

Chapter 2

Bacillus spp.

Genomics, Morphologies, and Growth Characteristics

- **Genomics:**

- *Bacillus anthracis* – chromosome, 5,227,293 bp; 5508 predicted ORFs (Read et al. 2003):
 - pOX1 plasmid: 181,677 bp; 217 predicted ORFs
 - pOX2 plasmid: 94,829 bp; 113 predicted ORFs
- *Bacillus cereus* – chromosome: 5,426,909 bp; 5366 predicted ORFs (Ivanova et al. 2003)

- **Cell morphology:**

- Large, boxy rod-shaped cells; usually in single short chains or long chains (Fig. 2.1)
- Endospore former; subterminal or central endospores that do not swell the cell

- **Gram stain:**

- Gram positive; older cells tend to stain Gram negative

- **Growth:**

- Obligate aerobes or facultative anaerobes; catalase positive
- Ubiquitous environmental pathogens found primarily in soil; also in water, dust, agricultural products, and invertebrates; primarily exist in endospore form
- Common laboratory contaminant
- Most species are highly motile (except *Bacillus anthracis*) with peritrichous flagella – involved in biofilm formation (*B. cereus*)

Fig. 2.1 *B. anthracis* cells
(From: Public Health
Image Library (PHIL)
#9826)



- >300 species; most are rarely associated with human disease:
 - Two major human pathogens: *B. anthracis* and *B. cereus*

Disease States Associated with *Bacillus* spp.

- ***B. anthracis*:**
 - Anthrax – cutaneous (most common), inhalation (most lethal), and gastrointestinal (rare):
 - Cutaneous: endospores enter cuts or abrasions through direct contact with infected animal, wool, or animal hides; results in eschar lesion (Fig. 2.2)
 - Inhalation: endospores enter the lungs and germinate in lung phagocytes; bacteria enter bloodstream and lead to septic shock symptoms
 - Gastrointestinal: endospores are ingested usually in undercooked meat; leads to gastrointestinal pain and bleeding
- ***B. cereus*:**
 - Two forms of food-borne intoxications:
 - Short incubation time emetic intoxication:
 - Nausea and emesis (vomiting)
 - Associated with heat-stable emetic toxin (ETE) cereulide
 - Long incubation time diarrheal intoxication:
 - Abdominal cramps and diarrhea; non-emetic
 - Associated with heat-labile enterotoxin Nhe and/or hemolytic enterotoxin HBL
 - Ocular infections; endocarditis; musculoskeletal infections

Fig. 2.2 Eschar lesion
(From: PHIL #1934)



Virulence Factors

- ***B. anthracis*** (Mikesell et al. 1983):
 - Virulence plasmids pXO1 and pXO2 are essential for virulence
 - Adherence to host cells:
 - **BsIA** (*Bacillus anthracis* S-layer protein A) (Kern and Schneewind 2008, 2010):
 - Major S-layer hydrophobic protein
 - Binds to host cells by interacting with host integrin $\alpha 2 \beta 1$ and complement component C1q
 - Negatively regulated by InhA protease (Tonry et al. 2012)
 - *bsIA* gene encoded on the pXO1 virulence plasmid
 - **Capsule:**
 - Poly- γ -D-glutamic acid capsule
 - Antiphagocytic factor; used to evade host immune system
 - Encoded by *capBCADE* operon on the pXO2 virulence plasmid
 - **S-layer:**
 - ~24 proteins that self-assemble into a crystalline sheet surrounding the cell; may contain other adhesins besides BsIA
 - Binds extracellular matrix (ECM) components
- Growth in host cells:
 - Iron acquisition – required for growth in host:

- Siderophores (Cendrowski et al. 2004; Hotta et al. 2010; Wilson et al. 2010):
 - **Petrobactin:**
 - Catecholate iron siderophore; predominate siderophore secreted early in infection
 - Encoded by *asbAB* genes
 - **Bacillibactin:**
 - Catecholate iron siderophore; secreted late in infection
 - Encoded by *bacACEBF* operon
- Fe-heme scavengers (Segond et al. 2014):
 - **IlsA** (iron-regulated leucine rich surface protein A):
 - Binds host heme, hemoglobin, and ferritin
 - Analogous to Isd (iron-regulated surface determinant) system in *Staphylococcus aureus*
- Biofilms: capable of forming biofilms, but unclear if biofilms of vegetative *B. anthracis* exist in the environment or in human hosts
- Damage to host cells:
 - **Anthrax toxins** (Fig. 2.3) (Friebe et al. 2016; Liu et al. 2014; Prince 2003):
 - A-B exotoxins – one common B subunit with two different A subunits:

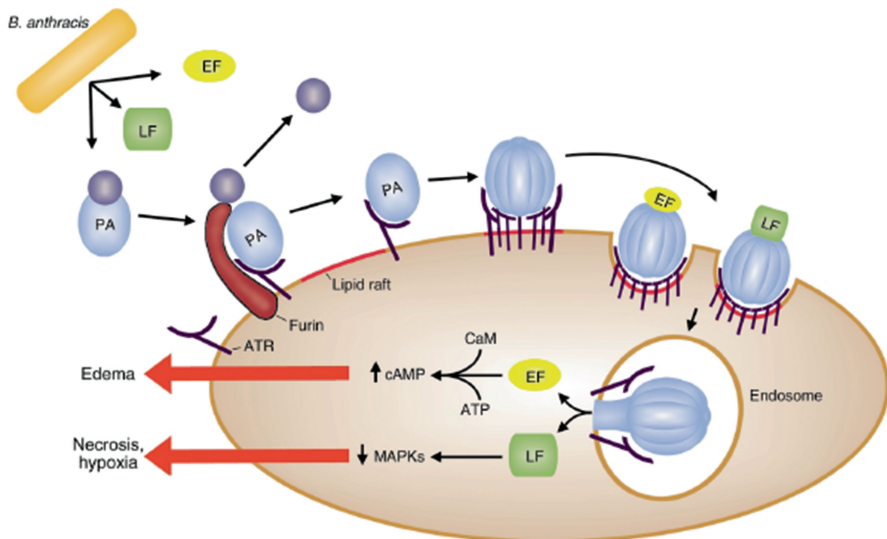
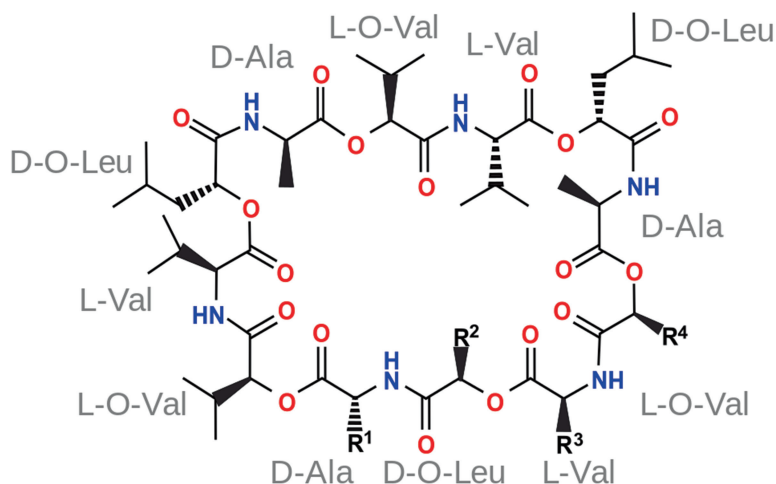


Fig. 2.3 Anthrax toxin effects (From: Prince 2003)

- B subunit:
 - **PA (protective antigen)**: forms homo-heptameric complex
 - Binds to host anthrax toxin receptor (ATR) and host capillary morphogenesis protein CMG2
 - Encoded by *pagA* gene on pOX1 virulence plasmid
- A subunits:
 - **EF (edema factor)**:
 - Calcium-/calmodulin-dependent adenylate cyclase; converts ATP to cAMP
 - Increases host intracellular cAMP levels; induces water and ion loss from cells and edema in surrounding tissues
 - Usually targets liver hepatocytes
 - **LF (lethal factor)**:
 - Zn metalloprotease
 - Cleaves host mitogen-activated protein kinases (MAPKs); loss of these signaling proteins leads to cell death
 - Usually targets cardiomyocytes and vascular smooth muscle cells
 - Encoded by *lef* (LF) and *cya* (EF) genes on pOX1 virulence plasmid
- **Anthrolysin O (ALO)** (Shannon et al. 2003):
 - Cholesterol-dependent cytolysin
 - Belongs to the β -pore-forming toxin (β -PFT) family of pore-forming toxins (Peraro and van der Goot 2016)
 - Lytic activity against host phagocytic cells
 - Encoded by *bas3109* gene
- ***B. cereus*** – most strains lack the pXO1 and pXO2 virulence plasmids:
 - Adherence to host cells: same as *B. anthracis*; capsule synthetic genes are encoded on the chromosome
 - Growth in host cells:
 - Iron acquisition: same as *B. anthracis*
 - Biofilms (Majed et al. 2016):
 - Excellent biofilm former in host and in environment
 - Plays key roles in persistence and ubiquitous distribution in the environment
 - Provides resistance to various stresses
 - Excellent adhesive capacity on biotic and abiotic substrates



- 1 cereulide ; $R^1 = \text{Me}$, $R^2 = i\text{-Bu}$, $R^3 = R^4 = i\text{-Pr}$
- 2 cereulide DAK1; $R^1 = (\text{CH}_2)_4\text{NH}_3^+$, $R^2 = i\text{-Bu}$, $R^3 = R^4 = i\text{-Pr}$
- 3 cereulide DOLK1; $R^1 = \text{Me}$, $R^2 = (\text{CH}_2)_4\text{NH}_3^+$, $R^3 = R^4 = i\text{-Pr}$
- 4 cereulide LVK1; $R^1 = \text{Me}$, $R^2 = i\text{-Bu}$, $R^3 = (\text{CH}_2)_4\text{NH}_3^+$, $R^4 = i\text{-Pr}$
- 5 cereulide LOVK1; $R^1 = \text{Me}$, $R^2 = i\text{-Bu}$, $R^3 = i\text{-Pr}$, $R^4 = (\text{CH}_2)_4\text{NH}_3^+$

Fig. 2.4 Cereulide structure (From: Thieme E-journals)

- Host cell damage:
 - A-B exotoxins: none; lack of pOX1 virulence plasmid
 - **Cereulide** (Ehling-Schulz et al. 2015):
 - Heat-stable emetic toxin (ETE); associated with short incubation time emetic intoxication
 - Pore-forming cytolytic toxin
 - Hydrophobic cyclic dodecapeptide (Fig. 2.4)
 - Encoded on a pOX-1-like plasmid
 - Synthesized by the nonribosomal cereulide synthetase (*ces*) complex (Ehling-Schulz et al. 2005)
 - **Hemolysin BL (HBL)** (Ramaraio and Sanchis 2013; Senesi and Ghelardi 2010):
 - Associated with long incubation time diarrheal intoxication
 - Tripartite toxin: B, L1, and L2 subunits; encoded by *hblA*, *hblC*, and *hblD* genes
 - Pore-forming cytolytic exotoxin; all three subunits are needed for toxicity

- **Nonhemolytic enterotoxin (Nhe)** (Senesi and Ghelardi 2010):
 - Associated with long incubation time diarrheal intoxication
 - Tripartite toxin: NheA, NheB, and NheC subunits; encoded by *nheA*, *nheB*, and *nheC* genes
 - Pore-forming cytolytic exotoxin; all three subunits are needed for toxicity
- **Hemolysin HlyI (cereolysin O)** (Ramarao and Sanchis 2013):
 - Homologous to *B. anthracis* anthrolysin O (ALO)
 - Heat-labile, cholesterol-dependent cytolysin; belongs to the β -PFT family of pore-forming toxins
 - Encoded by *hlyI* gene
- **Hemolysin HlyII** (Ramarao and Sanchis 2013):
 - Heat-labile, cholesterol-independent cytolysin; belongs to the β -PFT family of pore-forming toxins
 - Encoded by *hlyII* gene
- **Hemolysin HlyIII** (Ramarao and Sanchis 2013):
 - Heat-labile cholesterol-independent cytolysin
 - Encoded by *hlyIII* gene; least characterized *B. cereus* hemolysin
- **Hemolysin IV (cytotoxin K, CytK)** (Ramarao and Sanchis 2013; Senesi and Ghelardi 2010):
 - Cytolysin; belongs to the β -PFT family of pore-forming toxins
 - Encoded by *cytK* gene
- **Phosphatidylcholine-specific phospholipase C (PC-PLC)** (Pomerantsev et al. 2003):
 - Responsible for the hemolytic properties of *B. cereus*
 - Encoded by *plc* gene
- **Sphingomyelinase (SPH)** (Pomerantsev et al. 2003):
 - Responsible for hemolytic properties of *B. cereus*
 - Encoded by *sph* gene

Regulation of Virulence Factor Expression

- **Quorum sensing:**

- **PlcR–PapR regulon** (Agaisse et al. 1999; Grenha et al. 2013):
 - **PlcR** – 34 kDa global transcriptional activator; helix-turn-helix (HTH) type regulator:
 - Inactive in *B. anthracis* due to nonsense mutation; hence, virulence factors activated in *B. cereus* are not expressed in *B. anthracis*
 - **PapR** – 48 aa peptide:
 - Secreted, reimported, and then truncated to a heptapeptide (PapR7)
 - Binding to PlcR activates its function
 - PlcR–PapR binds to PlcR-box palindromic DNA sequences upstream of target genes
 - At least 45 genes are under PlcR–PapR regulation: environmental sensors, enterotoxins, cytolytic exotoxins, and hemolysins – these virulence factors are not expressed in *B. anthracis*

- **Iron regulon:**

- **Fur (Fe utilization repressor)** – Fe⁺²-binding repressor protein:
 - Regulates all aspects of iron regulation
 - Fe⁺²-binding activates Fur repressor activity
 - Fe⁺²-Fur binds to Fur-box DNA sequences upstream of its negatively regulated target genes
 - Low Fe⁺² concentrations lead to derepression of siderophore genes; also regulated by temperature, oxidative stress, and CO₂ atmosphere

- **Anthrax toxin synthesis** – *pagA*, *lef*, and *cya* regulon (*B. anthracis*):

- **AtxA**: transcriptional activator (Dai et al. 1995; Fouet and Mock 2006; Uchida et al. 1993; Kolsto et al. 2009)
- **PagR**: transcriptional repressor
- Encoded on pXO1 pathogenicity island (PAI)

- **Capsule synthesis** – *capBCADE* operon (*B. anthracis*):

- **AcpA** and **AcpB** (Fouet and Mock 2006):
 - Transcriptional activators; regulated by AtxA activator
 - Encoded on pXO2 pathogenicity island (PAI)

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