

Microbial sporulation and its relationship with food

1. What is sporulation?

- ❖ **Sporulation** (or sporogenesis) is a cell differentiation process that allows certain bacteria (primarily Firmicutes, such as the genera *Bacillus* and *Clostridium*) to form spores (endospores) in response to unfavorable environmental conditions (lack of nutrients, dehydration, extreme temperatures, etc.).

A spore is a structure that is:

- Dormant: No active metabolism.
- Extremely resistant: To high temperatures (pasteurization, moderate cooking), desiccation, UV radiation, chemicals (disinfectants), and high pressure.
- Long-lasting: Can survive for years, even decades, in the environment.

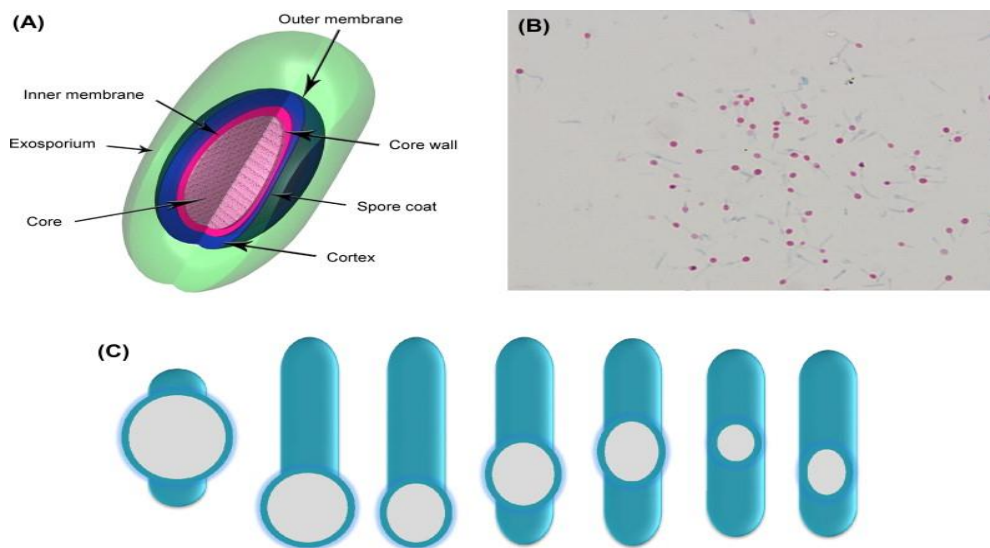


Figure : (A) Schematic representation of the bacterial spore. (B) Spore stain of *Clostridium tetani* (fuchsin-methylene blue stain). (C) Size, morphology, and location of bacterial spores.

- ❖ **Germination:** When conditions become favorable again (presence of water, nutrients, heat), the spore can reactivate (germinate) and give rise to an active vegetative cell, capable of multiplying and, for pathogens, producing toxins.

2. Relationship with food

2.1. The danger: safety issues and spoilage

Spores represent a major challenge for the food industry.

1. Spore-forming pathogens: the food "super-bacteria"

-*Clostridium botulinum* : produces the botulinum neurotoxin, one of the most potent known, responsible for botulism (a severe foodborne illness). Its spores, present in soil, contaminate

vegetables and fish. They survive boiling and can germinate in canned, vacuum-packed, or low-acid foods ($\text{pH} > 4.6$) in the absence of oxygen.

-*Clostridium perfringens* : Causes foodborne toxico-infections (gastroenteritis), often linked to cooked dishes (sauces, meats) that are reheated or held warm for too long, allowing rapid spore germination.

-*Bacillus cereus* : Responsible for two types of poisoning: diarrheal (meat and vegetable dishes) and emetic (vomiting) linked to cooked rice left at room temperature. Spores survive rice cooking and germinate if cooling is too slow.

2. Food spoilage

-*Bacillus subtilis* and related species: Their spores resist moderate heat treatments and can germinate in a wide range of foods (UHT milk, bread, pasta, ready meals), causing spoilage: off-flavors, slimy textures, "flat sour" spoilage of low-acid canned vegetables.

3. Heat treatment

Pasteurization (72°C for a few minutes) does not destroy spores. Only a drastic heat treatment (canning/sterilization: 121°C for several minutes) can eliminate them. However, such heat alters the sensory qualities of many foods.

2.2. The potential: beneficial applications

Some spores are exploited beneficially:

2.2.1. Probiotics:

Bacillus coagulans, *B. clausii*: Used as probiotics in dietary supplements or certain functional foods. Their spore form allows them to survive stomach acidity and arrive intact in the intestine, where they germinate and exert their beneficial effects.

2.2.2. Starter cultures in biotechnology:

Bacillus spp. are used to produce enzymes (amylases, proteases) used in the food industry (baking, cheese-making, syrup production).

2.2.3. Biocontrol agent:

Bacillus thuringiensis (Bt): Its spores, associated with an insecticidal toxin, are used as a bio-pesticide in agriculture to protect crops.

3. Control strategies in the food industry

The fight against spores relies on a combination of barriers (the hurdle technology concept):

1. Sterilization (canning): Very high-temperature treatment ($\geq 121^{\circ}\text{C}$) to destroy all spores. For "low-acid" canned foods ($\text{pH} > 4.6$).

2. Tyndallization: Fractional sterilization (multiple cycles of moderate heat) aimed at inducing spore germination between cycles to kill them in their vegetative form.

3. Preservation parameters:

- Acidic pH (<4.6): Prevents the germination and growth of most pathogenic spores (principle of pickles, jams).

- Low water activity (a_w): Addition of salt, sugar to dehydrate the medium and inhibit germination.

- Low temperatures (refrigeration): Slow or prevent germination.

- Antimicrobial additives: Nitrites (in cured meats), nisin (a bacteriocin) to inhibit *C. botulinum* and others.

4. Good hygiene practices: Reduce initial contamination of raw materials (spices, herbs, soil on vegetables are often heavily contaminated with spores).

5. Rapid cooling of cooked foods to avoid the "danger zone" (between 4°C and 63°C) where surviving spores can germinate and cells can multiply rapidly.