Ministry of Higher Education
& Scientific Research
Al Muthanna University
College of Veterinary Medicine
Branch of Microbiology

Subject: Microbiology II
Grade: 3
Lecture : 1

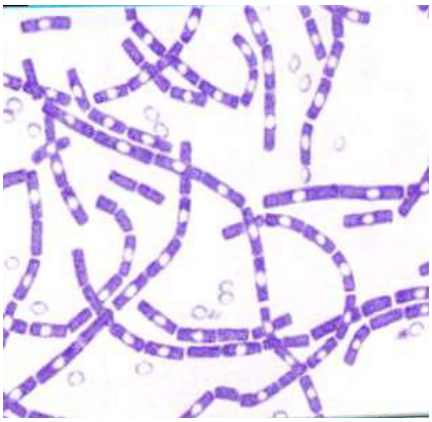
Microbiology II

Bacillus

Introduction

General Characteristics:

- Strict Aerobic
- Spore forming (Central, Sub - terminal of Terminal)
- Capsulated
- 1 X (3-4) µ in size
- Arranged in long chains
- Spores are resistant to heat.



Gram stain of Bacillus anthracis, the cause of anthrax

Classification

Domain:	Bacteria
Division:	Firmicutes
Class:	Bacilli
Order:	Bacillales
Family:	Bacillaceae
Genus:	*Bacillus*

* Early attempts at classification of Bacillus species were based on two characteristics:

- aerobic growth and

- endospore formation.

* Resulted in tethering together many bacteria possessing different kinds of physiology and occupying a variety of habitats.

* Hence, the heterogeneity in physiology, ecology, and genetics, made it difficult to categorize the genus Bacillus or to make generalizations about it.

* Bacilli includes the Order Bacillales and the Family Bacillaceae.In this family there 37 new genera on the level with Bacillus.

* The phylogenetic approach to Bacillus taxonomy has been accomplished largely by analysis of 16S rRNA molecules by oligonucleotide sequencing.

* Surprisingly, Bacillus species showed a kinship with certain nonsporeforming species, including Enterococcus, Lactobacillus, and Streptococcus at the Order level, and Listeria and Staphylococcus at the Family level.

* some former members of the genus Bacillus were gathered into new Families, including

- Acyclobacillaceae,
- Paenibacillaceae

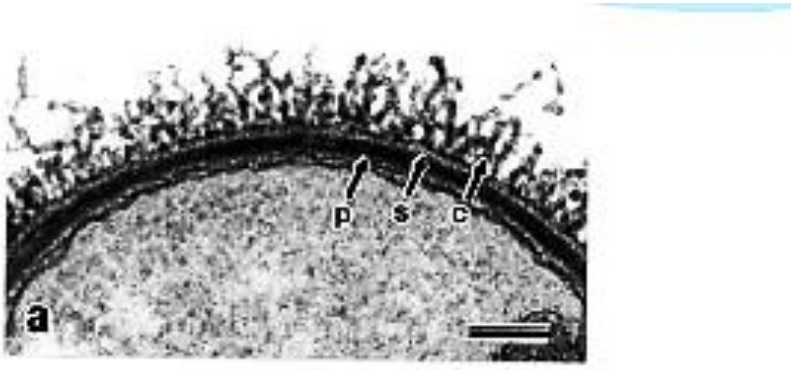
Bergey's Manual of Systematic Bacteriology (1st ed. 1986)	Bergey's Manual of Systematic Bacteriology (2nd ed. 2004)
Bacillus acidocalderius	*Acyclobacillus acidocalderius*
Bacillus alvei	*Paenibacillus alvei*
Bacillus brevis	*Brevibacillus brevis*

STRUCTURE

Surface of Bacillus:

- The surface of the Bacillus is complex and is associated with their properties of adherence, resistance and tactical responses.
- The vegetative cell surface is a laminated structure that consists of

- a capsule,
- a proteinaceous surface layer (S-layer),
- several layers of peptidoglycan sheeting
- the proteins on the outer surface of the plasma membrane.



Surface of a *Bacillus*. Transmission E.M. C=Capsule; S=S-layer; P=Peptidoglycan. Pasteur Institute

S-Layer:

- Crystalline surface layers of protein or glycoprotein subunits.
- As with S-layers of other bacteria, their function in Bacillus is unknown, but they have been presumed to be involved inadherence.
- It has been demonstrated that the S-layer can physically mask the negatively charged peptidoglycan sheet in some Gram-positive bacteria and prevent autoagglutination.
- It has also been proposed that the layer may play some role in bacteria-metal interactions.

Capsule:

- The capsules of many bacilli, including B. anthracis, B. subtilis, B. megaterium,and B. licheniformis, contain poly-D or L-glutamic acid.

- Other Bacillus species, e.g., B. circulans, B. megaterium, B. mycoides and B. pumilus, produce carbohydrate capsules. Dextran and levan are common, but more complex polysaccharides are produced, as well.
- The capsule of B. anthracis is composed of a poly-D-glutamic acid.
- The capsule is a major determinant of virulence in anthrax.
- The capsule is not synthesized by the closest relatives of B. anthracis, i.e., B. cereus and B. thuringiensis, and this criterion can be used to distinguish the species.

FA stain of the capsule of *Bacillus anthracis*. CDC

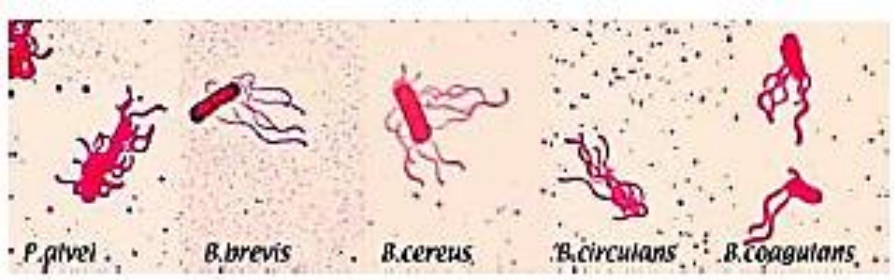
Cell wall

- The vegetative cell wall of almost all Bacillus species is made up of a peptidoglycan staining meso-diaminopimelic acid (DAP).
- (The cell walls of Sporosarcina pasteurii and S. globisporus, contain lysine in the place of DAP.)
- In all bacillus species, peptidoglycan in the cell wall, contain large amounts of teichoic acids which are bonded to muramic acid residues.
- The types of glycerol teichoic acids vary greatly between Bacillus species and within species.

- As in many other Gram-positive bacteria, lipoteichoic acids are found associated with the cell membranes of Bacillus species.

Flagella

- Most aerobic sporeformers are motile by means of peritrichous flagella. Chemotaxis has been studied extensively in B. subtilis.
- The flagellar filament of B. firmus, an alkaliphile, has a remarkably low content of basic amino acids, thought to render it more stable in environmental pH values up to 11



Flagellar stains (Leifson's Method) of various species of bacilli from CDC.

Individual cells of motile bacilli photographed on nutrient agar. About 15,000X. magnification. U.S. Dept. of Agriculture. A. B. subtilis; B. P. polymyxa; C. B. laterosporus; D. P. alvei.

Endo spore

• First discovered by Cohn in Bacillus subtilis and later by Koch in pathogen Bacillus Anthracis.

• Cohn demonstrated heat resistance of endospores.

• Koch described the developmental cycle of spore formation in B. anthracis.

• Endospores are formed intracellularly, although they are eventually released from this mother cell or sporangium as free spores

• Endospores do not form normally during active growth and cell division.

• Their differentiation begins when a population of vegetative cells passes out of the exponential phase of growth, usually as a result of nutrient depletion.

- Endospores have proven to be the most durable type of cell found in Nature, and
- In their cryptobiotic state of dormancy they can remain viable for extremely long periods of time, perhaps millions of years

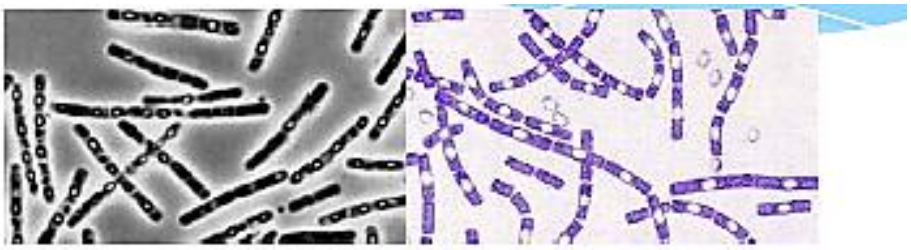
Staining of Endospore

• When viewed unstained, endospores of living bacilli appear edged in black and are very bright and refractile.

• Endospores strongly resist application of simple stains or dyes and hence appear as non-staining entities in Gram-stain preparations.

• Once stained, endospores are quite resistant to decolorization.

• This is the basis of several spore stains such as the Schaeffer-Fulton staining method which also differentiates the spores from sporangia and vegetative cells.



Left. *Bacillus thuringiensis* phase micrograph. Endospores can be readily recognized microscopically by their intracellular site of formation and their extreme refractility. Right. *Bacillus anthracis* Crystal violet stain viewed by light microscopy. Endospores are highly resistant to application of basic aniline dyes that readily stain vegetative cells.

Eco-physiological Groups:

S.N.	Groups	Organism	S.N.	Groups	Organism
1.	Acidophiles	*Bacillus coagulans*	5.	Thermophiles	*Bacillus schlegelii*
2.	Alkaliphiles	*Sporosarcina pasteurii*	6.	Denitrifiers	*Bacillus cereus*
3.	Halophiles	*Virgibacillus pantothenticus*	7.	Nitrogen-fixers	*Paenibacillus polymyxa*
4.	Psychrophiles	*Bacillus megaterium*	8.	Antibiotic Producers	*Bacillus licheniformis* (bacitracin)

Pathogens of Animals & Human:

- Bacillus anthracis and B. cereus are the predominant pathogens of medical importance.
- Paenibacillus alvei, B. megaterium, B. coagulans, Brevibacillus laterosporus, B. subtilis, B. sphaericus, B. circulans, Brevibacillus brevis, B. licheniformis, P. macerans, B. pumilus and B. thuringiensis have been occasionally isolated from human infections.
- B. anthracis is the causative agent of anthrax, and B. cereus causes food poisoning.

- Nonanthrax Bacillus species can also cause a wide variety of other infections, and they are being recognized with increasing frequency as pathogens in humans.

Microbiology II

Anthrax

BACILLUS ANTHRACIS

• Anthracis from the Greek word anthrakos for coal (black colour of the eschar).

• The historical interest is attached to the anthrax bacillus.

• 1st to Observe under Microscope. (Pollender, 1849)

• 1st to communicable disease. (Davaine, 1850)

• 1st to be isolated in pure culture and observe the spores. (Robert Koch, 1876)

• 1st to prepare for attenuated vaccine. (Louis Pasteur, 1881)

• Primarily a disease of domesticated & wild animals

– Herbivores such as sheep, cows, horses, goats

• Natural reservoir is soil

– Does not depend on an animal reservoir making it hard to eradicate

– Cannot be regularly cultivated from soils where there is an absence of endemic anthrax

– Occurs sporadically throughout US

– South Dakota, Arkansas, Texas, Louisiana, Mississippi, California recognized endemic areas

• Anthrax was for long feared as a potential tool in biologicalwarfare.

• This fear became an actual fact in 2001, when anthrax in the form of weapons grade spores having enhanced dispersability and virulence was sent by mail to various destinations in the USA, causing disease and death in many persons.

MORPHOLOGY

• The anthrax bacillus is one of the largest of pathogenic bacteria, measuring 3-10 µm x 1-1.6 µm.

• In tissues, it is found singly, in pairs or in short chains, the entire chain being surrounded by a capsule.

• The capsule is polypeptide in nature.

• Capsules are not formed under ordinary conditions of culture but only if the media contain added bicarbonate or are incubated under 10-25 % CO2.

• If grown in media containing serum, albumin, charcoal or starch, capsule formation may occur in the absence of CO2.

• In cultures, the bacilli are arranged end to end in long chains like 'bamboo stick' appearance.

• Spores are formed in culture or in the soil but never in the animal body during life.

• Spores are central or subterminal, elliptical or oval in shape, and are of the same width as the bacillary body so that they do not cause bulging of the vegetative cell.

• The anthrax bacillus is Gram positive and non-acid fast.

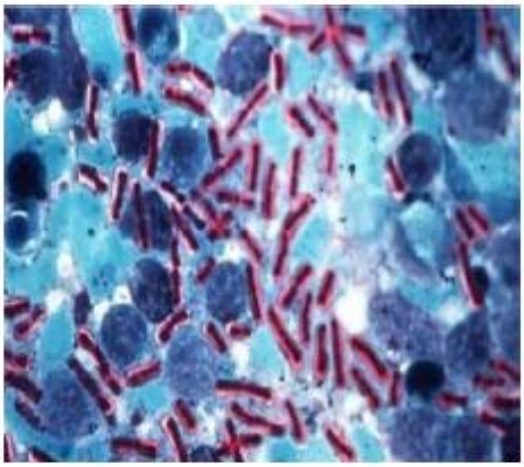
• The spores do not stain by ordinary methods but can be stained differentially by special techniques When stained with Sudan black B, fat globules may be made out within the bacilli.

• M"Fadyean's reaction: When blood films containing anthrax bacilli are stained with polychrome methylene bluefor a few seconds and examined under the microscope, an amorphous purplish material is noticed around the bacilli. This represents the capsular material and is characteristic of the anthrax bacillus.

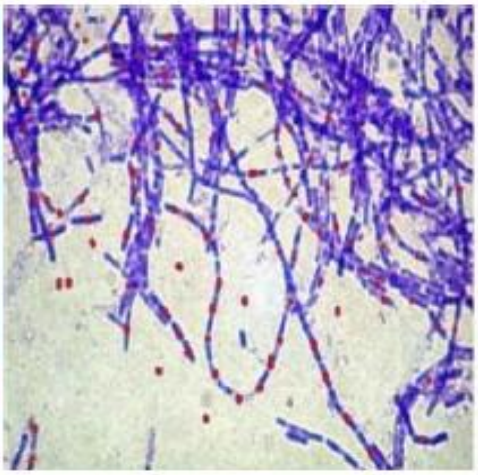
• This is called the M"Fadyean's reaction is the presumptive diagnosis of anthrax in animals.

• The anthrax bacillus is nonmotile, unlike most other members of this genus.

B. anthracis in McFadyean Reaction



B. anthracis in Spore stain



CULTURAL CHARACTERISTICS

- It is an aerobe, and facultative anaerobe, with a temperature range for growth of 12-45 °C (optimum 35-37 °C).
- Good growth occurs on ordinary media. On agar plates, irregularly round colonies are formed, 2-3 mm in diameter, raised, dull, opaque, greyish white, with a frosted glass appearance.
- Under the low power microscope, the edge of the colony is composed of long, interlacing chains of bacilli, resembling locks of matted hair. This is called the 'Medusa head appearance'
- On blood agar, the colonies are nonhemolytic.
- In broth growth occurs as floccular deposit.
- When B anthracis is grown on the surface of a solid medium containing 0.05-0.50 units of penicillin/ml, in 3-6 hours the cells become large, spherical, and occur in chains on the surface of the agar, resembling a string of pearls. This 'string of pearls reaction' differentiates clearly B anthracis from B cereus and other aerobic spore bearers (ASB's)
- A selective medium (PLET medium), consisting of polymyxin, lysozyme, ethylene diamine tetra acetic acid (EDTA) and thallous acetate added to heart infusion agar has been devised to isolate B anthracis from mixtures containing other spore bearing bacilli.

- **BIOCHEMICAL REACTIONS**

- Glucose, maltose and sucrose are fermented producing acid but no gas.
- Nitrates are reduced to nitrites.
- Catalase is formed.
- On gelatin stab culture, a characteristic 'inverted fir tree' appearance is seen, with slow liquefaction commencing from the top.

RESISTANCE

- The vegetative bacilli are not particularly resistant and are destroyed at 60°C in 30 minutes.
- In the carcasses of animals which have died of anthrax, the bacilli remain viable in the bone marrow for a week and in the skin for two weeks.
- The spores are highly resistant to physical and chemical agents.
- They have been isolated from naturally infected soil after as long as 60 years.
- They resist dry heat at 140°C for 1-3 hours and boiling for 10 minutes.
- They survive in 5 % phenol for weeks.
- $HgCl_2$ in a 1/1000 solution may fail to kill anthrax spores in less than 70 hours.
- 4% potassium permanganate kills them in 15 minutes.
- Destruction of the spores in animal products imported into nonendemic countries is achieved by ‘Duckering' in which 2% formaldehyde solution is used at 30-40 °C for 20 minutes for disinfection of wool and as 0.25 % at 60°C for six hours for animal hair and bristles.

- The anthrax bacillus is susceptible to sulphonamides, penicillin, erythromycin, streptomycin tetracycline and chloramphenicol.
- Occasional strains resistant to penicillin are encountered.

PATHOGENICITY

• In nature, anthrax is primarily a disease of cattle and sheep, and less often of horses and swine but experimentally most animals are susceptible to a greater or lesser degree.

• Rabbits, guinea pigs and mice are susceptible.

• Following the subcutaneous inoculation of a culture into a guinea pig, the animal dies in 24-72 hours showing a local, gelatinous, haemorrhagic edema at the site of inoculation, extensive subcutaneous congestion and characteristically, an enlarged, dark red, friable spleen.

• The bacilli are found in large numbers in the local lesion, heart blood and spleen (more than 108 bacilli/ ml)

VIRULANCE FACTORS

• Two virulence factors have been identified- Capsular polypeptide and Anthrax toxin.

• The capsular polypeptide aids virulence by inhibiting phagocytosis.

• Loss of the plasmid (pX 02) which controls capsule production leads to loss of virulence.

• This is how the live attenuated anthrax spore vaccine (Sterne strain) was obtained.

• The anthrax toxin was identified by the finding that the injection of sterile plasma of guinea pigs dying of anthrax into healthy guinea pigs killed them and that death could be prevented by immune serum.

• The toxin is a complex of three fractions:

• The edema factor (OF or Factor I)

• The protective antigen factor (PA or Factor II) and

• The lethal factor (LF or Factor III).

• They are not toxic individually but the whole complex produces local edema and generalized shock

• The three factors have been characterised and cloned.

• PA is the fraction that binds to the receptors on the target cell surface, and in turn provides attachment sites for OF or LF, facilitating their entry into the cell.

General concepts

• Anthrax is a zoonosis.

• Animals are infected by ingestion of the spores present in the soil.

• Direct spread from animal to animal is rare.

• The disease is generally a fatal septicemia but may sometimes be localised, resembling the cutaneous disease in human beings.

• Infected animals shed in the discharges from the mouth, nose and rectum, large numbers of bacilli, which sporulate in soil and remain as the source of infection.

• Human anthrax is contracted from animals, directly or indirectly. The disease may be 1. cutaneous; 2. pulmonary; or 3. intestinal, all types leading to fatal septicemia or meningitis.

CUTATEOUS ANTHRAX

• This follows entry of the infection through the skin.

• The face, neck, hands, arms and back are the usual sites.

• The lesion starts as a papule 1- 3 days after infection and becomes vesicular, containing fluid which may be clear or bloodstained

• The whole area is congested and edematous, and several satellite lesions filled with serum or yellow fluid are arranged round a central necrotic lesion which is covered by a black eschar (The name anthrax, which means coal, comes from the black colour of the eschar.)

- The lesion is called a malignant pustule.
- The disease used to be common in dock workers carrying loads of hides and skins on their bare backs and hence was known as the hide porter's disease.
- Cutaneous anthrax generally resolves spontaneously, but 10-20 per cent of untreated patients may develop fatal septicemia or meningitis.

PULMONARY ANTHRAX

• This is called the wool sorter's disease because it used to be common in workers in wool factories, due to Inhalation of dust from infected wool.

• This is a hemorrhagic pneumonia with a high fatality rate.

• Hemorrhagic meningitis may occur as a complication

INTESTINAL ANTHRAX

• This is rare and occurs mainly in primitive communities who eat the carcasses of animals dying of anthrax.

• A violent enteritis with bloody diarrhea occurs, with high case fatality.

Interesting notes

• Human anthrax may be industrial or non- industrial (agricultural).

• The former is found in workers in industries such as meat packing or wool factories.

• Non-industrial anthrax is often an occupational disease in those who associate frequently with animals, such as veterinarians, butchers and farmers. It may also be found in the general population.

• Cutaneous anthrax used to be caused by shaving brushes made with animal hair.

• Stomoxys calcitrans and other biting insects may occasionally transmit infection mechanically.

PROPHYLAXIS

• Prevention of human anthrax is mainly by general methods such as improvement of factory hygiene and proper sterilisation of animal products like hides and wool.

• Carcasses of animals suspected to have died of anthrax are buried deep in quicklime or cremated to prevent soil contamination.

• Prevention of anthrax in animals is aided by active immunisation.

• The original Pasteur's anthrax vaccine is of great historical importance.

• It was Pasteur's convincing demonstration of the protective effect of his anthrax vaccine in a public experiment at Pouillyle- Fort in 1881 that marked the beginning of scientific immunoprophylaxis.

• Pasteur's vaccine was the anthrax bacillus attenuated by growth at 42-43 °c.

• The spore is the common infective form in nature, vaccines consisting of spores of attenuated strains were developed.

• The Sterne vaccine contained spores of a noncapsulated avirulent mutant strain.

• The Mazucchi vaccine contained spores of stable attenuated Carbazoo strain in 2 % saponin.

• The spore vaccines have been used extensively in animals with good results. They give protection for a year following a single injection.

• They are not considered safe for human use, though they have been used for human immunisation in Russia.

• Alum precipitated toxoid prepared from the protective antigen has been shown to be a safe and effective vaccine for human use It has been used in persons occupationally exposed to anthrax infection.

• Three doses given intramuscularly at intervals of six weeks between first and second, and six months between second and third doses induce good immunity, which can be reinforced if necessary with annual booster injections.

TREATMENT

• Antibiotic therapy is effective in human cases but rarely succeeds in animals as therapy is not started sufficiently early.

• Antibiotics have no effect on the toxin once it is formed.

• Penicillin and streptomycin are no longer used for treatment.

• They have been replaced by doxycycline and ciprofloxacin, which are effective in prophylaxis and treatment.

Dr. Naer Alkaabi