Manual of Training Programme on Seafood Quality Assurance,CIFT Cochin 14-26 Sep.:8 (1998),(17-24)

FUNDAMENTALS OF BACTERIOLOGY

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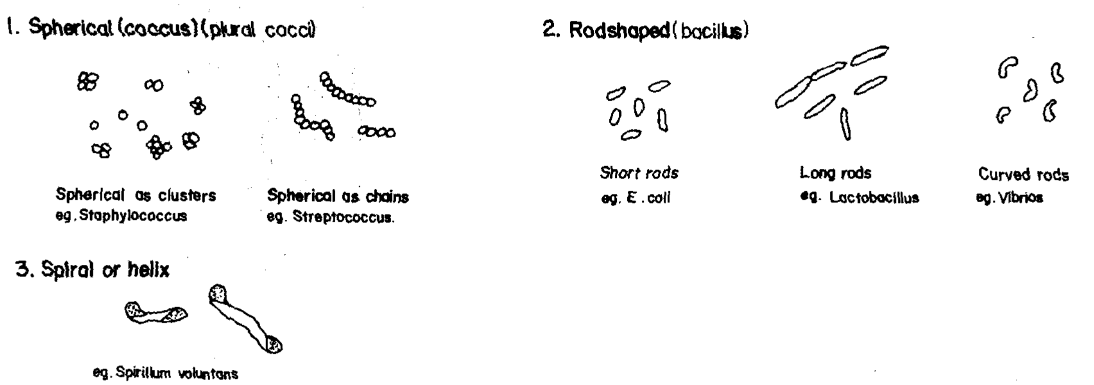
Microorganisms are living things, which are individually too small to see without the aid of microscope. They can be classified into five general groups.

1. Viruses
2. Bacteria
3. Fungi
4. Algae
5. Protozoa

Bacteria(bacterium singular) are widely distributed in nature. They are present in the intestinal tract and body surface of animals, plants, soil, air and the natural waters such as ponds, lakes, rivers and the sea. There are both useful and disease producing bacteria. Useful bacteria bring changes including decomposition of dead tissues of plants and animals. The harmful bacteria cause various types of diseases in man, animals and plants. Bacteria decompose almost all animal and vegetable food products, if they are not properly preserved.

Morphology of bacteria

Figure 1 Presents the morphology of bacteria. The bacterial cell can have several shapes, but the most commonly found ones are: Spherical (coccus) (plural-cocci), rod shaped (Bacillus) and spiral (or helix) forms



Size

The diameter of coccus forms may vary from 0.5 - 2.5 microns (m). One micron is equivalent to 1/1000 of a millimeter or 1/25,000 of an inch. In rod-like species the variations are much greater ranging from 0.2 to 2.0m in width and from 1 to 15m in length. A few rods and spiral forms are much larger measuring up to 100m.

Bacterial cell

It is generally accepted that living cells of all kinds have at least three things in common; some form of outer wall or membrane, the cytoplasm and the nuclear material, each making its own contribution to the life of the cell.

The outer part of the bacterial cell is made up of three definite, demonstrable structures, which are considered as cell wall, cytoplasmic membrane and the slime layer or capsule.

Fimbriae

Fimbriae are filamentous structures on the surface of gram-negative organisms composed largely of protein . Some Organisms have one or two large fimbriae per cell, which are involved in sexual conjugation and others have numerous small fimbriae involved in adhesion and pathogenicity.

Flagella

Flagella are organs of locomotion found in most of the bacteria. But some bacteria for example, *Myxobacteria* and *Cyanobacteria* although, motile do not posses flagella. They can move up to 18 cm/hour. In some bacteria they occur as one or two polar flagella as in the case of *Pseudomonas* spp. In others they occur in large numbers over the whole cell surface (peritrichous). They are semi rigid and can rotate.

Nuclear material: the nucleoid

Unlike eucaryotic cell, a membrane does not surround bacterial DNA. Only a single nuclear body is present in some cells where as in others, as a result of nuclear division preceding cell division, two, four or even more nuclear bodies are present and their shape can be round, ovoid or irregular. The nucleoid is revealed by special staining procedures. The nuclear bodies replicate by growth and simple fission and not by mitosis. They have no nucleolus. The genetic information of a bacterial cell is contained in a single long molecule of double stranded deoxyribonucleic acid (DNA).

Cytoplasm

The cytoplasm of the bacterial cell is a viscous watery solution or soft gel containing a variety of organic and inorganic solutes and numerous small granules called ribosomes. The cytoplasm of bacteria differs from that of the higher eukaryotic organisms in that it does not contain endoplasmic reticulum or mitochondria.

Spores

Under certain conditions some bacteria produce endospores for example, *Bacillus* spp. and *Clostridia* spp. They are formed within the cell. The spores survive adverse conditions. They are more resistant, than the vegetative cell, to heat and certain types of radiation and chemicals. Usually some of the rod-shaped bacteria produce such spores. When favourable conditions restore, the spores germinate to form the original vegetative cell. Spore forming organisms are of particular importance in heat-processed foods.

Bacterial growth

When a small number of organisms are taken from a culture and inoculated into a fresh growth medium the number of cells inoculated may multiply a million-fold or more during growth. The number of cells present at different times is measured and plotted in relation to time as a growth curve. Two types of growth curves can be drawn based to the cell number. A total count is based on the number of cells present, irrespective of whether they are living or not. Viable count measures only those cells capable of growing and producing a colony on suitable growth medium.

A typical growth curve is shown below, from which four main phases of growth can generally be recognized. (Fig 3)

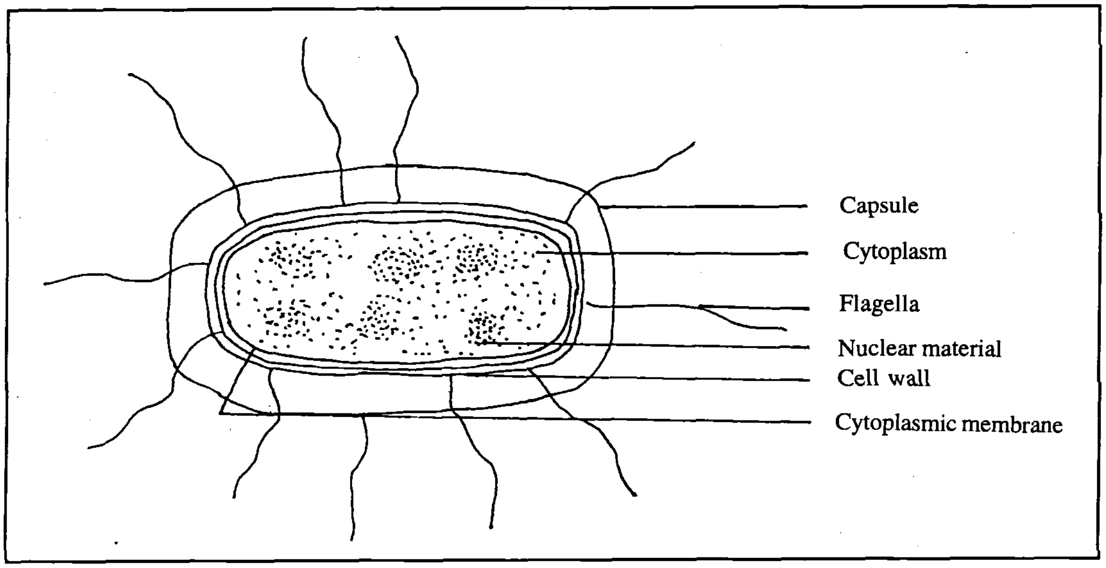


Fig.2 A cross section of a bacterial cell

Cell wall

The shape of the bacterial cell is due to the rigid cell wall. It surrounds the delicate cytoplasmic membrane, which encloses the cytoplasm. Cell wall protects the cell from osmotic lysis. It may also participate in the uptake of ions and nutrients and act as a barrier to loss and entry of some molecules.

Bacteria can be classified into two categories on the basis of the Gram's staining reaction. They are:

a) Gram-positive organisms : Organism that retains the basic dye - crystal violet. for example, *Staphylococcus* spp.

b) Gram-negative organisms : Organism does ~~not take~~ take up the counter stain usually safranin dye is used. For example, *Vibrio* spp.

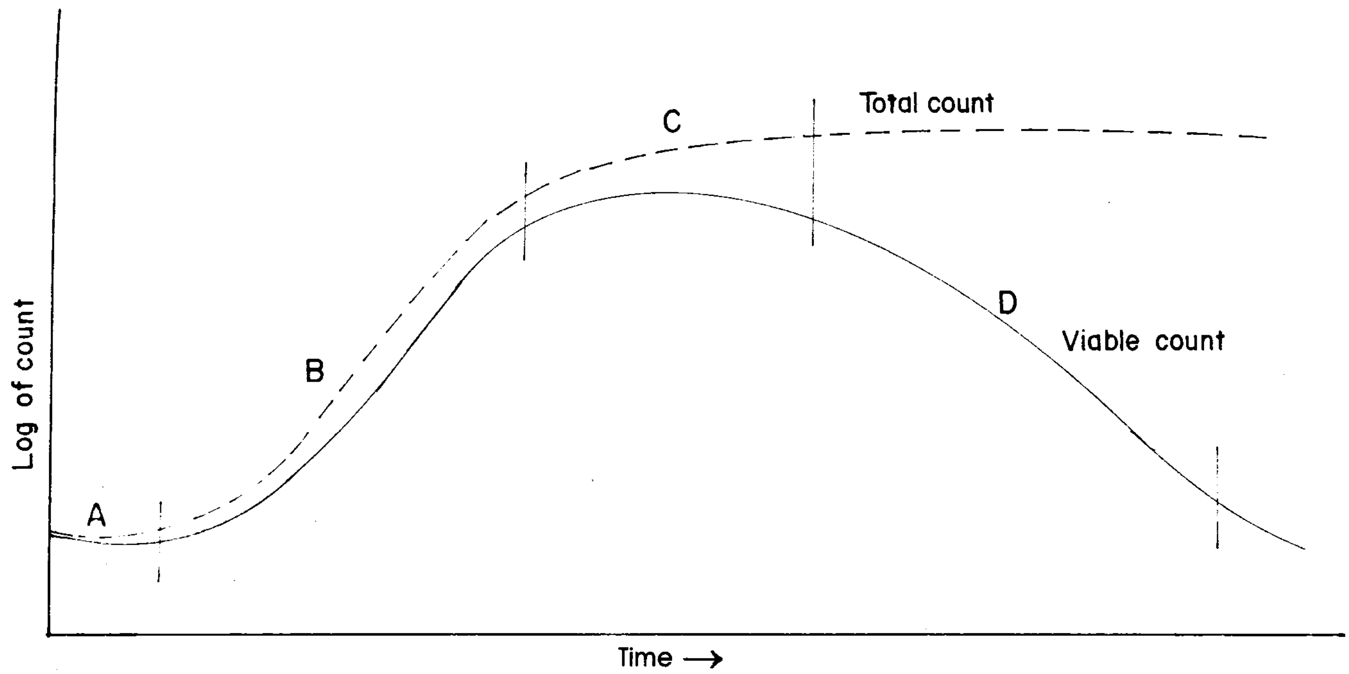
The response of an organism to Gram stain reflects the fundamental difference between the cell wall of gram-positive and gram-negative organisms.

Cytoplasmic membrane

Cytoplasmic membrane constitutes an osmotic barrier that is impermeable to many small molecular solutes and is responsible for maintaining the difference in solute content between the cytoplasm and the external environment. It permits the inward and outward passive diffusion of water and certain other molecular substances especially lipid soluble ones. The selective transport of specific nutrient solutes into the cell and the waste products out of it is actively controlled by the cytoplasmic membrane. In addition to the enzyme responsible for the active uptake of nutrients, the membrane contains many other enzymes, notably respiratory enzymes and pigments. It has little mechanical strength and is supported on the outside by the cell wall.

Capsule or slime layer

In many gram-positive and gram-negative bacteria the outermost protective layer is a mucilaginous material known as capsule, which is composed of 99% water and 1% carbohydrate. The capsule material has several functions such as imparting, virulence, prevention of phagocytosis, protection against desication, adhesion and as a reserve food material.



A - Lag phase
B - Log phase
C - Stationary phase
D - Death phase or phase of decline

Fig. 3 The four faces of the growth curve

a. Lag phase: In this phase there is no appreciable multiplication of cells, although they may increase considerably in size and show marked metabolic activity. In this phase the organisms become accustomed to their environment.
b. Log phase: (logarithmic) or exponential phase: In this period, the cells divide at rapid and constant rate. Growth is maximum during this phase.
c. Stationary phase: After the log phase the exponential growth declines. The rate of multiplication decreases until it is balanced by the death rate. Cell number reaches the maximum that the environment can support. This is known as the stationary phase.
d. Death phase or phase of decline: The cells in a culture begin to die at this phase. This is because of the depletion of essential nutrients in the medium and accumulation of toxic metabolic products.

Factors affecting bacterial growth

Growth of bacteria is influenced by the environmental conditions and availability of nutrients. The environmental conditions include temperature, pH, presence or absence of oxygen and moisture.

Effect of temperature

Bacterial growth is influenced by temperature. Each species of bacteria has a preferred temperature regemen. Accordingly they are divided into the following groups.

a. Psychrophiles or Psychrotrophs: As the names indicate, these are cold-loving bacteria and can grow below 20°C with an optimum growth temperature of about 15°C, for example *Pseudomonas*. Psychrophilic bacteria are important in the spoilage of refrigerated food items and these are very often gram-negative organisms.

b. Mesophiles: These bacteria prefer atmospheric temperature, between 20 - 45°C with the optimum lying between 30 - 37°C, depending on the species. All pathogens come under this group, for example, *Staphylococcus aureus and Salmonella*.

c. Thermophiles: They require higher temperature for growth, say about 45 - 65°C which can go even up to 90°C. Optimum temperature for growth is about 55°C, for example, *Clostridium.*

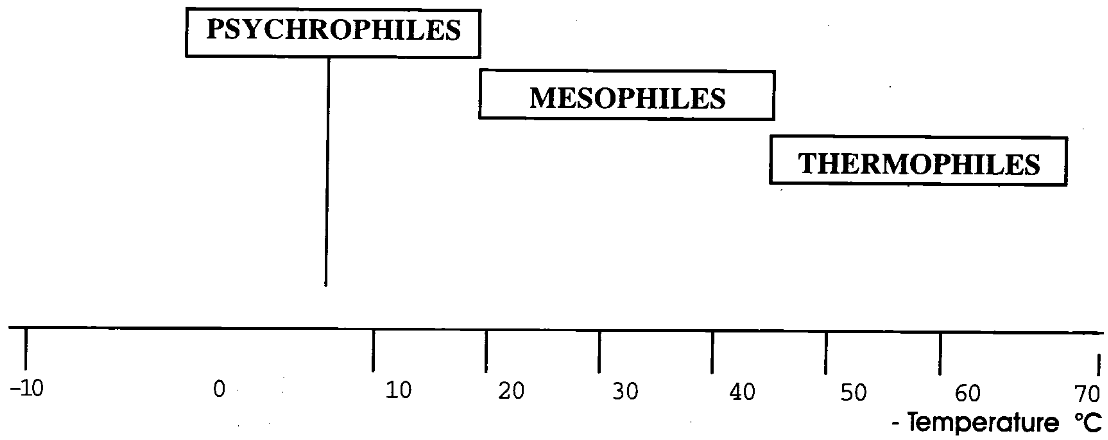


Fig. 4 Classification of bacteria based on temperature

Effect of pH : Hydrogen ion concentration is usually expressed as pH. Each organism has its own optimum, minimum and maximum pH for growth. Most bacteria grow best at a pH near neutrality, but some are favoured by an acid reaction and a few can grow both in acidic or alkaline media.

Minimum pH for growth of some food borne pathogens.

Salmonella spp.	4.0 - 4.5
S. aureus	4.0
C. botulinum	4.7
V.parahaemolyticus	4.8

Approximate pH of some foods.

Shrimp	6.8 - 8.2
Cod (fish)	6.5 - 7.1
Pork and beef	5.3 - 6.4
Bread	5.0 - 6.0
Banana	4.5 - 5.2
Tomato	3.7 - 4.9
Lemon	2.2 - 2.4

Oxidation - reduction potential On the basis of their respiration process bacteria are classified as:

a. Aerobic bacteria or aerobes : Bacteria which can grow only in the presence of atmospheric oxygen, for example, *Pseudomonas.*

b. Anaerobic bacteria or anaerobes: Bacteria which can grow only in the absence of atmospheric oxygen, for example,*Clostridium.*

c. Facultative anaerobes: Organisms which can grow both in the presence or absence of atmospheric oxygen, for example *Escherichia coli*.

d. Microaerophilic bacteria: Bacteria which can grow best at partial presence of oxygen, considerably at a lower level than in air for example, *Lactobacillus casei*.

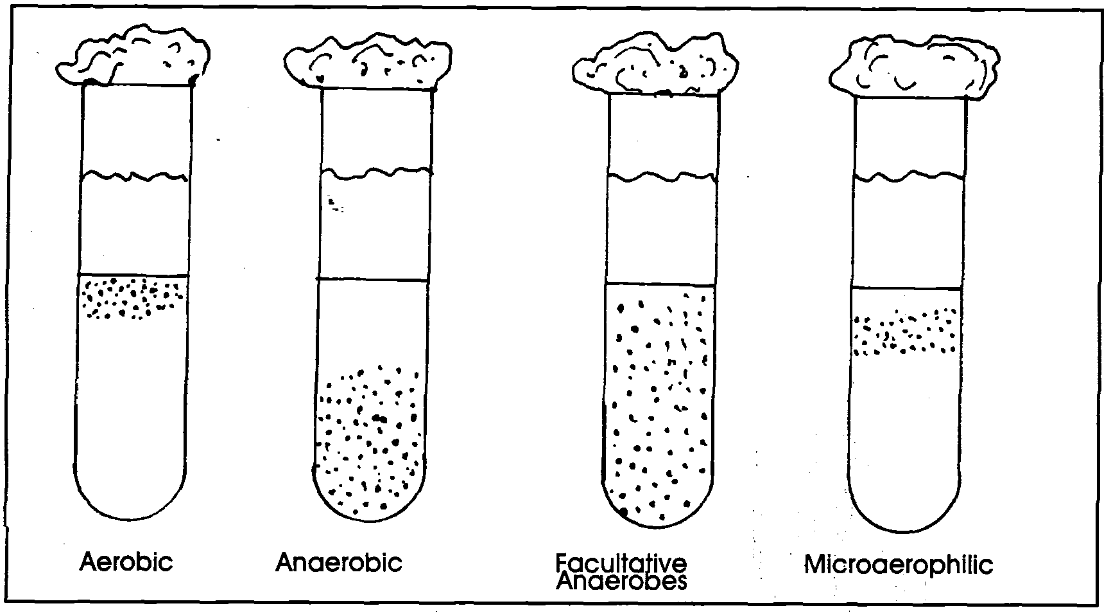


Fig. 5 Growth of aerobic, anaerobic, facultative and microaerophilic organisms in nutrient broth tubes, showing the relationship between the growth of these organisms and atmospheric oxygen

Moisture

In general bacteria require more moisture for growth than yeast or molds. The free moisture required for microbiological growth is often expressed as water activity (a_w). The moisture content of a food material is the sum total of free moisture and bound water. The a_w of pure water is 1.00. Each organism has a maximum, optimum and minimum a_w for growth. No microbial growth can take place below a_w 0.60. Generally bacteria require higher a_w (0.98- 0.99). Growth of most bacteria, moulds and yeasts occurs at a_w above 0.90. Most of the spoilage and pathogenic organisms do not grow well below a_w 0.93. Approximate minimum a_w for growth of some food borne pathogens are given below:

	a_w range
Salmonella	0.93 - 0.96
C.botulinum	0.90 - 0.98
V. parahaemolyticus	0.94 - 0.98
S. aureus	0.83 - 0.92

S. aureus is the most drought resistant pathogenic bacteria.

Approximate a_w of some foods are given below:

Food	a_w range
Fresh fish and poultry	0.98 - 1.00
Fresh meat	0.95 - 1.00
Bread	0.95 - 0.96
Cake	0.90 - 0.94
Biscuit	0.30
Sugar	0.10

Based on the requirement of salt and moisture bacteria can be classified in to two.

1. Halophiles

Halophiles usually require substantial quantities of salt for growth, They can not grow in the absence of salt. This group of organisms is responsible for the spoilage of salted fish, for example, *Serratia salineria.*

2. Xerophiles

These organisms can grow at a_w of less than 0.85. They are usually yeast and moulds.

Inhibitory substances

Substances produced by bacteria during growth, will at times slow down or stop the growth, and may be inhibitory to the multiplication of other organisms. Natural foods may contain compounds that inhibit the growth of some organisms. Inhibitory substances added during the processing of foods may check the growth of most of the organisms, particularly the undesirable ones. For instance, propionate added to bread inhibits moulds and rope bacteria.

Bacterial reproduction

Multiplication of bacteria takes place by simple binary fission. Individual cell grows and after reaching a critical size divides into two cells with identical physiological properties. Each cell subsequently grows and divides at approximately the same rate as the parent cell. Consequently the number of cells doubles at regular intervals. This interval is known as generation time. Under favourable growth conditions some bacteria, for example, *E. coli*, can divide themselves in every twenty minutes and thus large populations of bacteria numbering into millions can be produced in a relatively short time.

Bacteriology of fish

The microflora of fish depends on the microbial types of the waters from which it is caught. Fish from unpolluted marine waters usually does not contain any of the common pathogenic bacteria except the *Clostridium botulinum* type E, *Vibrio parahaemolyticus* and *V. vulnificus*. However, fish does carry a significant number of spoilage organisms. A freely swimming fish harbours bacteria mainly at three sites, namely, the surface slime, the gills and the gut. The muscle flesh or other internal organs such as liver and heart of a healthy fish is generally sterile. As long as the fish is alive, because of its defence mechanism, the activity of these bacteria will be under check. Once the fish is dead, these bacteria start attacking the flesh, thereby initiating the process of bacterial spoilage.

Bacterial load of fish

The number of bacteria per gram of fish muscle or per unit area of the fish surface is called bacterial load of fish. In ocean fresh sardine the bacterial load on different parts of the body are as follows:

Skin & muscle	10^3 - 10^5/g
Gills	10^5 - 10^6/g
Gut	10^5 - 10^8/g

The important characteristics of bacterial flora of ocean fresh fish is that they generally consist of gram-negative rods. Once the fish is landed on the boats, deck or taken to harbour and factories the proportion of gram-positive organisms increases depending on the extent of terrastrial contamination. A typical general distribution of bacteria on fresh sardine is, *Achromobacter (Acinetobacter Moraxella), Vibrio, Pseudomonas, Flavobacterium, Corynebacterium, Micrococcii, Bacillus, and Aeromonas.* In case of prawns, the bacterial load is little higher than that in fish.