), functions, design and control; sterilization; growth rate analysis, estimation of biomass; difference in chemostat and turbidostat. Microbial production of industrial products: Immobilization of enzymes/cells; Microorganisms and processes involved in the production of single cell protein and industrial alcohol, beer and wine; organic acids (citric and lactic), enzymes (protease, lipase and rennet), vitamin (B12), antibiotics and bacteriocins; and fermented whey beverages.

**Practical**

Microbiological examination of: 1) fresh and canned fruits, vegetables and juices; 2) flour and bread; and 3) eggs and meat. Isolation of psychrophilic, salt and sugar tolerant microorganisms from foods. Isolation of industrially important microorganisms from environment. Determination of Z, D and F values. Production and assaying of microbial enzymes (protease/ lipase). Production of lactic acid from whey. Production of nisin and assaying the antimicrobial activity of the culture. Design and control of a table-top and 10 liter lab fermenter (Demonstration). Production of ethyl alcohol from molasses and whey by yeasts. Production of fermented whey beverages. Educational tour to food processing/ fermentation industries.