**Bacillus**

*Bacillus* is a genus of Gram-positive, rod-shaped bacteria and a member of the division Firmicutes. *Bacillus* species can be obligate aerobes or facultative anaerobes, and test positive for the enzyme catalase.\(^\text{[1]}\) Ubiquitous in nature, *Bacillus* includes both free-living and pathogenic species. Under stressful environmental conditions, the cells produce oval endospores that can stay dormant for extended periods. These characteristics originally defined the genus, but not all such species are closely related, and many have been moved to other genera.\(^\text{[2]}\)

**Clinical significance**

Two *Bacillus* species are considered medically significant: *B. anthracis*, which causes anthrax, and *B. cereus*, which causes a foodborne illness similar to that of *Staphylococcus*.\(^\text{[3]}\)

An easy way to isolate *Bacillus* is by placing nonsterile soil in a test tube with water, shaking, placing in melted mannitol salt agar, and incubating at room temperature for at least a day. Colonies are usually large, spreading and irregularly-shaped. Under the microscope, the *Bacillus* cells appear as rods, and a substantial portion usually contain an oval endospore at one end, making it bulge.
**Scientific classification**

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

**Species**

Numerous, including:
- *B. alcalophilus*
- *B. alvei*
- *B. aminovorans*
- *B. amyloliquifaciens*
- *B. aneurinolyticus*

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B. anthracis
B. atrophaeus
B. aquaemaris
B. boroniphilus
B. brevis
B. caldolyticus
B. centrosporus
B. cereus
B. circulans
B. coagulans
B. firmus
B. flavothermus
B. fusiformis
B. globigii
B. infernus
B. larvae
B. laterosporus
B. lentus
B. licheniformis
B. megaterium
B. mesentericus
B. mucilaginosus
B. mycoides
B. natto
B. pantothenticus
B. polymyxa
B. pseudoanthracis
B. pumilus
B. schlegelii
B. sphaericus
B. sporothermodurans
B. stearothermophilus
B. subtilis
B. thermoglucosidasius
B. thuringiensis
B. vulgatis
B. weihenstephanensis
**Selected Species:** *anthracis ; cereus*

1- *Bacillus anthracis*

*Bacillus anthracis* is the pathogen of the anthrax acute disease. It is a Gram-positive, spore-forming, rod-shaped bacterium, with a width of 1-1.2µm and a length of 3-5µm. It can be grown in an ordinary nutrient medium under aerobic or anaerobic conditions.\(^4\)

![Photomicrograph of Bacillus anthracis](image)

Photomicrograph of *Bacillus anthracis*
(fuchsin-methylene blue spore stain)

![Structure of Bacillus anthracis](image)

Structure of *Bacillus anthracis*
CapD protein crystal structure of *Bacillus anthracis*

It is one of few bacteria known to synthesize a protein capsule (D-glutamate). It forms a calmodulin-dependent adenylate cyclase exotoxin known as (edema factor), along with lethal factor.

*Bacillus anthracis* spores in particular are highly resilient, surviving extremes of temperature, low-nutrient environments, and harsh chemical treatment over decades or centuries.

**Pathogenesis**

Three forms of anthrax disease are recognized based on their form of inoculation.

- **Cutaneous**, the most common form (95%), causes a localized, inflammatory, black, necrotic lesion (eschar).
- **Pulmonary**, the highly fatal form, is characterized by sudden, massive chest edema followed by cardiovascular shock.
- **Gastrointestinal**, a rare but also fatal (causes death to 25%) type, results from ingestion of spores.
**Treatment**

Infections with \( B. \text{ anthracis} \) can be treated with \( \beta \)-lactam antibiotics such as penicillin, and others which are active against Gram-positive bacteria.\[^5\] Penicillin-resistant \( B. \text{ anthracis} \) can be treated with fluoroquinolones such as ciprofloxacin or tetracycline antibiotics such as doxycycline.

**Laboratory research**

Components of tea, such as polyphenols, have the ability to inhibit the activity both of \( Bacillus \text{ anthracis} \) and its toxin considerably; spores, however, are not affected. The addition of milk to the tea completely inhibits its antibacterial activity against anthrax.\[^6\] Activity against the \( B. \text{ anthracis} \) in the laboratory does not prove that drinking tea affects the course of an infection, since it is unknown how these polyphenols are absorbed and distributed within the body.

**Host interactions**

As with most other pathogenic bacteria, \( B. \text{ anthracis} \) must acquire iron to grow and proliferate in its host environment. The most readily available iron sources for pathogenic bacteria are the heme groups used by the host in the transport of oxygen. To scavenge heme from host hemoglobin and myoglobin, \( B. \text{ anthracis} \) uses two secretory siderophore proteins, IsdX1 and IsdX2. These proteins can separate heme from hemoglobin, allowing surface proteins of \( B. \text{ anthracis} \) to transport it into the cell.\[^7\]

**Therapeutic Agents \( B. \text{ anthracis} \) Vaccines**

Several types of vaccines are currently under development. These include: recombinant Protective Antigen (rPA)-based...
anthrax vaccines; protective antigen (PA) plus capsular poly-
gamma-D-glutamic acid (PGA) based anthrax vaccine (designed
to attack both bacilli and toxin); and epicutaneous and intranasal
vaccine (developed for needle-free administration). Anthrax
vaccine research is working toward developing a vaccine that
will confer simultaneous protection against spores, bacilli, and
toxins.

**Inhibitors**

Metalloprotease inhibitors composed of peptides and small
molecules are being identified and developed to neutralize
anthrax toxin and treat Bacillus anthracis infection. Inhibitors of lethal factor may rescue cells by inhibiting toxin
actions such as receptor binding, mitogen-activated protein
kinase (MAPK) kinase cleavage, enzymatic functions, blocking
of pore formation or translocation.

**2-Bacillus cereus**

*Bacillus cereus* is an endemic, soil-dwelling, Gram-positive,
rod-shaped, beta hemolytic bacterium. Some strains are harmful
to humans and cause foodborne illness, while other strains can
be beneficial as probiotics for animals.⁸ *B. cereus* bacteria are
aerobes, and like other members of the genus *Bacillus* can
produce protective endospores. Its virulence factors include
cereolysin and phospholipase C.
**Pathogenesis**

*B. cereus* is responsible for a minority of foodborne illnesses (2–5%), causing severe nausea, vomiting and diarrhea.\(^9\) *Bacillus* foodborne illnesses occur due to survival of the bacterial endospores when food is improperly cooked.\(^10\) Cooking temperatures less than or equal to 100 °C (212 °F) allows some *B. cereus* spores to survive.\(^11\) This problem is compounded when food is then improperly refrigerated, allowing the endospores to germinate.\(^12\) Cooked foods not meant for either immediate consumption or rapid cooling and refrigeration should be kept at temperatures above 60 °C (140 °F).\(^11\) Germination and growth generally occurs between 10–50 °C (50–122 °F),\(^11\) though some strains are psychrotrophic.\(^13\)

Bacterial growth results in production of enterotoxins, one of which is highly resistant to heat and to pH between 2 and 11,\(^14\) ingestion leads to two types of illness, diarrheal and emetic (vomiting) syndrome.\(^15\)

- The diarrheal type is associated with a wide-range of foods, has an 8- to 16.5-hour incubation time and is associated with
diarrhea and gastrointestinal pain. Also known as the long-incubation form of *B. cereus* food poisoning, it might be difficult to differentiate from poisoning caused by *Clostridium perfringens*.[14]

- The emetic form is commonly caused by rice that is not cooked for a time and temperature sufficient to kill any spores present, then improperly refrigerated. It can produce a toxin, cereulide, which is not inactivated by later reheating. This form leads to nausea and vomiting 1–5 hours after consumption. It can be difficult to distinguish from other short-term bacterial foodborne pathogens such as *Staphylococcus aureus*.[14]

The diarrhetic syndromes observed in patients are thought to stem from the three toxins Hemolysin BL Hbl, Nonhemolytic Enterotoxin Nhe and Cytotoxin K CytK.[16] These enterotoxins are all produced in the small intestine of the host, thus thwarting the issue of digestion by host endogenous enzymes. The Hbl and Nhe toxins are pore-forming toxins closely related to ClyA of *E. coli*. The proteins exhibit a conformation known as "beta-barrel" that can insert into cellular membranes due to a hydrophobic exterior, thus creating pores with hydrophilic interiors. The effect is loss of cellular membrane potential and eventually cell death. CytK is a pore-forming protein more related to other hemolysins.

It was previously thought that the timing of the toxin production might be responsible for the two different courses of disease, but in fact the emetic syndrome is caused by a toxin called cereulide that is found only in emetic strains and is not part of the "standard toolbox" of *B. cereus*. Cereulide is cyclic polypeptide containing 3 repeats of 4 amino acids: D-Oxy-Le—D-Ala—L-Oxy-Val—L-Va (similar to Valinomycin produced by *Streptomyces griseus*) produced by nonribosomal peptide
synthesis (NRPS). Cereulide is believed to bind to 5-hydroxytryptamine 3 (5-HT3) serotonin receptors activating them and leading to increased afferent vagus nerve stimulation.\[^{[17]}\]

*B. cereus* is also known to cause chronic skin infections that are difficult to eradicate though less aggressive than *necrotizing fasciitis*. *B. cereus* can also cause keratitis.\[^{[18]}\]

**Diagnosis**

In case of foodborne illness, the diagnosis of *B. cereus* can be confirmed by the isolation of more than $10^5$ *B. cereus* organisms per gram from epidemiologically implicated food, but such testing is often not done because the illness is relatively harmless and usually self-limiting.\[^{[19]}\]

**Prevention**

Total prevention is probably not possible, however properly stored, heated and cooked foods are generally safe for the non-emetic type. The largest risk is cross-contamination, where cooked material comes into contact with raw produce or contaminated materials.

The emetic type is generally associated with improperly stored starchy products (rice). Proper storage (below 7 °C and for only a few days) will prevent outgrowth and toxin production.
What sanitation methods are used to prevent the contamination of foods?

*Bacillus cereus* spores are extremely heat resistant, so while cooking at proper temperatures would destroy most foodborne pathogens including the vegetative cells of *B. cereus*, it does not destroy the spores. While heat resistance is increased by high salt concentrations and gradual heating, the spores lose their heat resistance in acidic environments. Spores can be activated by heat and or improper handling; therefore the 2001 Food Code recommends that hot foods be maintained at a temperature of 140°F or above.

According to the National Institutes of Health (NIH), the National Institute of Allergy and Infectious Diseases (NIAID), and the National Food Processors Association (NFPA), the suggestions below are good examples of how to destroy *B. cereus*:

- Steaming under pressure, roasting, frying and grilling foods can destroy the vegetative cells and spores.
- Foods infested with the diarrheal toxin can be inactivated by heating for 5 minutes at 133°F.
- Foods infested with the emetic toxin need to be heated to 259°F for more than 90 minutes. Reheating foods until they're steaming is not enough to kill the emetic toxin.
In meat processing facilities, to prevent contamination and toxin formation:

- Assure current Good Manufacturing Practices (cGMP) (21 CFR 110), are being used in the slaughterhouses and processing units.
- Apply approved treatments of carcasses to remove fecal bacteria.
- Use proper cleaning and disinfection of food contact surfaces (FCS) with hypochlorite or other approved sanitizers.
- Utilize a heat process to destroy the vegetative cells and a rapid cooling process to prevent the spores from germinating.
- Keep hot foods above 60°C and cold foods below 4°C to prevent the formation of spores.
- Wash hands, utensils, FCSs with hot soapy water after they touch raw meat or poultry, or before food preparation, and after using the bathroom.
- Cook beef and beef products thoroughly.
- Properly refrigerate leftovers.

**References**


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