



SPORE FORMING ANAEROBES

35.1 INTRODUCTION

Spore-forming bacteria produce a unique resting cell called an endospore. They are Gram-positive and usually rod-shaped, but there are exceptions. Bacteria are a large group of microscopic, unicellular organisms that exist either independently or as parasites. Some bacteria are capable of forming spores around themselves, which allow the organism to survive in hostile environmental conditions. Bacterial spores are made of a tough outer layer of keratin that is resistant to chemicals, staining and heat. The spore allows the bacterium to remain dormant for years, protecting it from various traumas, including temperature differences, absence of air, water and nutrients.

The two medically-important genera are *Bacillus*, the members of which are aerobic spore formers in the soils, and *Clostridium*, whose species are anaerobic spore formers of soils, sediments and the intestinal tracts of animals.

Some spore formers are pathogens of animals, usually due to the production of powerful toxins. *Bacillus anthracis* causes anthrax, a disease of domestic animals (cattle, sheep, etc.), which may be transmitted to humans. *Bacillus cereus* causes food poisoning. *Clostridium botulinum* causes botulism, a form of food poisoning, and *Clostridium tetani* is the agent of tetanus. *Clostridium perfringens* causes food poisoning, anaerobic wound infections and gas gangrene, and *Clostridium difficile* causes a severe form of colitis called pseudo-membranous colitis. Whenever the spore-formers act as pathogens, it is not uncommon or surprising that their spores are somehow involved in transmission or survival of the organism between hosts.



OBJECTIVES

After reading this chapter, the student will be able to

- explain why bacteria produce spores

- identify various types of spore forming bacteria
- describe the structure of Spores
- explain Sporulation Cycle



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35.2 STRUTURE OF SPORES

It consists of following layers

Exosporium - A thin delicate covering made of protein.

Spore coats - Composed of layers of spore specific proteins.

Cortex - Composed of loosely linked peptidoglycan and contains dipicolinic acid (DPA), which is particular to all bacterial endospores. The DPA cross links with calcium ions embedded in the spore coat. This cross linkage greatly contributes to the extreme resistance capabilities of the endospores because it creates a highly impenetrable barrier. The calcium DPA cross linkages compose 10% of the dry weight of the endospores.

Core - The core contains the usual cell wall and, cytoplasmic membrane, nucleoid, and cytoplasm. The core only has 10-30% of the water content of vegetative cells; therefore the core cytoplasm is in a gel state. The low water content contributes to the endospores success in dry environments. However, the low water concentration and gel cytoplasm contributes to the inactivity of cytoplasmic enzymes. The core cytoplasm is also one unit lower in pH than the vegetative cell, thus conferring acidic environment survival. SASPs, small acid soluble spore proteins, are formed during sporulation and bind to DNA in the core. SASPs protect the DNA from UV light, desiccation, and dry heat. SASPs also serve as a carbon energy source during germination, the process of converting a spore back to a vegetative cell.

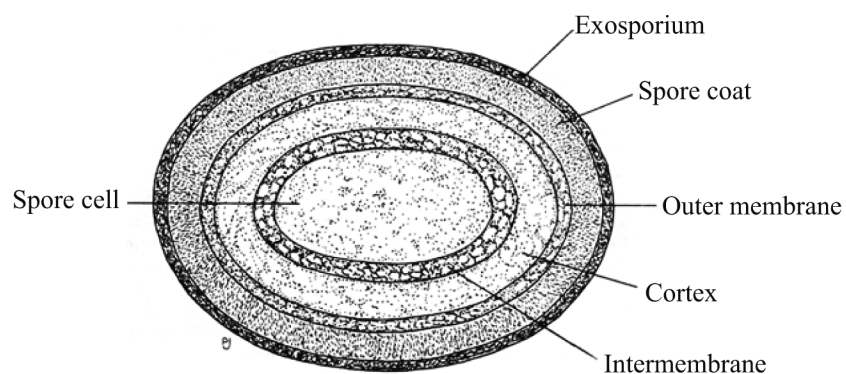


Fig. 35.1: Endospore

Sporulation Cycle: You may have read elsewhere in this site that bacteria sometimes form protective spores to help them survive through tough times. Some other kinds of microbes do, too. Here's how that transformation takes place.

First of all, you might think of a bacterial spore roughly as a mummified bacterium. The spore has a hard protective coating that encases the key parts of the bacterium – think of this coating as the sarcophagus that protects a mummy. The spore also has layers of protective membranes, sort of like the wrappings around a mummy. Within these membranes and the hard coating, the dormant bacterium is able to survive for weeks, even years, through drought, heat and even radiation. When conditions become more favorable again – when there's more water or more food available – the bacterium “comes to life” again, transforming from a spore back to a cell. Some bacterial spores have possibly been revived after they lay underground for more than 250 million years!

Ok, so how do bacteria turn themselves into spores? First, the bacterium senses that its home or habitat is turning bad: food is becoming scarce or water is disappearing or the temperature is rising to uncomfortable levels. So it makes a copy of its chromosome, the string of DNA that carries all its genes.

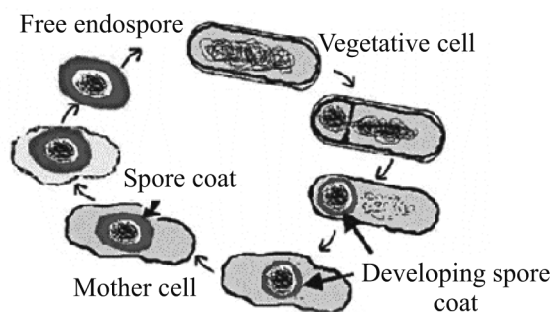


Fig. 35.2: ASM Digital image collection merkel

Then, the rubbery cell membrane that surrounds the bacterial cell fluid begins pinching inward around this chromosome copy, until there's a little cell within the larger bacterial cell. This little cell is called the “daughter cell” and the bigger, original one, what starts out as the “vegetative cell” in this illustration, is now called the “mother cell.” Next, the membrane of the mother cell surrounds and swallows up the smaller cell, so that now two membrane layers surround the daughter cell. Between these two membranes a thick wall forms made out of stuff called peptidoglycan, the same stuff found in bacteria's rigid cell walls. Finally, a tough outer coating made up of a bunch of proteins forms around all this, closing off the entire daughter cell, which is now a spore. As the mother cell withers away or gets blasted by all kinds of environmental damage, the spore lies dormant, enduring it all, just waiting for things to get better. Not all bacteria



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can form spores. But several types that live in the soil can. Bacteria in the *Bacillus* and *Clostridium* groups are spore-formers. Their spores are called endospores.

Another group of bacteria called *Methylosinus* produces spores called exospores. The difference between endospores and exospores is mainly in how they form. Endospores form inside the original bacterial cell, as described above. Exospores form outside by growing or budding out from one end of the cell. Exospores also don't have all the same building blocks as endospores, but they're similarly durable.

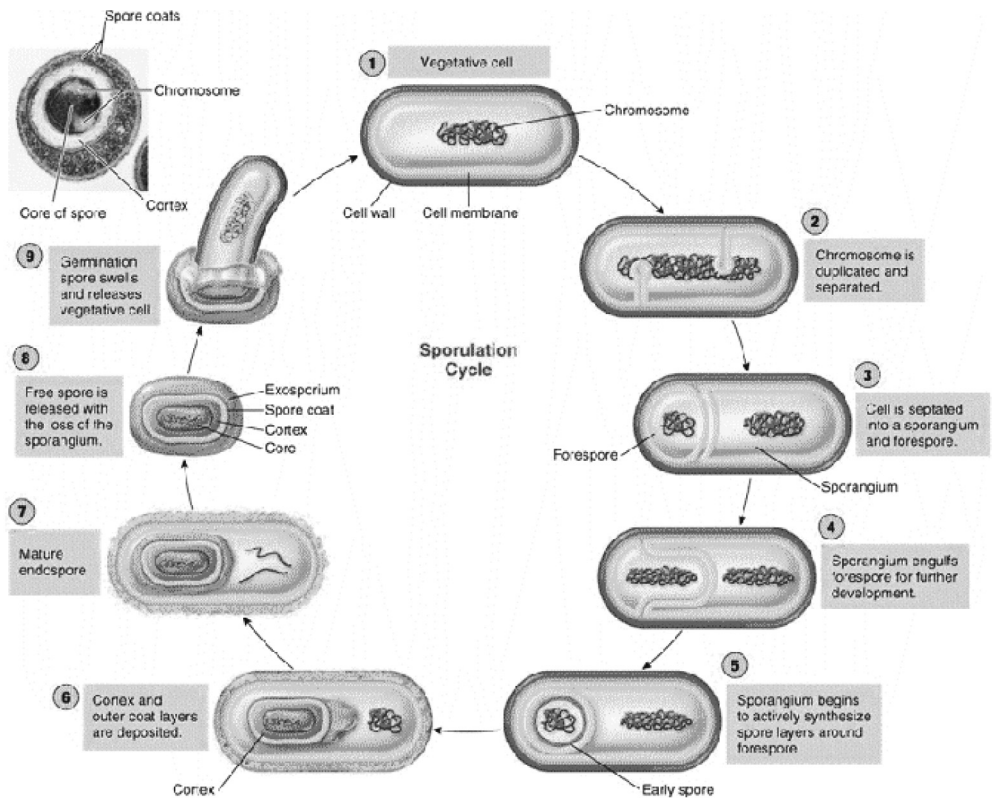


Fig. 35.3: Sporulation Cycle



INTEXT QUESTIONS 35.1

1. Spore forming bacteria produce unique cell called
2. Bacillus groups produce spores
3. Methylosinus produces spores
4. Endospores forms the bacterial cell
5. Exospores forms the bacterial cell.

35.3 DEMONSTRATION OF SPORES

Endospores can be examined with both light and electron microscopes. Because spores are impermeable to most stains, they often are seen as colourless areas in bacteria treated with Methylene blue and other simple stains; special spore stains are used to make them clearly visible. Spore position in the mother cell or sporangium frequently differs among species, making it of considerable value in identification. Spores may be centrally located, close to one end (subterminal), or definitely terminal. Sometimes a spore is so large that it swells the sporangium.

Electron micrographs show that endospore structure is complex. The spore often is surrounded by a thin, delicate covering called the exosporium. A spore coat lies beneath the exosporium, is composed of several protein layers, and may be fairly thick. It is impermeable and responsible for the spore's resistance to chemicals. The cortex, which may occupy as much as half the spore volume, rests beneath the spore coat. It is made of a peptidoglycan that is less cross-linked than that in vegetative cells. The spore cell wall (or core wall) is inside the cortex and surrounds the protoplast or core. The core has the normal cell structures such as ribosomes and a nucleoid, but is metabolically inactive.

Bacterial Endospore: Endospores or spores are highly resistant dormant structure produced by number of Gram negative bacteria. Eg. *Sporosacrium spp*, *Bacillus spp*, *Clostridium spp*. Bacteria normally grow, matured, & reproduce by somatic cells. When there is nutrient depletion or environmental stress (heat, UV radiation, chemical disinfection desiccation), spore former bacteria begin spore formation. After return of suitable environmental spores produce vegetative cell. Some endospores remain viable for 100 yrs. Since spores often survive boiling for an hour or more therefore, autoclave must be used to sterilize any bacteria.

Bacillus: Bacillus is a specific genus of rod-shaped bacteria that are capable of forming spores. They are sporulating, aerobic and ubiquitous in nature. Bacillus is a fairly large group with many members, including *Bacillus cereus*, *Bacillus clausii* and *Bacillus halo denitrificans*. Bacillus spores, also called endospores, are resistant to harsh chemical and physical conditions. This makes the bacteria able to withstand disinfectants, radiation, desiccation and heat. Bacillus are a common cause of food and medical contamination and are often difficult to eliminate.

Sporolactobacillus: Sporolactobacillus is a group of anaerobic, rod-shaped, spore forming bacteria that include *Sporolactobacillus dextrus*, *Sporolactobacillus inulinus*, *Sporolactobacillus laevis*, *Sporolactobacillus terrae* and *Sporolactobacillus vineae*. Sporolactobacillus are also known as lactic-acid bacteria for



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they are capable of producing the acid from fructose, sucrose, raffinose, mannose, inulin and sorbitol. Sporolactobacillus are found in the soil and often in chicken feed. According to “Fundamentals of Food Microbiology,” the spores formed by Sporolactobacillus are less resistant to heat than those formed by the Bacillus genus.

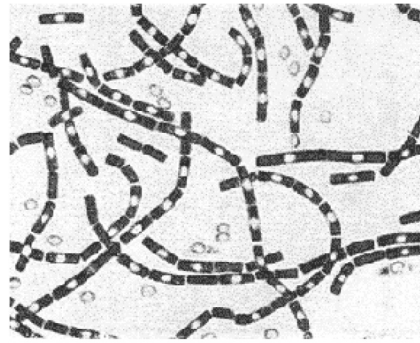


Fig. 35.4: *Bacillus anthracis*

Sporosarcina: Sporosarcina are a group of round-shaped (cocci) aerobic bacteria that include Sporosarcina aquimarina, Sporosarcina globispora, Sporosarcina halophila, Sporosarcina koreensis, Sporosarcina luteola and Sporosarcina ureae. According to “Antibiotic Resistance and Production in Sporosarcina ureae,” Sporosarcina is thought to play a role in the decomposition of urea in the soil.

Clostridium

Clostridium are rod-shaped, Gram-positive (bacteria that retain a violet or dark blue Gram staining due to excessive amounts of peptidoglycan in their cell walls) bacteria that are capable of producing spores. According to the Health Protection Agency, the Clostridium genus consists of more than a hundred known species, including harmful pathogens such as Clostridium botulinum, Clostridium difficile, Clostridium perfringens, Clostridium tetani and Clostridium sordellii. Some species of the bacteria are used commercially to produce ethanol (Clostridium thermocellum), acetone (Clostridium acetobutylicum), and to convert fatty acids to yeasts and propanediol (Clostridium diolis).

We would study in detail about the Clostridium species. Clostridium species forms the important

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Clostridia are Gram-positive, spore-forming and strictly anaerobic rod shaped bacteria. The genus consists of a number of medically important pathogens,

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including *Clostridium perfringens*, *C. tetani*, *C. botulinum* and *C. difficile*. They can be differentiated on the basis of their biochemical activities, including saccharolysis and proteolysis.

Pathogenesis

The key virulence factors in pathogenic clostridia are the powerful toxins released from vegetating (growing) bacteria.

Epidemiology

Clostridia live commensally in the human and animal gut or as saprophytes in the soil.

Clinical infections

C. perfringens causes gas gangrene and food-poisoning. *C. tetani* causes tetanus, *C. botulinum* causes botulism and *C. difficile* causes antibiotic-associated diarrhea.

Laboratory diagnosis

Gas gangrene is diagnosed by Gram stain and culture of body specimens. Tetanus and botulism are often diagnosed clinically. Antibiotic-associated diarrhea is proven by isolation of the organism and demonstration of the presence of toxins in stool samples.

Treatment

Gas gangrene is treated with antibiotics and surgical debridement of gangrenous wounds. Tetanus and botulism require antibiotics, antitoxins and additional life-supporting measures. Antibiotic-associated diarrhea is treated by the withdrawal of antibiotics from patients and the use of metronidazole if necessary. *C. perfringens* food-poisoning is self limiting.

Prevention and control

Good care of wounds can prevent gas gangrene. Vaccination against tetanus is highly successful. The risk of botulism can be minimized by avoiding expired or inadequately sterilized canned meat. Antibiotic-associated diarrhea can be prevented by avoiding unnecessary use of antibiotics and isolation of patients who have acquired the disease.

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The clostridia are a group of strictly anaerobic large (4–6µm~1µm) Gram-positive bacilli. They can be pleomorphic. Some tolerate small amounts of oxygen. They form spores which can be centrally or terminally positioned

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causing bulging within the cell. They are soil saprophytes or normal commensals of the human and animal gut. However, they are capable of causing deadly diseases, which are invariably mediated by potent exotoxins.

In addition to their colonial and microscopic morphologies, *Clostridium* species can be differentiated on the basis of their biochemical activities. Many species break down sugar (saccharolysis) and/or protein (proteolysis) molecules. These activities can be detected and used for species differentiation. The ability to produce aromatic fatty acid end-products (detected by gas-liquid chromatography) can also be used for species differentiation. Toxicogenicity of isolates is often demonstrated in the laboratory using various appropriate techniques, e. g. observing cytopathic effect on human cells, or neutralization of enzymatic effect on substrates using antibodies.

The clostridia, like all other medically important anaerobic organisms, are sensitive to metronidazole and clindamycin. They are also sensitive to many other antibiotics, including penicillin and erythromycin, but resistant to aminoglycosides, which are therefore added to culture media to enhance the selective isolation of these organisms.

Clostridium perfringens

C. perfringens rarely forms spores under normal laboratory conditions. On blood agar, it produces a characteristic **double zone of β -hemolysis**, a narrow transparent zone and a wide shadowy zone. It is mainly saccharolytic and produces acid and gas from milk, producing a 'stormy clot' in a test tube (litmus milk test).

C. perfringens produces a **number of potent toxins**, the most important of which is the α -toxin (phospholipase C) which causes host cell lysis. This toxin is produced by all isolates of *C. perfringens*.

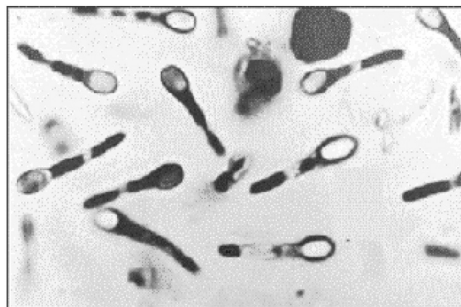


Fig. 35.5: *Clostridium perfringens*

Different strains of the organism produce any of another 11 well-known toxins, including collagenase, proteinase, hyaluronidase and deoxyribonuclease. Based on these toxins, the *C. perfringens* strains are divided into five types, A-E. *C.*

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perfringens uses these toxins to kill human muscle cells, causing necrosis (**myonecrosis**). Death of these cells creates an even more suitable anaerobic condition where the organism can grow rapidly and release gas, hence the disease is known as '**gas gangrene**'. Infected and discolored blood inside the wound and under the skin turns the infected area to black. Gas gangrene can be caused by other, less common clostridia, including *C. novyi*, *C. septicum*, *C. histolyticum* and *C. sordelli*. Gas gangrene is treated by surgical debridement of infected areas and the use of high doses of penicillin given intravenously.

Some strains of *C. perfringens* also produce enterotoxins which, when ingested in large numbers, can cause food-poisoning (diarrhea, vomiting and abdominal pain). This is self-limiting and does not require treatment.

Clostridium tetani

C. tetani is a straight, slender, anaerobic Gram-positive bacillus with a rounded terminal spore which looks like a 'drumstick' under the microscope. It is Gram positive, but readily loses the Gram stain. The organism is motile, lives in the human and animal gut and contaminates soil, mainly from cattle feces. *C. tetani* produces an **extremely potent single toxin** (tetanus toxin) which underlies the pathogenesis of tetanus. Tetanus toxin consists of two components, including the neurotoxic 'tetanospasmin' and the hemolytic tetanolysin. The toxin **prevents muscle relaxation**, leading to persistent contraction of facial and body muscles. Simultaneous contraction of opposite groups of muscles leads to a characteristic grin on the face (*risus sardonicus*) and spastic posture of the limbs and body trunk.

The organism is rarely isolated from the site of bacterial entry, which may not even be visible. Therefore, diagnosis is made mostly on clinical grounds, the features of which are characteristic. Tetanus is fatal in the absence of rapid and high quality management. The organism is sensitive to penicillin which is administered along with a specific anti-tetanus antibody (**antitoxin**). The infected wound should be debrided and the patient given a booster dose of tetanus vaccine.

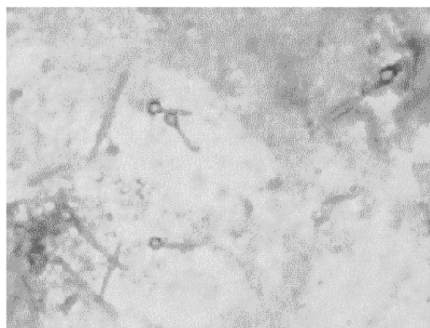


Fig. 35.6: Clostridium tetani terminal spores

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Tetanus is now extremely rare in industrialized countries, due to the availability of a safe and highly effective vaccine offered to all children as part of a childhood immunization programme. Booster doses are given to toddlers and young adults.

Clostridium botulinum

C. botulinum strains are motile, anaerobic, Gram-positive bacilli which form subterminal spores. They are ubiquitous and contaminate water and meat-containing canned food, including canned fish, liver pâté and sausages. *C. botulinum* causes botulism which is a severe, **usually fatal, form of food-poisoning**.

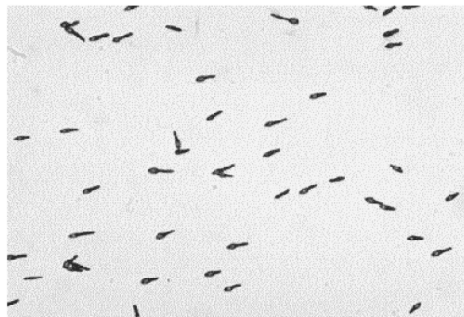


Fig. 35.7: *C. botulinum* sub-terminal spores

The bacterium produces **the most powerful toxin** known to man, which is the key virulence factor responsible for the pathogenesis of disease. Its action is the reverse of that of tetanus toxin, i.e. it prevents muscle contraction, leading to flaccid paralysis of important muscles. It inhibits the release of acetylcholine at **motor nerve endings** in the **parasympathetic nervous system**. This potent toxin is relatively heat-resistant (although destroyed by temperatures > 60°C), hence it is considered by the military as a suitable bioweapon. There are seven serotypes of botulinum toxin, named A-G. These act in almost identical ways; however, only types A, B and E cause human botulism.

C. botulinum can be isolated from left-over (suspect) food items. Isolates can be tested in the laboratory for toxin production. Traces of toxin can be found in food items or patient's serum. Laboratory animals are occasionally used to confirm the diagnoses.

Treatment is by removal of undigested food, injecting antitoxins and intensive therapy. Patients will require assistance with breathing and eating due to muscular paralysis. Botulinum toxin is sometimes used as a medical treatment for spastic paralysis of facial or bladder muscles.

Clostridium difficile

C. difficile is found in the feces of 3–5% of humans, in the gut of several animals and in the environment. The organism produces at least two potent toxins that are responsible for severe and occasionally fatal diarrhea. The organism is harmless in the normal gut where it cannot compete successfully against the resident gut flora. This competitive environment provides a useful barrier (**colonization resistance**) against *C. difficile* and many other pathogens. Administration of broadspectrum antibiotics in vulnerable patients, e.g. elderly inpatients, removes the competitive barrier in the gut, allowing *C. difficile* to grow and produce toxins. The latter then cause ‘**antibiotic-associated**’ colitis, ranging in severity from mild diarrhea to overwhelming **pseudomembranous colitis**.

Treatment is by withholding antibiotics where possible, replacing body fluids, administration of oral metronidazole or vancomycin and isolating the patient to prevent further spread of disease. Widespread dispersal of *C. difficile* spores in hospital environment can lead to nosocomial infection.



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INTEXT QUESTIONS 35.2

1. Spores are seen as area in simple strain
2. Lactic acid bacteria is also known as
3. *C. perfringens* causes &
4. *C. tetani* causes



WHAT HAVE YOU LEARNT

- Spore forming bacteria produce a unique resting cell called endospore, which allows them to survive in hostile environmental conditions
- Two medically important genera are bacillus & clostridium
- Bacillus & clostridium groups produce endospores
- Endospores forms inside of the bacterial cell
- Exospores forms outside the bacterial cell
- Endospores are seen as colourless area in simple strain
- Spore is surrounded by this delicate covering caused exosporium

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- Bacillus spores also called endospores are resistant to harsh chemical and physical conditions
- Sporolactobacillus are also known as Lactic acid bacteria
- Clostridia live commensally in the human gut
- C. Perfringens cause gas gangrene & food poisoning
- C. tetani causes tetanus



TERMINAL QUESTIONS

1. What are endospores
2. Describe sporulation cycle
3. Describe the structure of spore



ANSWERS TO INTEXT QUESTIONS

35.1

1. Endospore
2. Endo
3. Exo
4. Inside
5. Outside

35.2

1. Colourless
2. Sporolactobacillus
3. Gas gangrene & food Poisoning
4. Tetanus