Chapter 2

Characteristics of Predominant Microorganisms in Foods

A. Objectives

It is mentioned in chapter 1 that foods harbor (beside sterile foods) many types of bacteria, yeasts, molds, and viruses. They have important roles in: food spoilage (except viruses), food-borne diseases (except yeasts), and production of fermented foods and food additives (except viruses). It is important to classify them and study their morphological and physiological characteristics. These are discussed in this chapter. These information will be helpful in controlling their growth or stimulating growth or their destruction.

[It is important to note that several types of algae, protozoa, and helminthes (worms) and prion (proteinaceous infective particles) are important in foods and are discussed in other chapters].

B. Discussion Topics

1. **Classification systems for bacteria and fungi (yeasts and molds)**

    a. **Cell nucleus:** Absent in prokaryotes (bacteria); present in eukaryotes (yeasts, molds)
    b. **Prokaryotes' wall:** Made of murein (Eubacteria or bacteria), without murein (*Archaebacteria* or Archaea).
    c. **Taxonomic basis:** Families, Genera (genus), Species, Strains (descendent of a single cell/colony): Species is the basic group.
    d. **Species identification methods**: (i) **Fungi** (molds & yeasts): morphology, reproduction, cell composition, metabolic pattern, etc. (ii) **Bacteria:** morphology, Gram + ve or Gram -ve, amino acid sequence of specific proteins, base composition, nucleic acid hybridization, computerized numerical taxonomy, etc. (iii) **Viruses (Phages):** Arbitrary methods: types of diseases produce (Hepatitis virus), nucleic acid present (DNA or RNA and single stranded or double stranded), morphology, etc.

2. **Nomenclature Systems: Molds, Yeasts, and Bacteria:** Latinized binomial name with Species name first and then an epithet. Example: *Lactococcus lactis* (in italics or underlined with first letter of species capitalized)

    a. Special situations: A Trinomial name for subspecies (ssp): *Lactococcus lactis* ssp *lactis*, *Lactococcus lactis* ssp *cremoris* (the two differ for a very few characteristics)
    b. Biovar: *Lactococcus lactis* ssp *lactis* biovar diacetylactis (produces diacetyl).
    c. Strain: A pure culture of a species, originating from a single cell (produced a colony); should have at the end a specific number (alphabetical, numerical or both): *Listeria monocytogenes* ATCC 19166 (ATTC: American Type Culture Collection)
    d. Name approval of bacteria: According to the International Code of Nomenclature of

Bacteria. Approved names are listed in the book: Bergey's Manual of Systemic Bacteriology.

e. **Name abbreviation:** When used more than once, species is abbreviate: in this book, a three letter system is used. *Lac. lactis* [*Lac.* for *Lactococcus*]

f. **Viruses (Phages):** In place of taxonomic system, a numerical, alphabetical or a combination is used: T4, Hepatitis A.

3. Morphology and Cell Structures:

a. **Morphology (Fig.2.1):** (i) **Molds**: Larger cells, filamentous, branched, multicellular, non-motile. Filaments (hyphae) are collectively called mycelium, form spores. (ii) **Yeasts:** Cell size medium, non-motile, single cell with or without buds, oval, spherical, or elongated (appearing as chains of cells). (iii) **Bacteria:** Small cells (spherical, rod-shaped bacillus, spiral or curved), unicellular (can be in clusters, chains, or tetrads), some are motile, some form spores.

b. **Cell Structures:** (i) **Molds:** Cell wall contains cellulose or chitin or both; membrane contains sterols; streaming cytoplasm has nucleus, mitochondria, vacuoles, 80S ribosomes and chromosomes. Reproductive hypha carries spores exospores in conidia or sporangium. (ii) **Yeasts:** Glycans, proteins, and lipid in rigid wall, nucleate, cytoplasm contain 80S ribosomes, and chromosomes; cells reproduce mostly by budding. (iii) **Bacteria:** [**Fig. 2.2**] In Gram positives, cell wall has peptidoglycan and teichoic acid, some with a surface layer protein, and the cytoplasmic membrane. In Gram negatives, cell membrane, middle membrane, and inner or cytoplasmic membrane. Each cell has a circular DNA and may have plasmids. Contain 70S ribosomes. **(iv) Viruses (phages):** Have protein structure and open ended RNA or DNA

4. Important Microbes in Foods:

a. **Mold genera:** examples: some are: *Aspergillus*, *Alternaria*, *Mucor*, *Penicillium*, and *Rhizopus*. Importance: most cause food spoilage; some species/strains are used to produce foods and food additives, some produce carcinogenic mycotoxins.

b. **Yeast Genera**: Examples: Some are *Saccharomyces*, *Torulopsis*, *Candida, Pichia,* and *Rhodotorula.* Importance: Cause food spoilage, many are used in food production and to produce additives.

c. **Viruses (Phages):** Examples: Hepatitis virus, Norovirus, lactic phages. Importance: Enteric diseases, fermentation failure by lactic phages, phage typing to detect foodborne pathogens, biocontrol of foods. Protozoan (not microorganisms) species: Enteric diseases caused by *Giardia, Cryptosporidium, Cyclospora, Toxoplasma*, etc.

d. **Bacterial Genera:** [Table 2.1] Bergey's manual]; Gram-negative aerobes: *Campylobacter, Pseudomonas, Acetobacter, Moraxella, Flavobacterium, Alcaligenes*, *Brucella*, etc. (contain some psychrotrophic spoilage and food-borne pathogenic species/strains); Gram-negative facultative anaerobes: *Escherichia, Enterobacter, Klebsiella, Proteus, Salmonella, Shigella, Yersinia*, *Vibrio*, *Aeromonas* etc. (contain many foodborne pathogenic and spoilage species/strains); Rickettsias: *Coxiella burnetii* associated with Q-fever; Gram- positive cocci: *Staphylococcus, Streptococcus, Enterococcus*, *Lactococcus*, *Leuconostoc*, *Pediococcus* etc. (many species/strains are used in food fermentation, some are pathogens). Gram-positive endospore forming rods: *Bacillus*, *Clostridium* (species/strains are important in spoilage and food-borne diseases); Gram-negative endospore forming rods: *Desulfotomaculum* (spoilage); Gram-positive non-sporulating regular rods: *Lactobacillus, Carnobacterium, Brochothrix, Listeria* (some species/strains are used in food fermentation, some are pathogens). Gram-positive non spore-forming irregular rods: *Corynebacterium, Propionibacterium,*

Bifidobacterium (some are used in food fermentation). New Genera: With the availability of better identification techniques, several new bacterial genera have been created from the existing genera. Some examples are: *Tetragonococcus* (from *Pediococcus*), *Vagococcus* (from N-group *Streptococcus*), *Oenococcus* (from *Leuconostoc*), *Shewanella* (from *Pediococcus)*

5. Important bacterial group in foods (for common characteristics; no taxonomic importance):

a. **Lactic acid bacteria:** produce large amounts of lactic acid; *Lactobacillus* species
b. **Proteolytic bacteria:** hydrolyze proteins; *Pseudomonas* species
c. **Thermophilic bacteria:** grow at moderately high temperature; *Bacillus* species
d. **Psychrotrophic bacteria:** grow at refrigerated temperature. Some *Leuconostoc* species
e. **Thermoduric bacteria:** survive high temperature. *Clostridium* species
f. **Gas producing bacteria:** produce gas. *Desulfotomaculum* species.
g. **Spore formers:** form intracellular spores. *Clostridium* and *Bacillus* species.
h. **Aerobes:** require oxygen for growth. *Bacillus* species
i. **Anaerobes:** cannot grow in oxygen. *Clostridium* species
j. **Facultative anaerobes:** can grow both in the presence and absence of oxygen; *Lactobacillus* spp.
k. **Coliforms:** *Escherichia* and related enteric species used as sanitary index.
l. **Fecal coliforms:** Mainly *Escherichia coli.*
m. Enteric pathogens: Pathogenic microbes that can inhabit in the digestive system and cause food-borne diseases through fecal contamination. *Salmonella* spp.

C. Summary

The following aspects are discussed in this chapter: Differentiation of microbes important in foods on the basis of their morphology and physiology and important roles in foods. One main aim is to control their access to food by knowing the sources from where microbes get in foods. This aspect is discussed in Chapter 3.

D. Answer to chapter questions

1. Morphology, Gram stain characteristics, biochemical profile, base composition, nucleic acid hybridization. In nucleic acid hybridization, homology over a stretch of 1500 bases in 16S rRNA of the strains is compared, as they are the most conserved sequence.

2. A family contains several related species of bacteria. *Enterobacteriaceae.* A genus contains several related species. *Lab. plantarum, Lab. acidophilus* etc. A species (or subspecies) can contain several strains with common characteristics: *Esc. coli.* A strain, originally isolated from a single cell (colony) of a species, is a pure culture. A species can have many strains, one of which is the Type strain, and is used to compare a new isolate; ex: *Listeria monocytogenes* ATCC 19116 (ATCC: American Type Culture Collection). Each species should be identified with a numerical or alphabetical or a combination of both: *Pediococcus acidilactici* LB42. When the same species /strain is being written more than once, three alphabets from genus part is used: *Ped. acidilactici* LB42.] Subspecies (ssp): Over a period a species may produce cells that differ in a few genetic and physiological characteristics. They are designated as subspecies (abbreviated: subsp. or ssp.): *Lactococcus lactis* ssp. *lactis; Lactococcus lactis* ssp. *cremoris.*

(a) **Biovar:** Two strains differ in one biological trait: diacetyl producer of *Lactococcus lactis* ssp. *lactis* is designated as *Lactococcus lactis* ssp. *lactis* biovar diacetylactis (others types: phagovar,

serovar)
(b) **Plural forms:** lactobacilli; staphylococci; enterococci; leuconostocs; listeriae; salmonellae (not in italics; unless in the beginning of a sentence, first letter is not capitalized).

3. **Morphology: (a)** Yeasts: Oval to elongated, non-motile cells (average size: 20 micron), nucleated (one per cell), **(b)** Molds: Large (100 or more microns), filamentous, non-motile hypha, septate / nonseptate. (c) Bacteria: Small; round (coccus), elongated (bacillus) or curved, non-nucleate cells (1 to 5 micron) motile or non-motile, single or in chains, forming spores (bacillus, one per cell). (d) Phages: Phage particles have less than 100 millimicron, each with a round or hexagonal head, containing single stranded DNA or RNA and a tail, multiply inside the infected bacterial cells.

4. **Chemical composition of bacterial cell wall differs.** Gram-positive cell walls have thick rigid layers mucopeptide and teichoic acid, and in some species, surface layer proteins. Gram-negative cell walls have lipopolysaccharide, lipoprotein, phospholipids containing outer membrane, and a thin mucopeptide layer. Gram stained cells will look different under microscope: Gram-positive: Purple, as the wall retains the crystal violet stain. When cells are washed with alcohol, Gram–negative appears as pink, as the cells loose crystal violet stain in alcohol wash and take pink color as a counter stain.

5. **Molds**: *Aspergillus flavus, Geotrichum candidum, Penicillium roquefortii, Mucor rouxii.* Yeasts: *Saccharomyces cerevisiae, Candida lipolyticum*.

6. **Protozoa:** *Cyclospora cayetanensis, Giardia lamblia, Cryptosporidium parvum, Toxoplasma gondii.*

7. **Importance of bacteriophages:** Fermentation failure; foodborne diseases, pathogen identification.

8. **Genera of: (a)** Gm - ve aerobic rods: *Acetobacter; Alcaligenes* (b) Gm – ve facultative anaerobic rods: *Escherichia*; *Enterobacter* (c) Gram positive cocci: *Lactococcus; Staphylococcus*. (d) Gram-positive endospore forming rods: *Bacillus, Clostridium* (e) Gram- positive nonsporulating rods: *Lactobacillus, Listeria*

9. **Bacterial groups: (a)** Lactic acid bacteria: Produce large amounts of lactic acid from simple carbohydrates. (b) *Lactobacillus plantarum* (c) Coliforms: Enteric origin. *Enterobacter aerogenes* (d) Proteolytic bacteria: Hydrolyze proteins by proteolytic enzymes. *Clostridium perfringens* (e) Psychrotrophic bacteria: Grow at refrigeration temperature. *Pseudomonas putrefaciens*. (f) Enteric pathogens: Pathogenic bacteria of enteric origin. *Salmonella* Enteritidis

E. Supplemental question bank with answers:

1. True or False:

a. Eukaryotes have nucleus, but prokaryotes do not have nucleus (T)
b. Molds, yeasts, bacteria , phages: all have defined nucleus (F)
c. Certain bacterial species can move in liquid media (T)
d. Microaerophiles can grow both in the presence and absence of oxygen (F)
e. Halotolerant microorganism can be and halophilic (T)

2. Fill the blanks:

a. A bacterial strain contains identical cells, as it is obtained from a :_____(single cell)
b. Cell wall of Gram-positive bacteria have:____,__,___(SLP, mucopeptide, TA)
c. While molds form exospores, spore forming bacteria form _____(endospores)
d. On the basis of oxygen need for growth bacteria are grouped as: _____(Aerobic, Anaerobic, Microaerophilic)
e. A Type strain should be written as follows: _____ (*Pediococcus acidilactici* LB42).

3. Multiple choices:

a. One group with 70S ribosomes: Molds, Yeasts, Bacteria, Bacteriophages, Protozoa
b. Which one is not known to cause food-borne diseases: Molds, Yeasts, Bacteria, Phage, Protozoa.
c. Among yeasts, molds, bacteria, & phages, selected strains can be used in food processing: From all; from only bacteria; from bacteria and yeasts; from bacteria, yeasts, molds.
d. Psychrotrophic bacteria are differentiated from psychrophiles by their ability to grow at at very low temperature, refrigeration temperature, room temperature
e. A bacterial strain, surviving in salt environment, is halophilic, halotolerant, halogenic

4. Differentiate bacterial groups: (answer in the last part in Chapter 2):

a. Thermoduric vs. thermophilic (at high temperature: able to survive vs. need for growth)
b. Psychrophilic vs. psychrotrophic (grow at: low temperature vs. refrigeration temp)
c. Aerobic vs. Facultative anaerobic (air: need for growth vs. can grow in presence/ absence)
d. Coliforms vs. fecal coliforms (enteric bacterial species in genera: *Escherichia, Enterobacter*, *Citrobacter, and Klebsiella* vs. mainly *Esc. coli* species)
e. Strain vs. Type strain: A species can have many strains, but a Type strain of a species is the one that conforms with all the characteristics of the species and to be used as a standard to compare a new isolate.

F. Case Study

Show growth and colony characteristics and cell morphology of molds, yeasts, and bacteria obtained from plates made in Chapter 1, using a microscope fitted with a projector.