

## Endospores and formation of cell aggregates

Endospores are metabolically dormant stages observed in certain Gram positive bacteria as a survival strategy to overcome unfavourable environmental conditions. Spore forming bacteria are of significance in food industry because of their ability to cause food spoilage and produce toxins which cause illness in humans. Among the bacteria, *Clostridium botulinum*, *C. perfringens* and *Bacillus cereus* are toxigenic while many species of spore formers cause spoilage of food. Endospores are produced inside bacterial cell by members of Gram positive *Bacillus*, *Clostridium*, *Desulfotomaculum*, *Sporolactobacillus* and *Sporosarcina*. Spore formation also called as sporulation or sporogenesis is part of the natural life cycle of bacteria. Spores differ from metabolically active and growing vegetative cells by their inert resting condition. Endospores vary in size, shape and position in the vegetative cells in different bacteria and are often useful in the identification of some species.

### 4.6.1. Endospore formation

Endospore formation is initiated by the vegetative cell under the conditions of nutrient depletion, especially the carbon and nitrogen source. The vegetative cell prepares for sporulation by transforming into a committed sporulating cell called sporangium. The sporangium actively involves in synthesizing compounds required for spore formation. Most spore formers develop mature spore of complex structure within 6-8 hours. Sporulation usually appears in the late logarithmic phase of growth possibly because of nutrient depletion and accumulation of toxic metabolites.

#### Structure of endospore

The spore released at the end of sporulation from the mother cell is structurally, biochemically and physiologically different from vegetative cells. The inner core of the spore containing proteins and nucleic acids is surrounded by several layers of varying composition. These include core, spore cortex, spore coat, and outer exosporium.

**Exosporium:** The outermost spore layer is the exosporium and it varies in size in different species. It is a thin, delicate covering made of protein, polysaccharide and some lipids.

**Spore coat:** Following exosporium is the thick and structurally complex spore coat of several layers consisting of proteins with unusual amino acids. The spore coat protects the inner spore cortex from attack by lytic enzymes, and serves as barrier to oxidizing agents. This layer is not involved in offering resistance to spores from heat or radiation.

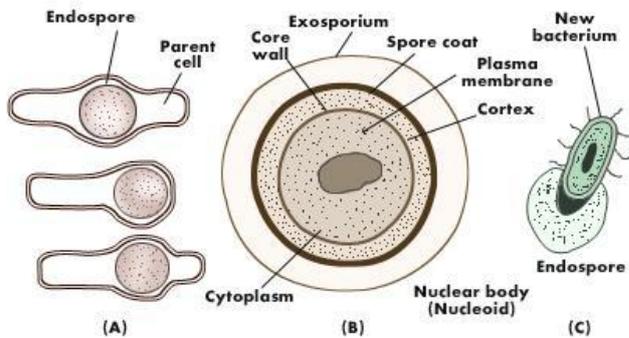
**Spore cortex:** It is made of several layers of loosely cross linked peptidoglycan with calcium and dipicolinic acid.

**Core:** Core is the central region of the spore and contains DNA, ribosomes, most enzymes, diaminopimelic acid and divalent cations and other macromolecules. Core is also characterized by low water content of 10-30% of vegetative cell which reduces the core cytoplasm to a gel like consistency. The dehydration is responsible for spore dormancy and offering resistance to variety of agents. The core cytoplasm also contains high concentration of small acid-soluble spore proteins (SASP) which bound tightly to DNA and protect it from damaging effect of ultraviolet light, desiccation, and dry heat. The SASPs also function as a source of carbon and energy during germination of endospore.

### 4.6.2. Germination of spores

The spores remain in dormant state for a varying period of time, even for several years. When

conditions become favourable the spores break dormancy and enter in to a process called spore germination. Presence of water and certain specific chemicals such as aminoacids or inorganic salts and environmental stimulus initiate germination process by activating dormant hydrolytic enzymes from spore membranes. These enzymes digest the spore cortex and expose the core to water. The rehydrated core utilizes nutrients and grows out of spore coat fully reverting back to vegetative cell. Generally spores germinate in to vegetative cells within a short period of about 90 minutes.



Endospore formation. A, Endospores according to their position in parent cells. B, An endospore in cross-section. C, Germination of endospore

#### 4.6.3. Resistance of spores

Bacterial endospores are highly hardy structures capable of withstanding extreme heat, drying, freezing, radiation and chemicals that would readily kill vegetative cells. Ability to survive such harsh environmental conditions is attributed to several factors.

- The high heat resistance of spores is due to high content of calcium and dipicolinic acid which removes water and makes it dehydrated. The absence of free water offers heat resistance and thus protective effect on proteins and nucleic acids.
- The spore is metabolically inactive due to non availability of water which makes it resistant to further drying.
- Presence of thick and impervious cortex and spore wall offer resistance against radiation and chemical substances.

#### • 4.6.4. Cell aggregates

Microorganisms occur either as single cells or as aggregates consisting of clumps or chains of cells. Microorganisms always grow attaching on to suspended organic and inorganic substances rich in nutrients. In food industry spoilage and pathogenic organisms grow on food residues and serve as continuous source of contamination to food. Also, the bacterial cell aggregates are more resistant to cleaning and disinfection in food industry. As the microbial cells occur in several layers on food residues and surfaces containing nutrients, the cells in the inner layers are not exposed to disinfection treatment and hence survive better. Formation of bacterial cell aggregates need to be prevented in any food handling and processing environment by following good sanitary measures.