Microbiology II

Bacillus

Introduction

General Characteristics:

• Strict Aerobic

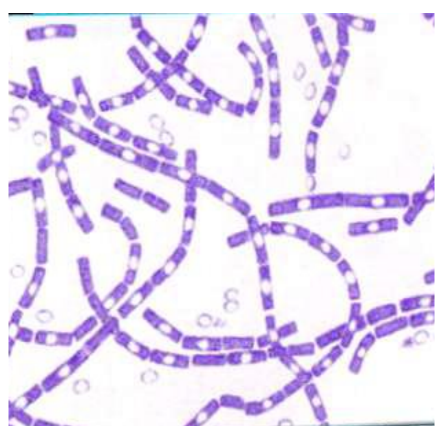
• Spore forming (Central, Sub - terminal of Terminal)

• 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

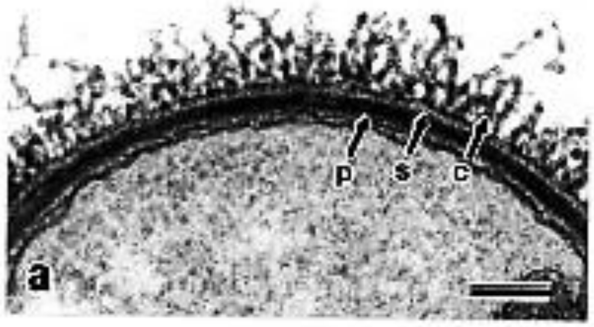
Bergey's Manual of Systematic Bacteriology (1st ed. 1986)	Bergey's Manual of Systematic Bacteriology (2nd ed. 2004)
Bacillus acidocalderius	*Acyclobacillus acidocalderius*
Bacillus alvei	*Paenibacillus alvei*
Bacillus brevis	*Brevibacillus brevis*

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

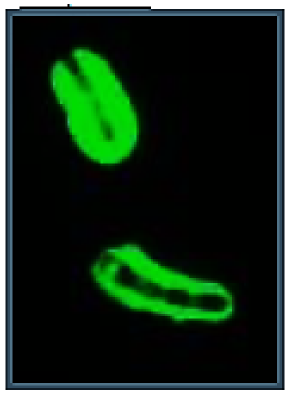
S-Layer:

- 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

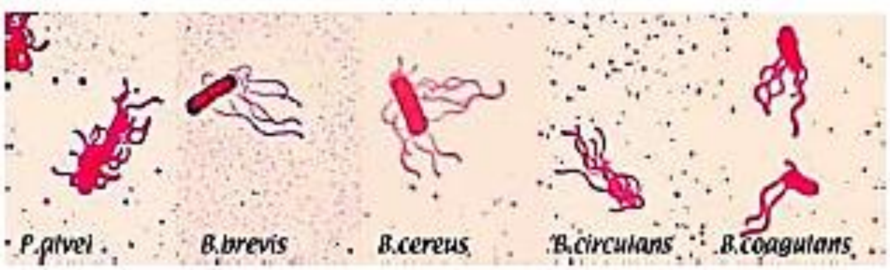
Cell wall

- 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

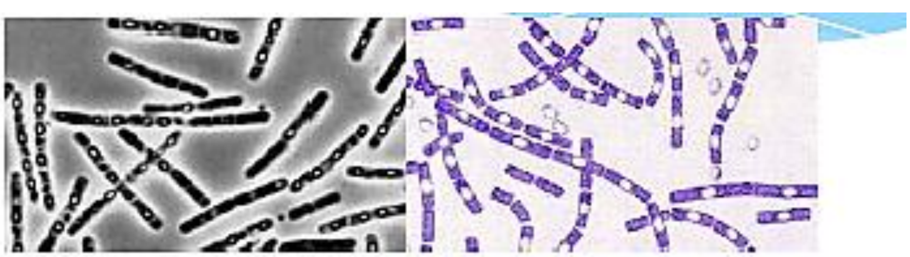
Staining of Endospore

• 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.

Dr. Naer Alkaabi