Characteristics of microorganisms in food

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Microorganisms play a major role in the foods. Either they can convert foods in value added foods like fermented foods or they can spoil the foods making unfit to eat. Some may also cause diseases by spreading through foods. Lactic acid bacteria are prominently used in food fermentations. Microorganisms are responsible for the preparation of many fermented foods like curd, dosa, idli and many more in India and throughout the world. Oxidative yeasts and molds like *Aspergillus*, *Penicillium Rhizopus* and bacteria like *Pseudomonas* are spoilage organisms. Microorganisms include prokaryotes and eukaryotes. Prokaryotes are microscopic single-celled organisms in which the nuclear material is not enclosed in a nuclear membrane. They have genetic material in the form of single continuous strands forming coils or loops, e.g., bacteria and cyanobacteria. Eukaryotes are the organisms that possess a definite or true membrane-bound nucleus. Genetic material is arranged in the form of chromosomes. Yeasts, molds, plants and animals are examples to eukaryotes. This unit is covered under the following sections.

1) Morphology of bacteria
2) Structure of the bacteria
3) Morphology of Fungi
4) Spores of Microorganisms
5) Significance of spores in food microbiology
6) Association of minerals in foods

1) Morphology of bacteria

Bacteria are very small creatures, which cannot be seen with naked eyes, have a diameter of 0.5 to 1.0 micron. These are the simplest of prokaryotes. Bacteria can survive extreme conditions of pH, temperature, oxygen tension, atmospheric pressures and osmotic conditions. Hence, can be found anywhere in this universe.

Bacteria have different shapes like rods, spherical and spiral shape. Rod shaped bacteria may appear as single or in chains. Very rarely they appear in pairs (ex: *diplobacilli*). Spherical shaped bacteria are also known as cocci or coccus, if single, and they may appear as single, in pairs (diplococci) or chains (streptococci). Sometimes appears as a group of four (tetrads- divide in 2 planes) or eight (*Sarcina*) or in bunches (staphylococci). Spiral shaped bacilli are known as spirilla. Rod shaped bacteria exhibit different forms. They may be small rods (Tularaemia bacillus) or long rods (Anthrax bacillus). Majority rods are with blunt ends, except few with tapered ends (diplobacilli: Pneumonia; streptobacilli: Anthrax). Some appear with pin headed thickening (Diphtheria bacteria) or lateral branching (Tuberculosis and Leprosy bacteria). Cells of *Corynebacterium diphtheriae* stay side by side (palisade arrangement).

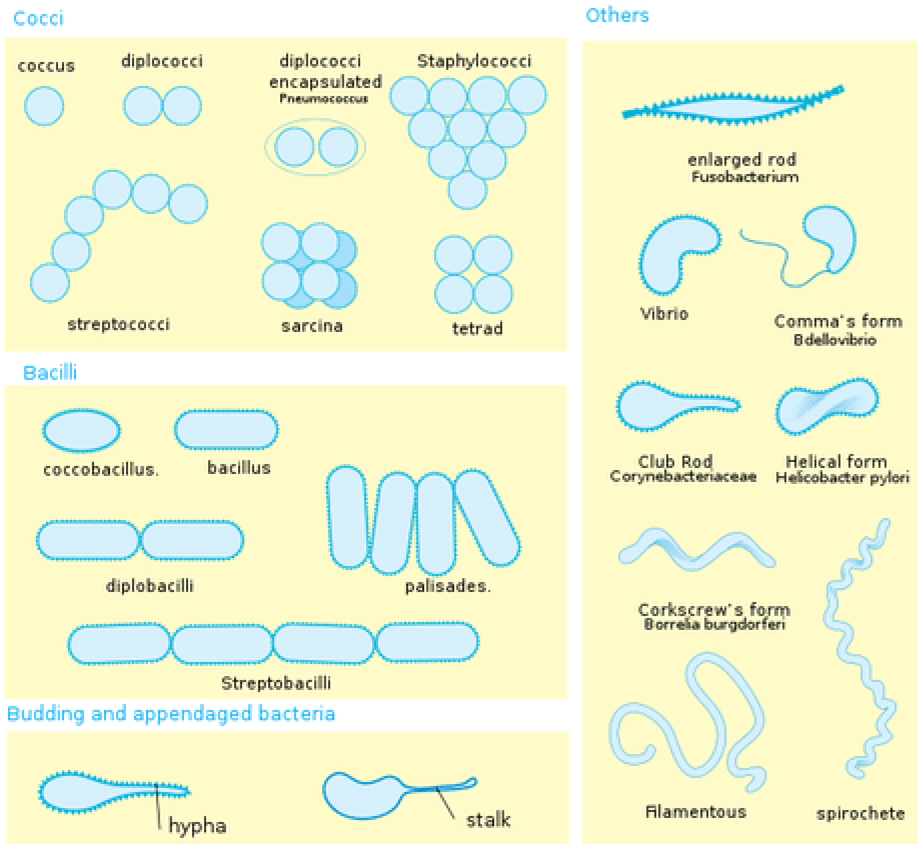


Fig. 2.1: Different shapes and arrangement of bacterial cells
(source: www.ocf.berkeley.edu)

The size of the bacteria varies from one to other. Some of the examples have been provided below. Due to small size bacteria occupies very high surface area/ volume ratio.

Name of the bacteria	**Size**: in microns (Micrometers)
Spiroplasma	: 0.1
Spirillum volutans	: 16-18
Most of the pathogenic bacteria	: 0.2-10
Neisseria meningitis	: 1.00
Streptococcus pnuemoniae	: 1.25
Clostridium botulinum	: 3.80
Vibrio cholera	: 1-5
Treponema pallidum	: 6-14

(2) Structure of the bacteria

Although bacteria are tiny creatures, they have very complex structure. Some have a capsule surrounded by them. Bacteria have cell wall which gives shape to it. Some have filamentous appendages known as fimbriae or pili and flagella (one or many).Cell membrane is located below the cell wall. Inside the cell ribosomes, mesosomes, nuclear material, storage granules, photosynthetic apparatus are present in the cytoplasm.

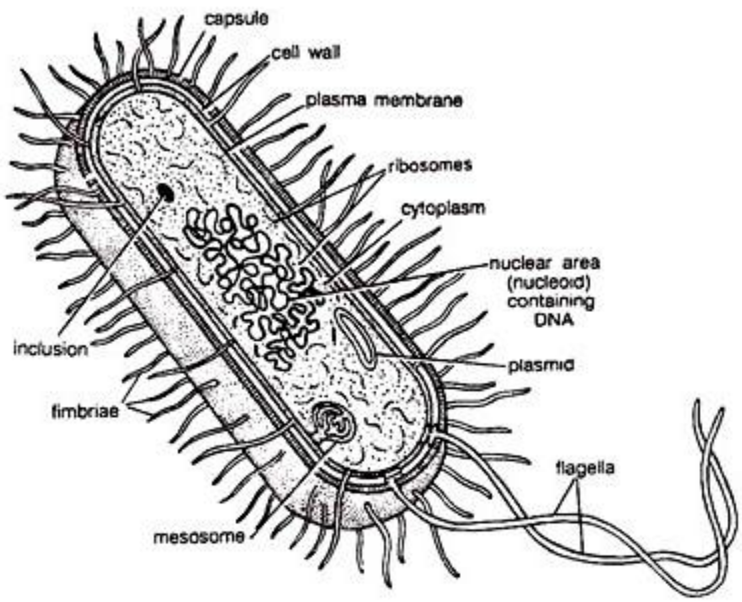


Fig.2. 2: A schematic diagram of a bacterial cell (Source: www.biologydiscussion.com)

The capsule is a loose aggregate of material which may not be part of an organism. It gives protection under unfavourable conditions and acts as a store house of food. Based on size it is classified as microcapsules (demonstrated immunologically). Microcapsules can't be seen under the microscope, ex. *Staphylococcus*, *Streptococcus*, BGA, etc.). Macrocapsules (0.2 µm) can be seen under microscope. The capsule forms a raft in which the actively growing cells float (Ex: *Acetobacter xylinum*, *Leuconostoc* sp.). Capsule is secreted by cell wall and composed of polysaccharides and polypeptides. Bacterial capsules are species specific and can be used for immunological distinction.

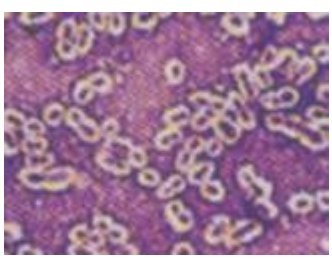


Fig. 2.3: Bacterial capsule (source: http://adistain.com)

Cell envelope in Gram-negative bacteria is composed of two unit membranes separated by a space called periplasmic region. The outer membrane is known as cell wall and the inner membrane as cytoplasmic membrane (CM). CM contains many proteins, lipids and enzymes in mosaic manner. It acts as a barrier. Its structure is best explained by Fluid Mosaic model of Singer and Nicholson. In Gram-positive bacteria the outer membrane is absent. However it has a thick peptidoglycan layer. In Gram negative bacteria outer layer contains a unique lipopolysaccharide (LPS) and lipoproteins.

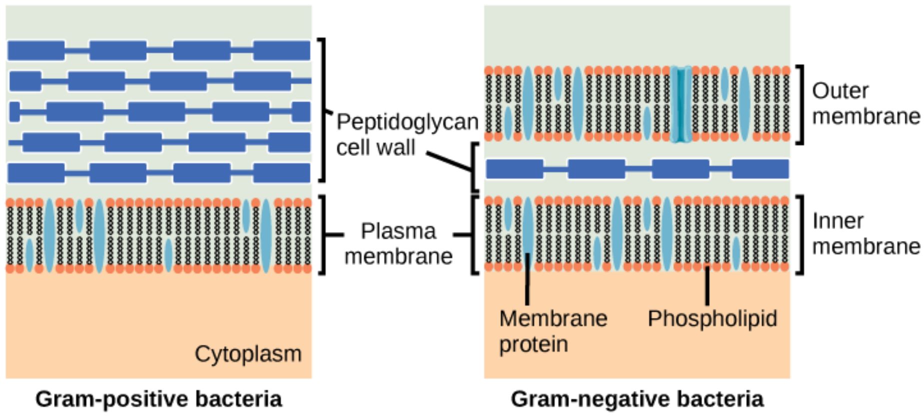


Fig. 2.4: Cell wall structure of Gram positive and Gram negative bacterial cell (Source: http://devarchive.cnx.org)

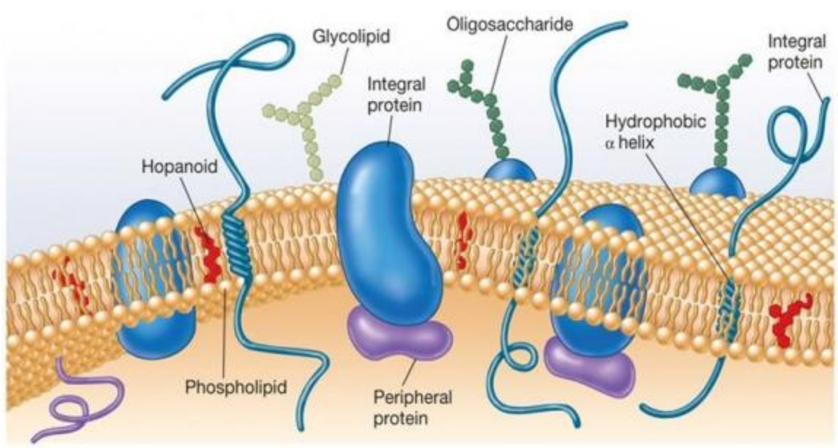


Fig. 2.5: Fluid mosaic model of cell membrane
(Source: http://studydroid.com)

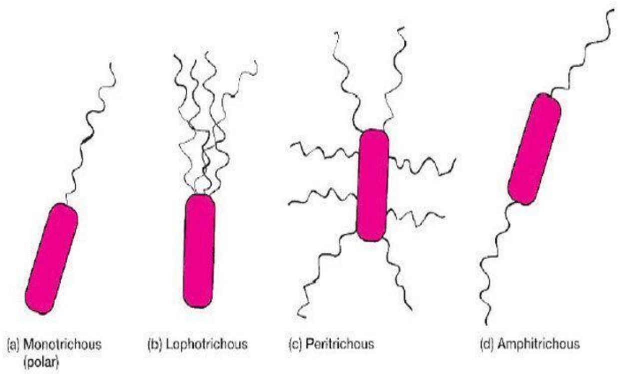
Cell wall provides structural integrity. It serves as a receptor. Cell wall is present in all prokaryotes except in *Halobacterium* and *Halococcus* sp. In Gram-negative bacteria, its content is very less (5-10%), whereas, in Gram-positive bacteria it is 40-90% of cell wall.

Amino sugars like N-Acetyl Muramic acid (NAM) and N-Acetyl Glucosamine (NAG) are present in cell wall. Pentaglycine bridges link adjacent amino sugars through tetra peptide side chains, which are attached to NAM. Mesosomes are more frequent in G +ve bacteria. All important cell functions (respiratory like mitochondria in eukaryotes) are attributed to it. It coordinates in septum formation during cell division.

Bacteria accumulate their energy reserve in the form of storage granules. These are of three types. 1)Polymetaphosphates (volutines/ metachromatic granules) 2) poly-β-hydroxy butyrates and 3) polyglucans. Fimbriae or pili are hair like structures and are present only in Gram –ve bacteria. Based on their functions, pili are of six types. Some pili help in transfer of genetic material during conjugation.

Flagella, a long filament like structure, help in creeping and swimming by bacteria.

Flagella are arranged in four different ways. Monotrichates- only one flagellum at one end of the cell, ex: *Vibrio;* lophotrichates - more than one at one end, ex: *Pseudomonas;* amphitrichates – many at both ends, ex: *Spirillum* and peritrichates - many, anywhere in the cell, ex: *Salmonella.*



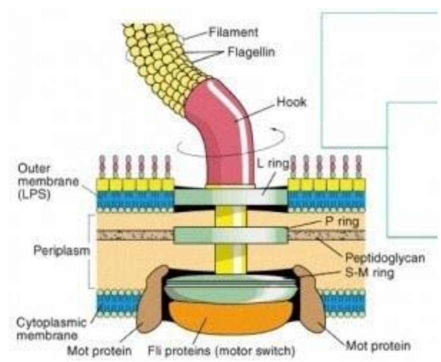


Fig. 2.6: Flagellar arrangement in bacteria **Fig. 2.7**: Structure of a flagellum

(Source: http://microbeonline.com)

In bacteria, nuclear envelop surrounding to nuclear material is absent and the nuclear material is known as nucleoid (chromatin body). It lacks histone proteins. It is folded or super coiled. Plasmids, also known as episomes, are extra-chromosomal genetic units present in bacteria. These are circular double stranded DNA that exists independently of nucleoid. They carry genes for various functions. These plasmids may be conjugate or non-conjugate type. Based on their functions, plasmids are classified into six types. Plasmids give resistance to antibiotics, transfer sex factors, carry genes to produce toxins, bacteriocins and tumors.

(3) Morphology of Fungi

Fungi belong to Eukaryota kingdom. These eukaryotic organisms lack plastids. Its cell wall contains chitin and β-glucans. Fungi may be unicelullar or filamentous in appearance. They reproduce by either sexually or asexually. Fungi are saprophytic, parasitic or mutualialistic in their nutrition requirement. The fungi that have filamentous structure are referred to as molds and most of the yeasts appear as unicellular organisms.

(i) Structure of molds

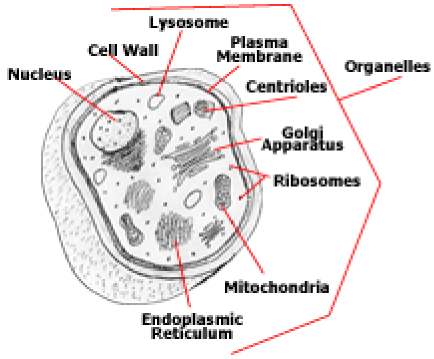


Fig. 2.8: A typical fungal cell and its components
(Source: www.peteducation.com)

The somatic structure of many molds looks like tubes or threads. These are known as hypha, which may be septate or non-septate. Its cell wall is very complex in nature. Cell wall contains cellulose and chitin, hemicellulose and lignin. Fungi have definite nucleus It may be single or many. A mass of hyphae is called mycelium (2-10 micron in diameter). Haustoria, special absorbing knob like, elongated and branched, can be seen in parasitic fungi. Haustoria are absent in *in vitro* cultures and in saprophytes. Some fungi produce sclerotium. It is a hard resting body, resistant to adverse conditions. Glycogen is the main storage material in fungi. The major components of fungal cells include, cytoplasm, cytoplasmic membrane, mitochondria, golgi apparatus, endoplasmic reticulum (ER), centrioles, ribosomes, lysosomes, nucleus and vacuoles. Lipids are the main storage compounds in fungi and these are placed in vacuoles. Mitochondria are site of respiration. ERs are sites of protein synthesis. Golgi apparatus help for import and export of proteins. Nucleus is relatively small and carries genetic material. The nucleus of eukaryotes contain histone proteins, whereas, histone proteins are absent in prokaryotes like bacteria. In fungi, the ribosomes are of 80S type.

(ii) **Structure of yeasts**

Yeasts are another predominant group of microorganisms in foods. Many yeasts are useful for food preparations, for example in bread. Some yeasts are responsible for food spoilage due to their metabolic activities. The shape and size of the yeasts varies depending on the genus it belongs to.

Ex: *Saccharomyces* sp. - 2.5- 10.5 μ width and 4.5-21 μ length
Candida tropicalis - 4-8 μ by 5-11 μ

Yeasts may be round, ovoid, ellipsoidal, filamentous, cylindrical, lemon shaped, etc.

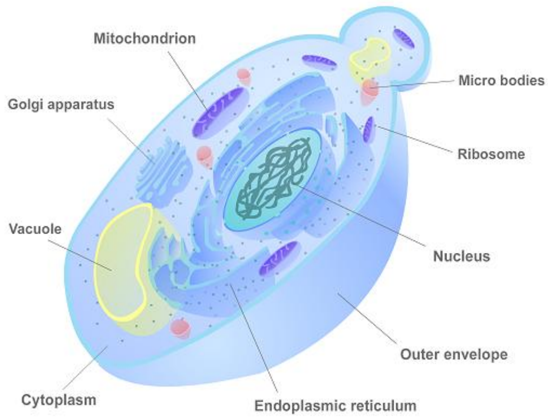


Fig. 2.9: Schematic diagram of a yeast cell
(Source: http://www.biocourseware.com)

Similar to bacteria and molds, yeast cell wall is also rigid and provides shape to the cell. It can be seen under light microscope. It accounts for 25% of dry cell weight. It also functions as a filter. The CW in bakers yeasts consists of 83% carbohydrates. The major carbohydrate in cell wall is glucans. It has a single birth scar and several bud scars on its surface. Birth scar is a structure on a daughter cell resulting from its separation from the mother cell during budding. Bud scar are formed when the mother cell produces daughter cells by budding. The number depends on the number of daughter cells produced by mother cell. Some yeasts have capsular material surrounding to its cells and it is antigenic in nature. The genus can be identified by the type of capsular material, which is secreted by CW. Fimbriae are present in both Ascomycotina & basidiomycotina yeasts. It is proteinaceous and 5-7 nm in dia. It helps in flocculation of cells.

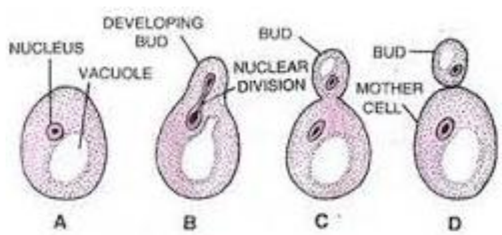


Fig. 2.10: Budding in yeasts
(source: http://www.yourarticlelibrary.com)

Cytoplasmic membrane is explained by fluid mosaic model. It contains various enzymes, neutral lipids, free & esterified sterols, neutral & acidic glycolipids, etc. The nucleus can be seen with phase contrast microscope. It has porous nuclear membrane, nucleolus, DNA and RNA. Other components include ribosomes, mitochondria, RNA polymerases, respiratory enzymes, Golgi bodies, etc. Microbodies are prominent in certain species of *Candida tropicalis*. They resemble peroxisomes in higher plants. RNA content is 5- 100 times more than DNA.

(4) Spores of Microorganisms

(A) Bacterial Endospores

Endospores are resistant bodies produced only by some Gram-positive bacteria like *Bacillus*, *Clostridium* and Sporobacillus, *Sporosarcina* and Desulfomaculum. Endospores have spore coat, cortex, and protoplast (core). Cortex separates spore coat and core. The core contains cytoplasm and nuclear material and m-RNA is absent in endospore. Cortex has many layers of peptidoglycan. Endospores contain dipicolinic acid, which binds to calcium and forms calcium dipicolinate. This gives more protection to bacterial cell. Spore coat protein is rich in cysteine and hydrophobic amino acids. In *Bacillus cereus*, an exosporium, an outer covering, is also present. Vegetative growth and cell division cease during sporulation. Asymmetric cell division initiates spore formation and produces 'forespore', within the mother cell. Endospores may be present at central, sub-terminal or terminal location of the bacterial cell. Endospores may be an oval or spherical in shape and each cell will produce only one endospore. Endospores are formed for the survival of the producing bacteria, but not for reproduction purpose. Very few bacteria produce cysts (ex: *Azotobacter* sp.). These are dormant, thick walled desiccation resistant forms. They can germinate and do not have high heat resistance.

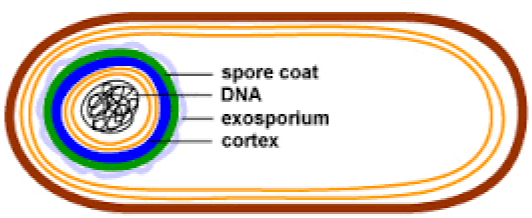


Fig. 2.11: Bacterial endospore

(Source: www.faculty.ccbcmd.edu)

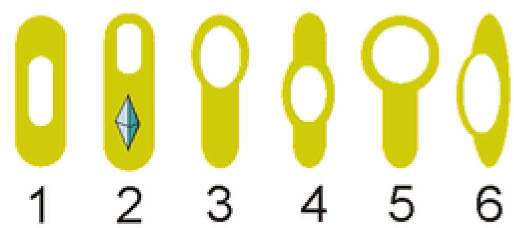


Fig. 2.12: Variations in endospore morphology: (1, 4) central endospore; (2, 3, 5) terminal endospore; (6) lateral endospore. (Source: https://en.wikipedia.org/wiki/Endospore)

(B) **Fungal spores**

Fungi produce spores by both asexual and sexual process. The spores produced by different fungi differ in their shape, colour and size. Hence, these are used for the identification and classification of fungi. These spores are formed on hyphae that involve in reproduction. Unlike bacterial endospores, fungal spores are not resting bodies. These are used for reproduction. Fungi produce spores both in sexual or asexual reproduction process.

In asexual reproduction fungi produce different types of spores in greater abundance. These are capable of dormancy. These spores help in disseminating the species for a long distance. Sometimes these spores may have a slimy fluid (attract insects and get transported to new habitat) or dry small size and spread by wind. These asexual spores may be in different colours like green, yellow, red brown, black and orange or colourless.

If hypha breaks into individual cells forming the spores, then these are known as oidia (exogenus). Arthrospores are another type of asexual spores produced by fungi that are surrounded by thick walls (in adverse conditions). Then these are called as chlamydospores (endogenous spores). Some spores are motile and some other non-motile. Motile spores are also known as zoospores (planospores) and they may have one or two flagella, which may be whip lash (unbranched) or tinsel type. Non motile spores are called as aplanospores. If spores are produced internally in a sporangium then they are called as sporangiospores. Conidiophores are the spores formed externally over a conidia.

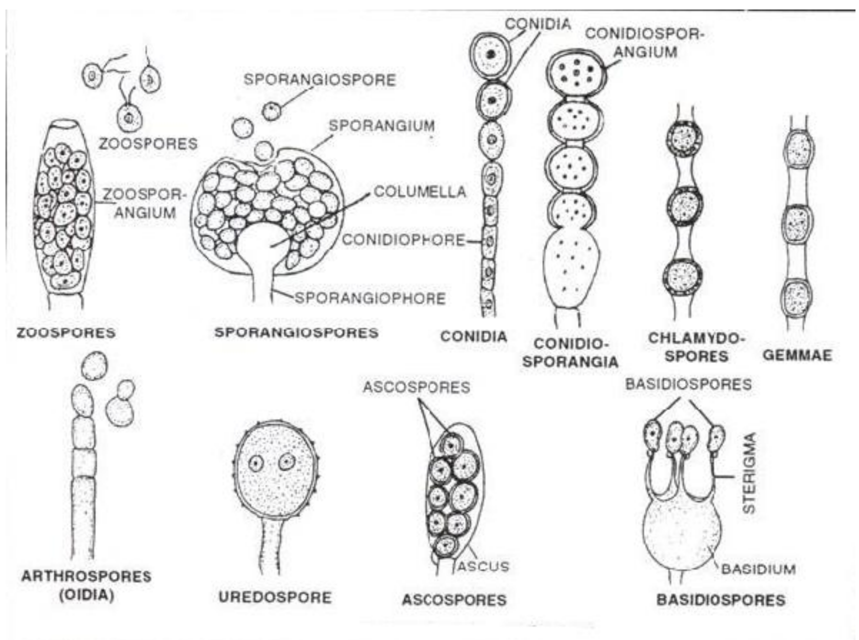


Fig. 2.13: Various types of fungal spores
(Source: www.biologydiscussion.com)

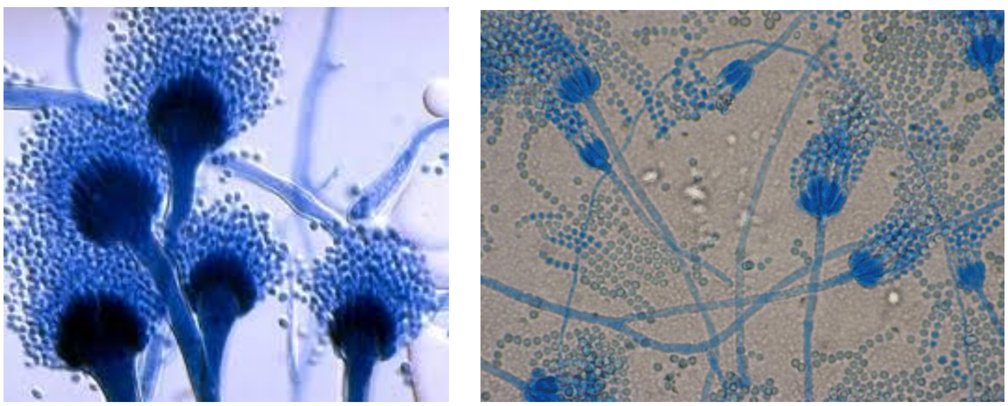
Ascospores and basidiospores are examples to spores produced by sexual reproduction process. These are produced inside the ascus and outside of the basidium, respectively. Eight ascospores are formed in one ascus, whereas, four basidiospores are formed exogenously over a special growth known as sterigmata, on a basidium.

(C) Yeasts spores

Yeasts also produce spore both by sexual and asexual reproduction methods. Asexual spores are of four types. These are blastospores (produced by *Candida tropicalis*), arthrospores (single cell fission products of mycelial hyphae, e.g. *Endomyces*), ballistospores (e.g. Sporobolomycetaceae) and chlamydospores (in *Candia albicans*).

Like in the case of fungal spores, yeast spores have different shapes. These are

a) Spherical and smooth- *Saccharomyces*
b) Bowler hat shaped- *Hansenula*
c) Saturn shaped- *Hansenula saturius*
d) Bean shaped- *Pichia membranaefaciens*
e) Spherical and warty- *Debaromyces*
f) Walnut shaped with equatorial rim and thick warty wall- *Schwanuriomyces*
g) Sickle shaped- *Eremothecium*
h) Bow shaped- *Coccidiascus*
i) Needle shaped- *Monosporella*
j) Needle shaped, with a non-motile flagellum- *Nematospora.*



(A) (B)

Fig. 2.14: Fungal spores. (A) *Aspergillus* sp.; (B) *Penicillium* sp.
(Source: Aspergillus-www.blackmould.me.uk; www.moldbacteria.com)

Table: Comparison of characteristic features of bacterial endospores and fungal spores

Parameters	**Bacterial endospores**	**Fungal spores (yeasts & molds)**
Type of spores	Endospores (exclusively inside the bacterial cell)	Both internal and external to the cell
Purpose of spore formation	To get protection from the harsh environmental conditions	To enhance its population numbers.
Resistance to heat, radiation, chemicals and freezing	Highly resistant to these conditions	Not resistant to these condition
Abundance	Only 5 genera of Gram positive bacteria produce endospores	All yeasts and molds produce spores
Means of spore formation	Endospores are not for reproduction Hence, not	Both sexual and asexual mode
Presence of dipicolinic acid	Present	Absent
Help in taxonomical identification	No	Yes.
Number of spores per cell	Only one per bacterial cell	Innumerable per species

(5) Significance of spores in food microbiology

Spores of either bacteria or fungi have several roles in food microbiology. Endospore-forming bacteria may enter foods through soil either directly or indirectly, through agriculture produce and at primary production level. Endospores are produced by both aerobic and anaerobic bacteria. Endospores can create spoilage in foods, especially in canned foods, if the heat treatment is improper. In fact, the heating process may kill the vegetative cells of bacteria. However, it activates the endospores leading to the growth of the spore forming bacteria and this in turn can grow in the food. Anaerobic spore forming bacteria like *Clostridium* spp. can cause havoc in insufficiently processed canned foods. Aerobic spore formers like *Bacillus* also can cause spoilage in canned foods. It can cause flat sour spoilage, wherein, cans remain flat without any gas production inside it. For example thermophilic *Bacillus coagulans* produces acidity in canned tomato products without any gas production. Some thermophilic anaerobic (TA) clostridia produce TA spoilage by producing both acid and gas leading to bulging of the cans, especially when stored at high temperatures. Spores of *Clostridium nigrificans* can cause sulfide stinker spoilage by producing hydrogen sulphide in low acid foods like peas and corn. Some of these spore-forming bacteria like *Bacillus cereus*, *Clostridium perfringens*, *Cl. botulinum*, etc., are also pathogenic and once growth occurs, it may also lead to production of various toxins. Some of these toxins may be heat stable, hence, can make foods unsafe for consumption. If high heat treatment is applied it may bring changes in sensory and textural properties of the foods. Hence, it is important to ensure that foods are not contaminated with spore forming bacteria. Alternatively, the processed foods should be kept in cold temperature so that the activated endospores cannot grow at this cold temperature.

With regard to fungal spores, they can easily be transported by wind currents and may enter the foods when they are exposed to open conditions. Most of the fungal spores enter the foods through air. Food being a rich source of nutrients, fungal spores, if entered into foods after processing can grow well leading to spoilage of the food. Like in the case of bacteria, some of the fungi can produce heat resistant mycotoxins, which can even cause cancers in human beings. For example, *Aspegillus flavus* produces aflatoxin and ochratoxin, *Penicillium expansum* produces petulin, *P. citrinum* produces citrinin, *Fusarieum roseum* produces zearalenone, etc.

(6) Association of minerals in foods

Many of the cereals based foods contain minerals such as phosphorous, zinc, iron, calcium, magnesium, manganese and copper. These are essential components required for human nutrition. Minerals are required to activate enzymes, to regulate the pH of the body fluids and to maintain osmotic balance between the environment and the cells. Deficiency of minerals can lead to severe metabolic disorders. More than 60% of phosphorous in corn meal is bound to phytate making it unavailable for the body. Phytate is considered as anti-nutritional factor as it gives a negative effect on mineral uptake from the cereal foods like finger millet, sorghum and corn. It can bind phosphorous, iron, zinc, copper, magnesium and calcium, making these minerals unavailable to the consumers.
One of the easy methods to reduce phytate content in the foods is fermentation. Microorganisms like *Aspergillus* and some lactic acid bacteria present in fermented foods can hydrolyse phytates by producing phytase. This step releases phosphorous, which can be absorbed by the body. Similarly, many animal and poultry feeds are being supplemented with phytase enzyme produced by *Aspergillus niger*, to increase the bioavailability of phosphorous in animals and birds. Natural fermentation of foods also improves HCI-extractability of several minerals like zinc, iron, calcium, copper and manganese in millet flour. Fermentation of cassava enhanced bioavailability of calcium, potassium and magnesium.

In conclusion, in this unit we have explained about different microorganisms, their classification into prokaryotes and eukaryotes, important features of bacteria, molds and yeasts. You also learned about different types of spores of microorganisms produced either by sexual or asexual reproduction methods. Bacterial endospores, which are heat and radiation resistant, can play a significant role in the spoilage of various foods, especially canned foods. Fermentation of foods through desirable microorganisms can enhance the bioavailability of several minerals for human and animal nutrition.

Microscopic observation

Staining of any living cell is carried out to know its morphology and cell components. This can be achieved either by simple staining or differential staining. Gram staining is a differential staining through which Gram positive and Gram negative bacteria can be distinguished.

Simple staining:

Take clean and dry glass slides.

↓

Mark an area in the middle of the slide and flame that surface.

↓

After cooling, sterilize the inoculation loop and transfer a loopful of water onto the heated surface.

↓

Take a loopful of the bacterial or fungal culture from the tube or the flask after flaming the mouth and spread it on the slide in the marked area.

↓

Make a thin and even smear using a drop of water.
Again sterile the loop and dry the smear and heat fix by passing on a flame two to three times with mild heat. Overheating may damage the cells.

↓

Stain the smear by flooding it with any one of the following staining solutions:
Crystal violet - 30 sec or Carbol fuchsin - 20 sec or Methylene Blue - 1 min.

↓

Rinse the slide with running tap water to remove excess stain. Wipe the back side of the slide with a tissue paper and place the slide on the stage of a microscope.

↓

Focus the smear with 10X objective. Once the smear is focused change the objective from 10 X to 40 X and sharpen the focus area.

↓

Put a drop of immersion oil and change to 100 X objective.

↓

Observe the smear for the morphological feature of the organism(s) present in the given sample. Cells will take up the colour of the stain used.

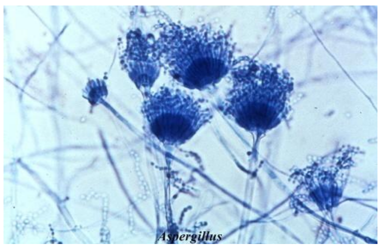


Fig 2.15: Simple staining of *Aspergillus* sp. with Lactophenol cotton blue (Source: http://microbeonline.com)

Gram staining

Christine Gram has developed a staining technique to differentiate the bacteria based on their cell wall structure. Hence, this method is known as Gram's staining. Gram-positive bacteria take up the crystal violet and appear in purple colour, whereas, Gram-negative bacteria appear as red cells.

Reagents: Crystal violet (primary stain); Grams iodine (mordant)
Ethyl alcohol (decolourizer); Safranin (counter stain)

Procedure:

Take clean and dry glass slides free of grease or oil.

↓

Make a marking at the center of on one side of the slide.

↓

Take a loopful of 18 to 24 hour grown culture of the test culture and apply in the maker area.

↓

Make a thin smear and heat fix the smear by passing on the flame with mild heat and allow it to dry.

↓

Flood the smear with crystal violet solution and allow for 1 min for staining the cells.

↓

Wash the slide using distilled water.

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Flood the smear with Gram's iodine for 1 min.

↓

Wash the slide with decolorizing solution for 5-10 sec or till alcohol runs clear.

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Rinse the slide with water.

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Apply safranin for 30 second and wash the slide with distilled water and allow to air dry.

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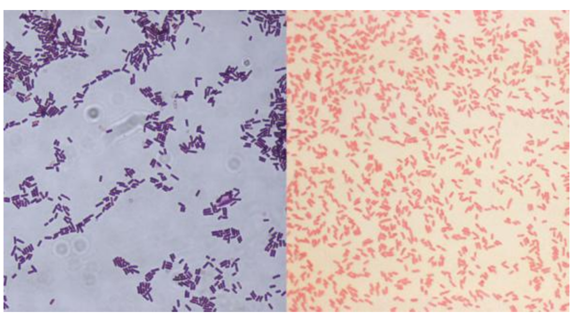
Observe under oil immersion (100 x objective) using a light microscope



(A) (B)

Fig.2. 16: Gram staining (A) Gram Positive (B) Gram negative bacterial cells
(Source: Heinrich Volschenk- https://plus.google.com/+HeinrichVolschenk)