## **CHARACTERISTICS OF MICROORGANISMS IN FOOD - FAQs**

1. Classify bacteria based on their shape.

The rigid cell wall of the bacteria gives bacteria its shape. It includes rods, spherical and spiral shape. Spherical shaped bacteria are also known as cocci or coccus, if single, and they may appear as single, in pairs (diplococci), chains (streptococci), a group of four (tetradsdivide in 2 planes) or eight (*Sarcina*) or in bunches, due to the division in different planes (staphylococci). Sarcinahas 8 or 16 cells in the form of a bunch by dividing into 3 planes at right angles to one another. Some bacteria may be pleomorphic by exhibiting many varieties of shapes. Rod shaped bacteria may appear as single or in chains. Very rarely they appear in pairs (ex: *diplobacilli*). 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), pin headed thickening (Diphtheria bacteria) or lateral branching (Tuberculosis and Leprosy bacteria). Cells of *Corynebacteriumdiphtheriae* stay side by side, which is known as palisade arrangement. Spiral shaped bacilli are known as spirilla.

2. What do you know about bacterial capsule?

The capsule is a loose aggregate of material which may not be part of an organism. It gives protection under unfavourable conditions and also acts as a storage material of food. It has significance in the disease causing organisms.Ex: non capsulated *Pneumococcus* destroyed by host phagocytes, but not the encapsulated ones. Based on size it is classified as microcapsules (demonstrated immunologically, can't be seen under the microscope, ex. *Staphylococcus, Streptococcus,* BGA, etc.) and macrocapsules (0.2  $\mu$ m), which 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.

3. Explain about cytoplasmic membrane.

The inner membrane of the cell envelop is known as cytoplasmic membrane. The proteins of the enzyme activity, molecule transfer and receptor function are located in cell membrane. It has a lipid bilayer, which acts as a permeable barrier. Cytoplasmic membrane is an important center of metabolic activity. It contains enzymes of the biosynthetic pathways of cell wall, permeases for the transport of organic and inorganic nutrients, components of electron transporting system and oxidative phosphorylation, photosynthetic apparatus of purple bacteria and components that control chemotaxis. It acts as an attachment site for chromosomal and plasmid DNA. Itsstructure is best explained by Fluid Mosaic model of Singer and Nicholson (1972). It is a quasi-fluid structure in which lipids and proteins are arranged in mosaic manner. The globular proteins are sandwiched by a phospholipid bilayer.

4. What is peptidoglycan layer? Explain its features in both Gram positive and Gram negative bacteria.

Peptidoglycan layer is an outer covering of the bacteria. It forms a rigid layer. In Grampositive bacteria it is more (40-90 % of cell wall) and in Gram-negative bacteria, its content is very less (5-10%). Peptidoglycans provide structural integrity to cell. Present in all prokaryotes except in *Halobacterium* and *Halococcus* sp. It has mucopeptides, glycopeptides and mureins. Amino sugar like N-acetyl muramic acid (NAM) is present in Gram-negative bacteria and techoic acids and teichuronic acids are present in Gram-positive bacteria. In *E. coli*, alternating residue of NAM and NAG (N-Acetyl Glucosamine) are linked by  $\beta$ -1-4glycosidic Linkages. Tetra peptide side chains are attached to NAM. Peptidoglycan in Grampositive bacteria is a linear glycan (alternating NAG and NAM). Each NAM carries a peptide chain of four Amino acids (L- alanine, D-glutamine, L-lysine and D-Alanine). Neighbouring tetrapeptides are linked by pentaglycine bridges between lysine and D-alanine. There is extensive cross linking. Teichoic acids, teichuronic acids and other polysaccharides are attached to peptidoglycan through a phosphodiester bond.

5. What are mesosomes and what is its role in a bacterial cell?

Mesosomes are in-folding of cell membrane and are complex localized bodies. More frequent in G +ve bacteria. They appear as vesicular or whorl type. All important cell functions (respiratory like mitochondria in eukaryotes) are attributed to it. It coordinates in septum formation during cell division.

6. In how many ways bacteria store its energy material and what are they?

Bacteria store its energy material in the form of granules, which have high molecular weight. These are osmotically inert and can be readily metabolized. These are of three types. <u>Polymetaphosphates</u> (volutine/ metachromatic granules): *Spirillum volutans*. It stains reddish violet with methylene blue or toluidine blue. These are very common in corynibacteria (Diphtheria and Mycobacteria). Poly- $\beta$ -hydroxybutyrates stain with Sudan black. Common in most bacteria and represents up to 60-80% of cell weight. Polyglucans are specific to certain bacteria (Clostridia, coliforms) and like starch it stain blue with iodine.

7. What is flagellum? In how many ways flagella are arranged in bacteria?

Flagellum is a long hair like appendage which has its base in cell membrane and helps the bacterium for creeping and swimming. Flagella are arranged in four different ways. If only one flagellum is present at one end of the cell, then it is called monotrichates. This type of arrangement can be seen in *Vibrio*. If more than one flagellum are present at one end, it is called as lophotrichates. This arrangement is present in *Pseudomonas fluorescens* and *Alcaligensfaecalis*. If flagella are present at both ends of the bacterial cell then it is referred to as amphitrichates. This type of arrangement can be seen in *Spirillumvolutans*. In some bacteria, several flagella are present all over the cell then it is called peritrichates. Coli bacterium and *Salmonella* have this type of arrangement.

8. What are plasmids? What is its role in bacteria?

Plasmids are extra-chromosomal genetic units present in bacteria. These are also known as episomes. These are circular double stranded DNA that exists independently of chromosome. Plasmids carry genes for various functions like production of bacteriocins, transfer sex factors, resistance to antibiotics, production of tumours, etc. These plasmids may be conjugate or non-conjugate type.

9. What are endospores? What is its role?

Endospores are resistant bodies produced by some Gram-positive bacteria (Sarcinae and Actinomycetes. Only bacteria belonging to the genus *Bacillus*, *Clostridium*, *Sporobacillus*, *Sporosarcina* and *Desulfatomaculum* produce endospores. Endospores may be present at central, sub-terminal or terminal location of the bacterial cell. Endospores may be on oval or spherical in shape and each cell will produce only one endospore. These are produced under conditions of limited supply of C, N and P. These are resistant to heat, chemical, freezing and radiation conditions. Endospores are formed for the survival of the producing bacteria, but not for reproduction purpose. Endospores protect the cell from extreme conditions like high temperature, radiation, etc.

10. Write a note on structure of endospores.

Endospores have spore coat, cortex, and protoplast (core).Cortex separates spore coat and core. The core contains cytoplasm and nuclear material. The m-RNA is absent in endospore core. Cortex has many layers of peptidoglycan, which gives the protection to the endospore. Spore coat protein is rich in cysteine and hydrophobic amino acids. Cortex also contains large amount of dipicolinic acid and calcium in the form of calcium dipicolinate. 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.

11. What do you know about asexual spores of fungi?

Fungi produce spores both in sexual or asexual reproduction process. However, 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 whiplash (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.

12. Write about the cell components of yeasts.

Like in the case of bacteria and molds, yeast cell wall is also rigid and provides shape to the cell. It accounts for 25% of dry cell weight. It also functions as a filter. The CW in bakers yeasts consists of 83% carbohydrates, 10% protein, 3% lipids, 0.45% sterols, 0.3% RNA, and 0.04% DNA. 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 scars are formed when the mother cell produces daughter cells by budding. The number depends on the number of daughter cells produced by mother cell. The major carbohydrate in cell wall is glucans (30-35%) and of this 85% is  $\beta$ -1-3 glucans and 15% is  $\beta$ -1-6-glucans. 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 Phosphomannans, secreted by CW. It contains β-linked mannans (ex: Hansenulacapsulata, Pitchia, Rhodotorula sp.) or heteropolysaccharides (ex. Cryptococcus sp.). Fimbriae are present in both Ascomycotina & basidiomycotina yeasts. It is proteinaceous and 5-7 nm in dia. It helps in flocculation of cells. It has porous nuclear membrane, nucleolus, DNA and RNA. Other components include ribosomes, mitochondria, RNA polymerases, respiratory enzymes, Golgi bodies, etc.

13. Write about asexual spores of yeasts.

Yeasts produce four different types of asexual spores. These are blastospores, which are produced by larger vegetative thallus body or pseudomycelium other than single cells. Ex: *Candidatropicalis*. Arthrospores are single cell fission products of mycelial hyphae. Ex: *Endomycesreessii*, *Trichosporons*p. Ballistospores are the spores borne one at a time on a pointed stalks, from which they are discharged with considerable force by a peculiar droplet mechanism. Ex: Sporobolomycetaceae members. Chlamydospores are produced by *Candiaalbicans*.

14. What is the significance of microbial spores in food industry?

Spores of bacteria and fungi have several roles in food microbiology. Endospore-forming bacteria may enter foods through soil either directly or indirectly. Bacterial endospores can create spoilage in foods, especially in canned foods. If the heat treatment is improper it can activate the endospore, while killing the vegetative cells of bacteria. Some of these spore-forming bacteria like *Bacillus cereus*, *Clostridium perfringens*, *Cl. botulinum*, etc., are also pathogenic and can produce 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 in it 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, *Penicilliumexpansum* produces petulin, *P. citrinum* produces citrinin, *Fusarieum roseum* produces zearalenone, etc.

15. What is the role of fermentation in the enhancement of mineral content in the foods?

Many of the cereals based foods contain minerals such as phosphorous, zinc, iron, calcium, magnesium, manganese and copper. However these are not available in free form and in many cases it is bound to phytic acid, which is an anti-nutritional factor present in many pant based foods. More than 60% of phosphorous in corn meal is bound to phytate making it unavailable for the body. However, fermentation can hydrolyse the phytic acid by phyatse enzyme produced by selected microbial cultures like *Aspergillus niger* and some lactic acid bacteria and release the minerals making it bioavailable to the consumers. 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.