2.4E: Endospores

Skills to Develop

1. Name 2 common genera of bacteria capable of producing endospores and state which is an obligate anaerobe.
2. Briefly discuss the function of a bacterial endospore.
3. Describe the structure of a bacterial endospore.
4. Define sporulation and germination.
5. Name three infections that may be transmitted to humans by endospores.

Highlighted Bacterium

1. Read the description of Clostridium tetani and match the bacterium with the description of the organism and the infection it causes.

Endospores are dormant alternate life forms produced by the genus Bacillus, the genus Clostridium, and a number of other genera of bacteria, including Desulfozymaculum, Sporosarcina, Sporolactobacillus, Oscillospira, and Thermoactinomyces. Bacillus species (see Figure 1) are obligate aerobes that live in soil while Clostridium species (see Figure 2) are obligate anaerobes often found as normal flora of the gastrointestinal tract in animals.

Figure 1: Endospore stain of Bacillus megaterium
Figure 2: Endospore stain of Clostridium tetani
Formation of Endospores

Under conditions of starvation, especially the lack of carbon and nitrogen sources, a single endospores form within some of the bacteria. The process is called sporulation.

First the DNA replicates (Figure 3, step 1) and a cytoplasmic membrane septum forms at one end of the cell (Figure 3, step 3). A second layer of cytoplasmic membrane then forms around one of the DNA molecules (Figure 3, step 4) - the one that will become part of the endospore - to form a forespore (Figure 3, step 5). Both of these membrane layers then synthesize peptidoglycan in the space between them to form the first protective coat, the cortex (Figure 3, step 6) that lies adjacent to the germ cell wall that will eventually form the cell wall of the bacterium upon germination.

Calcium dipicolinate is also incorporated into the forming endospore. A spore coat composed of a keratin-like protein then forms around the cortex (Figure 3, step 7). Sometimes an outer membrane composed of lipid and protein and called an exosporium is also seen (Figure 3, step 8).

Finally, the remainder of the bacterium is degraded and the endospore is released (Figure 3, step 9). Sporulation generally takes around 15 hours. The process is summarized in Figure 3.

GIF animation showing endospore formation

GIF animation showing endospore germination
Endospore Structure (see Figure 3, step 10)

The completed endospore consists of multiple layers of resistant coats (including a cortex, a spore coat, and sometimes
an exosporium) surrounding a nucleoid, some ribosomes, RNA molecules, and enzymes.

- To view an electron micrograph of an endospore of *Bacillus stearothermophilus*, see the Microbe Zoo web page of
Michigan State University.

(Some bacteria produce spore-like structures distinct from endospores. Exospores are heat resistant spores produced
by a budding process in members of the genus *Metylosinus* and *Rhodomicrobium*. Cysts are resistant to drying and are
formed singly within vegetative cells by *Azotobacter*, *Myxococcus*, and *Sporocytophaga*. Conidia are heat-susceptible
asexual reproductive spores produced by various genera of branching bacteria belonging to the group Actinomycetes.)

Function of Endospores

An endospore is not a reproductive structure but rather a resistant, dormant survival form of the organism. Endospores
are quite resistant to high temperatures (including boiling), most disinfectants, low energy radiation, drying, etc. The
endospore can then survive until a variety of environmental stimuli trigger germination, allowing outgrowth of a single
vegetative bacterium as shown in Fig 3, step 11 and step 12 and in Figure 4. Viable endospores have reportedly been
isolated from the GI tract of a bee embedded in amber between 25 and 40 million years ago. Viable endospores of a
halophilic (salt-loving) bacterium have also reportedly been isolated from fluid inclusions in salt crystals dating back over
250 million years!

Bacterial endospores are resistant to antibiotics, most disinfectants, and physical agents such as radiation, boiling, and
drying. The impermeability of the spore coat is thought to be responsible for the endospore's resistance to chemicals.
The heat resistance of endospores is due to a variety of factors:

- Calcium-dipicolinate, abundant within the endospore, may stabilize and protect the endospore's DNA.
- Small acid-soluble proteins (SASPs) saturate the endospore's DNA and protect it from heat, drying, chemicals, and
radiation. They also function as a carbon and energy source for the development of a vegetative bacterium during
germination.
- The cortex may osmotically remove water from the interior of the endospore and the dehydration that results is
thought to be very important in the endospore's resistance to heat and radiation.
- Finally, DNA repair enzymes contained within the endospore are able to repair damaged DNA during germination.

Exercise: Think-Pair-Share Questions
Botulism is caused by Clostridium botulinum, a bacterium that is found in the intestinal tract of many grazing animals and is an obligate anaerobe. When growing at a near neutral pH, the bacterium synthesizes and secretes an exotoxin that prevents acetylcholine from being released from the neural motor end plate of neurons at the synapse between the neuron and the muscle to be stimulated. As a result, the affected muscles don't contract or contract very weakly.

A person grows some green beans in a garden fertilized with manure. The beans are washed, boiled, placed in glass jars, and sealed with a lid. A couple of months later, that person heats one of the jars of beans, eats them, contracts botulism, and dies of respiratory failure.

Thinking of what we know about the genus Clostridium, its oxygen requirements, where it normally lives, and what its exotoxin does, explain the sequence of events that led to the person contracting botulism and dying.

Endospores and Infectious Disease

Although harmless themselves until they germinate, they are involved in the transmission of some diseases to humans. Infections transmitted to humans by endospores include:

- Anthrax, caused by Bacillus anthracis; endospores can be inhaled, ingested, or enter wounds where they germinate and the vegetative bacteria subsequently replicate.
- Tetanus, caused by Clostridium tetani; endospores enter anaerobic wounds where they germinate and the vegetative bacteria subsequently replicate.
- Botulism, caused by Clostridium botulinum; endospores enter the anaerobic environment of improperly canned food where they germinate and subsequently replicate.
- Gas gangrene, caused by Clostridium perfringens; endospores enter anaerobic wounds where they germinate and the vegetative bacteria subsequently replicate.
- Pseudomembranous colitis (Clostridium difficile); antibiotics destroy the normal microbiota of the intestines that keep the growth of C. difficile in check while the endospores of C. difficile survive and subsequently germinate and replicate before the microbiota is restored.

Highlighted Bacterium: Clostridium tetani

Click on this link, read the description of Clostridium tetani, and be able to match the bacterium with its description on an exam.

Concept map for Bacterial Endospores

E-Medicine article on infections associated with organisms mentioned in this Learning Object. Registration to access this website is free.

- Bacillus anthracis
- Clostridium tetani
Summary

1. Endospores are dormant alternate life forms produced by a few genera of bacteria.
2. The genus Bacillus (an obligate aerobe often living in the soil) and the genus Clostridium (an obligate anaerobe living in the gastrointestinal tract of animals) produce endospores.
3. Under conditions of starvation, a single endospore forms within a bacterium through a process called sporulation, after which the remainder of the bacterium is degraded.
4. The completed endospore consists of multiple layers of resistant coats (including a cortex, a spore coat, and sometimes an exosporium) surrounding a nucleoid, some ribosomes, RNA molecules, and enzymes.
5. Endospores are quite resistant to high temperatures (including boiling), most disinfectants, low energy radiation, and drying.
6. The endospore survives until a variety of environmental stimuli trigger germination, allowing outgrowth of a single vegetative bacterium.
7. Infectious diseases such as anthrax, tetanus, gas gangrene, botulism, and pseudomembranous colitis are transmitted to humans by endospores.

Questions

Study the material in this section and then write out the answers to these questions. Do not just click on the answers and write them out. This will not test your understanding of this tutorial.

1. Name 2 common genera of bacteria capable of producing endospores and state which is an obligate anaerobe. (ans)
2. Briefly discuss the function of a bacterial endospore. (ans)
3. The emergence of a vegetative bacterium from an endospore is called _________________. (ans)
4. Name three infections transmitted to humans by bacterial endospores. (ans)
5. Botulism is caused by Clostridium botulinum, a bacterium that is normal flora of the intestinal tract of grazing animals. A person home-canned some green beans by boiling the beans and placing them in jars and screwing on lids. The lids popped down indicating a vacuum had formed within the jar. Upon ingesting these beans the person contracted botulism. Based on what was learned about Clostridium, explain. (ans)

6. Multiple Choice (ans)

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