
UNIT 1 CLASSIFICATION OF MICROORGANISMS IMPORTANT IN THE FOOD INDUSTRY: BACTERIA, YEASTS AND MOLD

Structure

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1.0 OBJECTIVES

The objectives of this unit are to enable you to understand the important genera of microorganisms associated with food. This unit gives a brief account of the morphological, physiological and cultural characteristics of various microorganisms. After going through this unit, you should be able to:

- know the various types of microorganisms;
- explain the requirements for their growth;
- learn the classification of these organisms based on their characteristics; and
- distinguish between the useful and harmful microorganisms.

1.1 INTRODUCTION

We already know that the microorganisms use our food as a source of nutrients for their own growth. This, of course can result in deterioration of the food. By increasing their numbers, utilizing nutrients, producing enzymatic changes and contributing off flavors by means of breakdown of a product or synthesis of new compounds they can “spoil” a food. When the microorganisms involved are pathogenic, their association with our food supply is critical from a public health point of view. Therefore a classification of different organisms and their growth requirements is required to prevent spoilage of foods.

1.2 VARIOUS TYPES OF MICROORGANISMS

Microbes are single-cell organisms so tiny that millions can fit into the eye of a needle. They are the oldest form of life on earth. Microbe fossils date back more than 3.5 billion years to a time when the Earth was covered with oceans that regularly reached the boiling point, hundreds of millions of years before

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dinosaurs roamed the earth. Without microbes, we couldn't eat or breathe. Without us, they'd probably be just fine.

Bacteria

Many of us know bacteria only as "germs," invisible to naked eyes that can invade our bodies and make us sick. Few know that many bacteria not only coexist with us all the time, but help us do an amazing array of useful things like make vitamins, break down garbage, and even maintain our atmosphere. These are unicellular microorganisms that are classed as plants. A bacterial cell is about $1\mu\text{m}$ in length and somewhat smaller in diameter. Bacteria are classified according to their shape. Cocci are spherical, bacilli are cylindrical and spirilla and vibrios are spiral. Bacterial spores are more heat resistant than yeast or mold spores to most processing conditions. Bacteria, with a few exceptions cannot grow in acid media in which yeasts and molds thrive. They multiply by 'binary fission'. When a bacterium becomes mature it divides into two, these two become four and so on. Bacteria can be found virtually everywhere. They are in the air, the soil, and water, and in and on plants and animals, including us. A single teaspoon of topsoil contains about a billion bacterial cells (and about 120,000 fungal cells and some 25,000 algal cells). The human mouth is home to more than 500 species of bacteria. Some bacteria (along with archaea) thrive in the most forbidding, uninviting places on Earth, from nearly-boiling hot springs to super-chilled Antarctic lakes buried under sheets of ice. Microbes that dwell in these extreme habitats are aptly called extremophiles. The growth of bacteria is very rapid and depends upon the nature of the food material, moisture, temperature and air. Some bacteria do not grow in air but temperature plays a major role in their growth, the optimum being generally 37°C for bacteria pathogenic to humans.

Bacteria are very sensitive to acids and are destroyed in their presence even at temperature of boiling water. Hence, most fruits being acidic can be easily sterilized at 100°C whereas vegetables being non-acidic require a higher temperature of 116°C .

A bacterium's genetic information is contained in a single DNA molecule suspended in a jelly-like substance called cytoplasm. In most cases, this and other cell parts are surrounded by a flexible cytoplasmic membrane that is itself surrounded by a tough, rigid cell wall. A few species, such as the mycoplasmas, don't have cell walls.

Even though bacteria have only one cell each, they come in a wide range of shapes, sizes, and colours.

The important groups of bacteria are:

- a) Bacillus: rod-shaped.
- b) Coccus: spherical.
- c) Coccobacillus: oval-shaped.
- d) Aerobes: require atmospheric oxygen for growth, e.g., *Acetobacter aceti*.
- e) Facultative anaerobes: can grow with or without atmospheric oxygen.
- f) Obligate anaerobes: do not grow in atmospheric oxygen.

- g) Mesophiles: require a temperature below 38°C for growth.
- h) Obligate thermophiles: grow between 38°C and 82°C.
- i) Facultative thermophiles: grow over a wide range of temperatures covered by mesophiles and obligate thermophiles and below.
- j) Psychrotrophs: grow fairly well at refrigeration temperatures and some can even grow slowly at temperatures below freezing.

Some bacteria have natural colours. Certain species contain pigments, such as various chlorophylls, that make them naturally green, yellow, orange, or brown. Colonies of millions of bacteria may appear pink, yellowish, or white.

Important Food Spoilage Bacteria

Group	Genus
Acetics	<i>Acetobacter</i> and <i>Gluconobacter</i>
Lactics	<i>Lactobacillus</i> , <i>Leuconostoc</i> , <i>Pediococcus</i> , <i>Streptococcus</i>
Butyrics	<i>Clostridium</i>
Propionics	<i>Propionibacterium</i>
Proteolytics	<i>Bacillus</i> , <i>Pseudomonas</i> , <i>Clostridium</i> , <i>Proteus</i> etc.

Some useful bacteria

The following bacteria are of great importance in the food processing industry.

Acetobacter sp.

These bacteria, also known as “vinegar bacteria”, cause significant spoilage in the wine industry but are necessary for vinegar production. The important species are *Acetobacter aceti*, *A. orleansis* and *A. schutzenbachi*. They are very small, usually non-motile and generally do not form spores. These bacteria are aerobes and in the presence of oxygen convert ethyl alcohol to acetic acid. These bacteria can be easily destroyed by heating to 65°C.

Lactobacillus sp.

Different organisms of this group, also known as “lactic acid bacteria”, have different properties but all of them produce lactic acid from carbohydrates. The important species include *Lactobacillus plantarum*, *Pediococcus cerevisiae*, *Leuconostoc mesenteroides*, *Streptococcus faecalis* and *Lactobacillus brevis*. These bacteria cause “lactic souring” and spoil wines, which can be easily prevented by maintaining a sulphur dioxide concentration of 0.007 per cent in wine.

Yeasts

Yeasts are unicellular fungi which are widely distributed in nature. They are somewhat larger than bacteria. The cell length is about 10µm and the diameter is about a third of this. Most yeasts are spherical or ellipsoidal. Yeasts that multiply by means of ‘budding’ are known as ‘true yeasts’. Yeasts grow luxuriously at a moderate temperature in a solution of sugar in plenty of water.

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Under suitable conditions the sugar is converted into alcohol and carbon dioxide is evolved. This is the reason that carbon dioxide is evolved from food materials spoiled by yeasts and pushes out corks from bottles with great force. Most of them do not develop in media containing more than 66% sugar or 0.5% acetic acid. Boiling destroys the yeast cells and spores completely. Some of the yeasts which grow on fruits are *Saccharomyces*, *Candida* and *Brettanomyces*.

Pseudo-yeasts

These are like true yeasts but do not form spores. All the members of this group are particularly unsuitable for fermentation purposes as they produce off-flavours and cloudiness.

Yeasts causing food spoilage

Yeast	Product Spoilage
<i>Saccharomyces</i>	Low sugar products
<i>Candida</i>	High-acid foods, salty foods, butter
<i>Brettanomyces</i>	Beers, wines
<i>Zygosaccharomyces (osmophillic)</i>	Honey, syrups, molasses, wines, soy sauce
<i>Pichia</i>	Wines
<i>Hansenula</i>	Beers
<i>Torulopsis</i>	Milk products, fruit juices, acid foods
<i>Rhodotorula</i>	Meat, sauerkraut

Fungi (Molds)

Fungi are eukaryotic organisms. This means that their DNA-containing chromosomes are enclosed within a nucleus inside their cells. (The chromosomes of bacteria and archaea are not walled off inside nuclei, making them prokaryotic organisms). Molds are multicellular, filamentous fungi which are devoid of chlorophyll. They are larger than yeasts. They are strict aerobes and require oxygen for growth and multiplication and tend to grow more slowly than bacteria.

Fungi are lower thallophytic plants but do not make their own food via photosynthesis like green plants. They feed on organic matter like rotting leaves, wood, and other debris, or upon the tissues of living plants and animals.

Fungi, along with bacteria, are the planet's major composters and recyclers. Although fungi may seem like a nuisance when they grow in your fruit bowl or refrigerator, their ability to degrade some of the toughest organic materials, including tree wood and insect exoskeletons, means that our planet is not cluttered with a mass of debris. Fungi secrete digestive enzymes in order to break down complex food sources, such as animal corpses and tree stumps, into smaller components they can absorb.

The principle parts of a mold are a web-like structure known as mycelium and the spore. The mycelium is often white and cottony and penetrates into the

attacked foodstuff. After fixing itself the mold produces viable spores which resist the favourable conditions after the dispersal and germinate when they get favourable conditions. They thrive best in closed, damp and dark situations with an adequate supply of warm, moist air but require less free moisture than yeasts and bacteria. They prefer sugar containing substances and may spoil jams, jellies and other sugar-based products. Acid medium favours their growth and, therefore, they grow well in pickles, juices etc. this is the main reason that fruit and fruit products are attacked by molds which not only consume nutrients present in the food thereby lowering its food value but also spoil the flavour, texture and appearance of the product. Molds are sensitive to heat; boiling quickly destroys molds and their spores. The most important molds are:

- a) *Penicillium* sp. (Blue moulds)
- b) *Aspergillus* sp. (Black moulds)
- c) *Mucor* sp. (Gray moulds)
- d) *Bysschlamyces fulva*

Classification of Microorganisms

A) On basis of temperature for growth

Microorganisms can be classified into:

- Thermophilic: Microbes who require high temperature for their growth and survival (optimum temperature=45-65°C).
- Thermotolerant: Microbes which do not grow at high temperatures but can survive in it.
- Mesophilic: Microorganisms which require optimum temperature of 20-50°C for growth and multiplication.
- Psychrophilic: Microorganisms requiring less than 20°C as optimal temperature for growth.
- Psychrotolerant: Microorganisms which do not grow at low temperature but can survive.

B) On basis of oxygen requirement for growth:

- Obligate Aerobes: Require oxygen for growth and multiplication e.g. molds.
- Obligate Anaerobes: Strictly grow only in absence of oxygen.
- Facultative: Microorganisms that can grow in both presence and absence of oxygen e.g. yeasts.
- Microaerophilic: Organisms which are able to grow at very low oxidation-reduction potential.

C) On basis of requirement of water activity.

In general, bacteria require more moisture than yeasts and yeasts more than molds.

The classification according to requirement of a_w is as follows:

Group of microorganism	Minimal a_w value
Bacteria	0.91
Yeast	0.88
Molds	0.80
Halophillic bacteria	0.75
Xerophillic fungi	0.65
Osmophillic Yeasts	0.60

- Halophillic bacteria: Bacteria which grow in high salt solutions
- Osmophillic Yeasts: Yeasts which can grow best in high concentrations of sugar
- Xerophillic Fungi: Fungi which can grow in low water activity

D) On basis of nutrient degradation capacity:

- Proteolytic: Microorganisms which are capable of protein degradation because of extracellular proteinases produced.
- Lipolytic: Microbes which catalyze the hydrolysis of fats to fatty acids and glycerol.
- Sacchrolytic: These microorganisms hydrolyse disaccharides or polysaccharides to simpler sugars.
- Pectinolytic: These microorganisms hydrolyse pectin.

E) On basis of staining:

On basis of staining the bacteria can be classified as:

- Gram positive: Those bacteria that stain violet after Gram stain test. In these the cell wall is mostly comprised of peptidoglycan layer.
- Gram negative: Those bacteria that do not stain violet after Gram stain test. Cell wall mainly comprised of lipopolysaccharides.



Check Your Progress Exercise 1

- Note:** a) Use the space below for your answer.
b) Compare your answers with those given at the end of the unit.

1. Classify bacteria according to their morphology.

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2. Differentiate between yeasts and molds.

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3. Classify microorganisms on basis of the temperature requirements, oxygen requirements, water activity requirement, staining procedure and nutrient degrading capability.

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1.3 CHARACTERISTICS (MORPHOLOGICAL, CULTURAL AND PHYSIOLOGICAL) OF MICROORGANISMS

1.3.1 Bacteria

Morphological Characteristics

One of the first step in the identification of bacteria in food is microscopic examination to ascertain the shape, size, aggregation, structure and staining

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reactions of the bacteria present. The following characteristics may be of special significance:

Encapsulation: The presence of capsules or slime may account for sliminess or ropiness of a food. Most capsules are polysaccharides of dextrin, dextran or levan and they serve as a source of reserve nutrients and increase the resistance of bacteria under adverse conditions.

Formation of Endospores: Bacteria of genera *Bacillus*, *Clostridium*, *Sporosarcina* etc have the ability to form endospores. Endospores are formed at an intracellular site and are resistant to heat, ultraviolet light and desiccation. Lysis of the vegetative cell releases the free endospore, which may remain dormant with no detectable metabolism for years. Sporulation usually appears in the late logarithmic phase of growth, possibly because of nutrient depletion or product accumulation. The acquisition of heat resistance is closely related to the formation of dipicolinic acid and the Ca^{2+} uptake. Germination is favoured by conditions that are favourable for growth.

Formation of Cell Aggregates: It is characteristic of some bacteria to form long chains or of others to clump under certain conditions. It is more difficult to kill all bacteria in intertwined chains or sizable clumps than to destroy separate cells.

Cultural Characteristics

Bacterial growth in and on foods often is extensive enough to make the food unattractive in appearance or otherwise objectionable. Pigmented bacteria cause discolouration on the surfaces of foods; films which may cover the surfaces of liquids; growth may make surfaces slimy; or growth throughout the liquids may result in undesirable cloudiness or sediment.

Physiological Characteristics

Most bacteria may be placed into one of three groups based on their response to gaseous oxygen. Aerobic bacteria thrive in the presence of oxygen and require it for their continued growth and existence. Other bacteria are anaerobic, and cannot tolerate gaseous oxygen, such as those bacteria which live in deep underwater sediments, or those which cause bacterial food poisoning. The third group are the facultative anaerobes, which prefer growing in the presence of oxygen, but can continue to grow without it.

Bacteria may also be classified both by the mode by which they obtain their energy. Classified by the source of their energy, bacteria fall into two categories: heterotrophs and autotrophs. Heterotrophs derive energy from breaking down complex organic compounds that they must take in from the environment – this includes saprobic bacteria found in decaying material, as well as those that rely on fermentation or respiration.

The other group, the autotrophs, fix carbon dioxide to make their own food source; this may be fueled by light energy (photoautotrophic), or by oxidation of nitrogen, sulfur, or other elements (chemoautotrophic). While chemoautotrophs are uncommon, photoautotrophs are common and quite diverse. They include the cyanobacteria, green sulfur bacteria, purple sulfur bacteria, and purple nonsulfur bacteria. The sulfur bacteria are particularly interesting, since they use hydrogen sulfide as hydrogen donor, instead of water like most other photosynthetic organisms, including cyanobacteria.

Microbe is a term for tiny creatures that individually are too small to be seen with the unaided eye. Microbes include bacteria (*back-tear-ee-uh*), archaea (*are-key-uh*), fungi (*fun-jeye*) and protists (*pro-tists*). You've probably heard of bacteria and fungi before. Archaea are bacteria-like creatures that have some traits not found in any true bacteria. Protists include primitive algae (*al-gee*), amoebas (*ah-me-buhs*), slime molds and protozoa (*pro-toe-zoh-uh*). We can also include viruses (*vye-rus-is*) as a major type of microbe, though there is a debate as to whether viruses can be considered living creatures or not.

1.3.2 Molds

General Characteristics

The term “mold” is a common one applied to certain multicellular, filamentous fungi whose growth on foods usually is readily recognized by its fuzzy or cottony appearance. The main part of the growth commonly appears white but may be coloured or dark or smoky. Coloured spores are typical of mature mold of some kinds and give colour to part or all of the growth. The thallus, or vegetative body, is characteristic of thallophytes, which lack true roots, stems and leaves.

Morphological Characteristics

The morphology, i.e. the form and structure, of molds, as judged by their macroscopic and microscopic appearance, is used in their identification and classification.

Hyphae and Mycelium: The mold thallus consists of a mass of branched, intertwined filaments called hyphae (singular hypha), and the whole mass of these hyphae are known as the mycelium.

Reproductive Parts or Structures: Molds can grow from a transplanted piece of mycelium. Reproduction of molds is chiefly by means of asexual spores. Some molds also form sexual spores.

Culture Characteristics

The gross appearance of a mold growing on a food often is sufficient to indicate its class or order. Some molds are loose and fluffy; others are compact. Some look velvety on the upper surface, some dry and powdery, and others wet or gelatinous. Some molds are restricted in size, while others seem limited only by the food or container. Pigments in the mycelium – red, purple, yellow, brown, gray black, etc. – are characteristic, as are the pigments of mass of asexual spores; green, blue-green, yellow, orange, pink, lavender, brown, gray, black, etc.

Physiological Characteristics

The physiological characteristics of molds will be reviewed only briefly here and will be discussed in more detail subsequently.

Moisture Requirements: In general most molds require less available moisture than do most yeasts and bacteria. It has been claimed that below 14 to 15 percent total moisture in flour or some dried fruits will prevent or greatly delay mold growth.

Temperature Requirements: Most molds would be considered mesophilic i.e. able to grow well at ordinary temperature. The optimal temperature for most

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molds is around 25 to 30°C, but some grow well at 35 to 37°C or above, e.g. *Aspergillus* spp. And some at still higher temperatures. A number of molds are psychrotrophic or psychroduric i.e. they grow fairly well at temperatures of refrigeration, and some can grow slowly at temperatures below freezing. Growth has been reported at as low as – 5 to 10°C. A few are thermophilic; i.e. they have a high optimal temperature.

Oxygen and pH Requirements Molds are aerobic; i.e. they require oxygen for growth; this is true at least for the molds growing on foods. Most molds can grow over a wide range of hydrogen-ion concentration (pH 2 to 8.5), but the majority are favoured by an acid pH.

Food Requirements: Molds in general can utilize many kinds of foods, ranging from simple to complex. Most of the common molds possess a variety of hydrolytic enzymes, and some are grown for their amylases, pectinases, proteinases, and lipases.

Inhibitors: Compounds inhibitory to other organisms are produced by some molds, such as penicillin from *Penicillium chrysogenum* and clavacin from *Aspergillus clavatus*. Certain chemical compounds are mycostatic, inhibiting the growth of molds (sorbic acid, propionates, and acetates are examples), or are specifically fungicidal, killing molds.

Initiation of growth of molds is slow compared to that of bacteria or yeasts, so that when conditions are favourable for all these organisms, molds usually lose out in the competition. After mold growth is under way, however, it may be very rapid.

1.3.3 Yeasts

Like mold, the term “yeast” is commonly used but hard to define. As used here it refers to those fungi which are generally not filamentous but unicellular and ovoid or spheroid and which reproduce by budding or fission.

Yeasts may be useful or harmful in foods. Yeast fermentations are involved in the manufacture of foods such as bread, beer, wines, vinegar, and surface-ripened cheese, and yeasts are grown for enzymes and for food. Yeasts are undesirable when they cause spoilage of sauerkraut, fruit juices, syrups, molasses, honey, jellies, meats, wine, beer, and other foods.

Morphological Characteristics

Form and structure: The form of yeasts may be spherical to ovoid, lemon-shaped, pear-shaped, cylindrical, triangular, or even elongated into a false or true mycelium. They also differ in size.

Reproduction: Most yeasts reproduce asexually by multilateral or polar budding, a process in which some of the protoplasm bulges out the cell wall; the bulge grows in size and finally walls off as a new yeast cell. A new species or yeasts reproduce by fission, and one reproduces by combination of fission and budding.

Sexual reproduction of “true” yeasts (*Ascomycotina*) results in the production of ascospores, the yeast cell serving as the ascus. The ascospores may differ in colour, in smoothness or roughness of their walls, and in their shape (round, oval, reniform, bean or sickle-shaped, hemispherical, angular, fusiform, or needle-shaped).

“False” yeasts, which produce no ascospores or other sexual spores, belong to the *Fungi Imperfecti*. Cells of some yeasts become chlamydospores by formation of a thick wall about the cell, for example, *Candida*, *Rhodotorula*, and *Cryptococcus*.

Cultural Characteristics

For the most part, the appearance of massed yeast growth is not useful in the identification of yeasts, although growth as a film on the surface of liquid media suggests an oxidative or film yeasts, and production of a carotenoids pigment indicates the genus *Rhodotorula*. However, the appearance of the growth is important when it causes coloured spots on foods.

Yeasts are oxidative, fermentative, or both. The oxidative yeasts may grow as a film, pellicle, or scum on the surface of liquid and then are termed *film yeasts*. Fermentative yeasts usually grow throughout the liquid and produce carbon dioxide.

Physiological Characteristics

Most common yeasts grow best with a plentiful supply of available moisture. But since many yeasts grow in the presence of greater concentration of solutes (such as sugar or salt) than most bacteria it can be concluded that these yeasts require less moisture than the majority of bacteria. Most yeast require more moisture than molds, however, on the basis of water activity or a_w yeasts may be classified as ordinary if they do not grow in high concentrations of solutes, i.e. in a low a_w , and as osmophilic if they do. However limits of a_w for ordinary yeasts tested thus far ranges from 0.88 to 0.94.

The range of temperature for growth of most yeasts is, in general, similar to that for molds, with the optimum around 25°C to 30°C and the maximum about 35°C to 47°C. Some kinds can grow at 0°C or less. The growth of most yeasts is favoured by an acid reaction in the vicinity of pH 4 to 4.5, and they will not grow well in an alkaline medium unless adapted to it. Yeasts grow best under aerobic conditions, but the fermentative types can grow anaerobically, although slowly.

In general, sugars are the best source of energy for yeasts, although oxidative yeasts, e.g., the film yeasts, oxidize organic acids and alcohol. Carbon dioxide produced by bread yeasts accomplishes the leavening of bread, and alcohol made by the fermentative yeasts is the main product in the manufacture of wines, beer, industrial alcohol, and other products. The yeasts also aid in the production of flavors or “bouquet” in wines.

Nitrogenous foods utilized vary from simple compounds such as ammonia and urea to amino acids and polypeptides. In addition, yeasts require accessory growth factors.

Microorganisms, namely, bacteria, yeasts and molds can be found in any environment. The eight environmental sources of organisms to foods are: soil and water, plants and plant products, food utensils, intestinal tracts of humans and animals, food handlers, animal feeds, animal hides, air and dust. Although we see that the microorganisms are beneficial to the humans in many ways, there are many microorganisms that are the causative agents for food borne diseases. e.g. *Staphylococcus aureus* and *Clostridium botulinum* cause food borne intoxication whereas *Salmonella*, *E.coli*, *Campylobacter*, *Listeria*,

Yersinia, *Bacillus* etc cause food borne infections. Molds are responsible for causing food intoxication by production of mycotoxins, which are lethal for the human body e.g. Aflatoxin produced by *Aspergillus flavus*, patulin produced by *Penicillium expansum*, ochratoxins produced by *Aspergillus ochraceus* etc. All these will be discussed in Unit 3.



Check Your Progress Exercise 2

Note: a) Use the space below for your answer.
b) Compare your answers with those given at the end of the unit.

1. Explain the formation of special structures by bacteria.

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2. What are the physiological requirements of molds?

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3. Yeasts maybe useful or harmful. Explain.

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1.4 LET US SUM UP

This unit briefly outlines the identification and classification of food microorganisms. After reading this unit, you will be able to classify microorganisms broadly into three categories: bacteria, yeasts and molds. Further you will be able to categorize these microorganisms on the basis of their requirements of temperature, oxygen, water activity for growth and also on the basis of their ability to degrade certain nutrients. To prevent the spoilage

of food products, appropriate measures have to be taken for preventing their growth and multiplication. Hence food microbiologists have to be well versed with the various morphological, cultural and physiological characteristics of the different microorganisms so as to prevent their growth and proliferation.

After reading this unit, you will get a knowledge of the factors that favour or inhibit the growth of microorganisms which are essential to give an understanding of the principles of food spoilage and preservation.

1.5 KEY WORDS

- Bacteria** : Unicellular microorganisms 1µm in length, lacking chlorophyll and multiply by binary fission.
- Yeasts** : Unicellular fungi, larger than bacteria, which multiply by budding.
- Molds** : Multicellular, filamentous fungi devoid of chlorophyll.
- Endospore** : Heat resistant structures formed by bacteria under unfavourable conditions (nutrient depletion or product accumulation) which remain dormant till exposed to favourable environment.
- Capsule** : Slimy material composed of polysaccharides that serve as a source of reserve nutrients and make cell resistant to adverse conditions.
- Mycostatic** : Substances which are inhibitory to mold growth.
- Fungicidal** : Substances capable of killing the fungi.
- Thermophillic organisms** : Microorganisms which require high optimum temperature for their growth and multiplication (45-60°C).
- Mesophillic organisms** : Microorganisms whose optimum temperature for growth is 25-40°C.
- Proteolytic microorganisms** : Microorganisms that have the capacity to break down complex proteins to amino acids due to production of extracellular protein degrading enzymes.
- Gram positive bacteria** : Bacteria that stain violet after Gram staining.
- Gram negative bacteria** : Bacteria that stain red after Gram staining.

- Psychrophilic microorganisms** : Microorganisms requiring less than 20°C as optimal temperature for growth.
- Obligate aerobes** : Microorganisms that grow and multiply only in presence of oxygen.
- Obligate anaerobes** : Microorganisms that grow and multiply only in absence of oxygen.
- Facultative microorganisms** : Those microorganisms that can grow and survive under both aerobic and anaerobic conditions.
- Microaerophilic microorganisms** : Those microorganisms that can grow and survive under low concentrations of oxygen.
- Thermotolerant microorganisms** : Those microorganisms that can survive at high temperatures but grows in mesophilic range.



1.6 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

- On basis of morphology bacteria can be classified as:
 - Bacilli: rod-shaped
 - Coccus: spherical
 - Coccobacillus: oval-shaped
 - Spirilla: spiral shaped
- Yeasts are unicellular fungi and are smaller in size whereas molds are multicellular and filamentous and are larger than yeasts. Yeasts multiply asexually by budding whereas molds reproduce sexually by spores. Yeasts can grow in anaerobic conditions but molds are strictly aerobic.
- Classification of microorganisms on basis of temperature requirement:
 - Thermophilic(45-60°C)
 - Thermotolerant
 - Mesophilic (25-40°C)
 - Psychrotolerant
 - Psychrophilic

Classification of microorganisms on basis of oxygen requirement:

- Obligate aerobes: Strictly grow in presence of oxygen
- Obligate anaerobes: Strictly grow in absence of oxygen
- Facultative: Can grow both in presence and absence of oxygen
- Microaerophilic: Grow at low oxygen concentrations

Classification of microorganisms on basis of a_w requirement:

- a) Bacteria ($a_w = 0.9$)
- b) Yeast (0.88)
- c) Mold (0.80)
- d) Halophilic bacteria (0.75)
- e) Xerophilic molds (0.65)
- f) Osmophilic yeasts (0.60)

Classification of microorganisms on basis of nutrient degradation:

- a) Lipolytic: Fat degrading
- b) Saccharolytic: Sugar degrading
- c) Pectinolytic: Pectin degrading
- d) Proteolytic: Protein degrading

Classification of microorganisms on basis of staining:

- a) Gram positive bacteria: Stain violet after staining
- b) Gram negative bacteria: Stain red after Gram staining

Check Your Progress Exercise 2

1. Bacteria form special structures such as endospores, capsules and cell aggregates to combat the adverse environmental conditions. Bacteria of genera *Bacillus*, *Clostridium*, *Sporosarcina* etc have the ability to form heat resistant endospores. Sporulation usually appears in the late logarithmic phase of growth, possibly because of nutrient depletion or accumulation of toxic products. Endospores may remain dormant with no detectable metabolism for years and germinate when exposed to favourable conditions for growth. Capsules serve as a source of reserve nutrients and increase the resistance of bacteria under adverse conditions. They are composed of dextran and levan and account for sliminess or ropiness of a food. Bacteria may also form aggregates which helps them to combat unfavourable environments.
2. Physiological requirements of molds: Molds are mesophilic and grow well at 25-30°C. Some are psychrotrophic and can grow at -5 to 10°C. They are aerobic in nature and can grow at wide range of pH 2-8.5. They require a minimum moisture range of 14-15 per cent to grow. Owing to a number of hydrolytic enzymes, molds can proliferate on complex media also.
3. Yeasts may be useful or harmful in foods. Yeast fermentations are involved in the manufacture of foods such as bread, beer, wines, vinegar, and surface-ripened cheese, and yeasts are grown for enzymes and for food. Yeasts are undesirable when they cause spoilage of sauerkraut, fruit juices, syrups, molasses, honey, jellies, meats, wine, beer, and other foods.

1.7 SOME USEFUL BOOKS

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