MICROBIOLOGY
BACILLUS CEREUS

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CONTENTS

INTRODUCTION

- ***Bacillus cereus*** is a Gram-positive rod-shaped bacterium commonly found in soil, food, and marine sponges.
- The specific name, *cereus*, meaning "waxy" in Latin, refers to the appearance of colonies grown on blood agar.
- Some strains are harmful to humans and cause foodborne illness due to their spore-forming nature, while other strains can be beneficial as probiotics for animals, and even exhibit mutualism with certain plants.
- *B. cereus* bacteria may be anaerobes or facultative anaerobes, and like other members of the genus *Bacillus*, can produce protective endospores.
- They have a wide range of virulence factors, including phospholipase C, cereulide, sphingomyelinase, metalloproteases, and cytotoxin K,etc.
- *B. cereus* strains exhibit flagellar motility.

Bacillus cereus on sheep blood agar (SBA)

Gram-positive *Bacillus cereus*

TAXONOMY

Domain:	Bacteria
Phylum:	Bacillota
Class:	Bacilli
Order:	Bacillales
Family:	Bacillaceae
Genus:	*Bacillus*
Species:	***B. cereus***

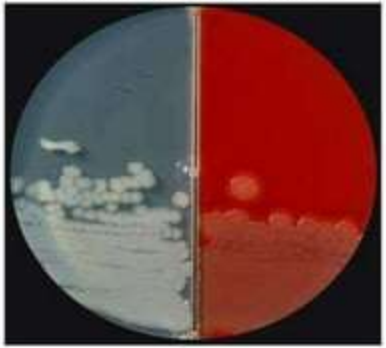
MORPHOLOGY

1. *Bacillus cereus* is gram-positive rod-shaped bacilli with square ends.
2. Occasionally may appear gram variable or even gram-negative with age.
3. They are single rod-shaped or appear in short chains.
4. Clear cut junctions between the members of chains are easily visible.
5. Tissue section staining may appear long and filamentous.
6. They are straight or slightly curved.
7. They are non-capsulated.
8. It contains spores with central spores.

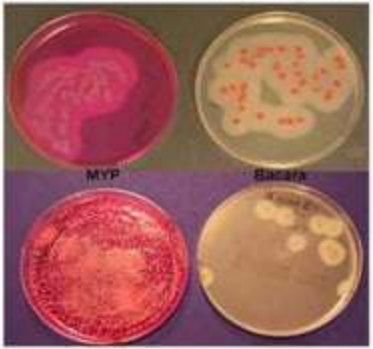
1. Spores are oval (ellipsoidal) and not formed in the animal's blood and tissues or in aerobic culture.
2. It is 1×3-4 μm in size.
3. It is motile and flagellated with peritrichous flagella.
4. Endospores are able to survive long periods of exposure to air and other adverse environmental conditions.
5. It is a beta-hemolytic bacterium that causes foodborne disease.
6. Its virulence factors include cerolysin and phospholipase.

CULTURAL PROPERTIES

Bicarbonate agar (left) and blood agar (right) plate cultures of Bacillus cereus.



Colonies of B. cereus grown on MYP are pink
Colonies of B. cereus grown on Bacara are pink-orange



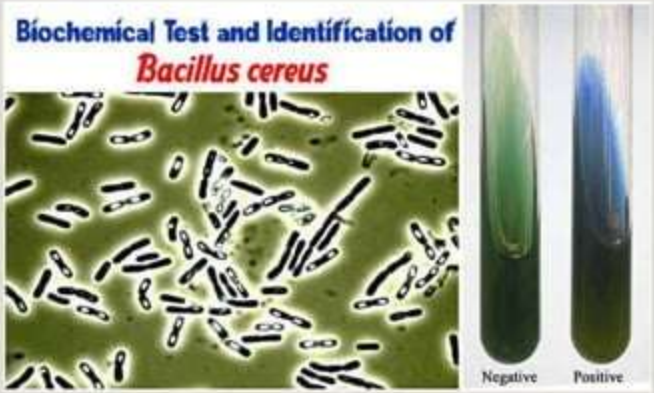
1. Growth on 5% sheep blood agar, chocolate agar, routine blood culture media, and nutrient broths.
2. Detectable growth within 24 hours following incubation on media incubated at 35° C, in ambient air, or in 5% carbon dioxide (CO_2).
3. Colony character on blood agar: Large, feathery, spreading, dull, gray, granular, spreading colonies and opaque with a rough matted surface and irregular perimeters, beta-hemolytic.
4. *Bacillus cereus* can be isolated from feces by using selective media such as MYPA (mannitol, egg yolk, polymyxin, phenol red, and agar), PEMBA (polymyxin, egg yolk, mannitol, bromothymol, blue agar).
5. These media take advantage of the phospholipase C positive reaction on egg yolk agar, no production of acid from mannitol, and incorporation of pyruvate or polymyxin as the selective agents.

1. Most *Bacillus* spp grow readily on nutrient agar or peptone media.
2. The optimum temperature for growth varies from 20°C to 40°C, mostly 37°C.
3. *B. cereus* is mesophilic and is capable of adapting to a wide range of environmental conditions.
4. On Nutrient Agar at 37°C, it forms large (2-5 mm) grey-white, granular colonies with a less wavy edge and less membranous consistency.
5. On 5% sheep blood agar at 37°C, *B. cereus* colonies are large, feathery, dull, gray, granular, spreading colonies, and opaque with a rough matted surface and irregular perimeters.

1. On blood agar, it is beta-hemolytic.
2. In some instances, smooth colonies develop either alone or in the midst of rough colonies.
3. When grown apart from the initial inoculum, smooth colonies are surrounded by a uniform zone of beta-hemolysis framing the centrally situated colony.
4. The MYP (Mannitol egg Yolk Polymyxin) agar has been the standard media for plating *B. cereus*, but it has little selectivity so background flora is not inhibited and can mask the presence of *B. cereus.*
5. *B. cereus* colonies are usually lecithinase-positive and mannitol-negative on MYP agar.

1. Bacara is a chromogenic selective and differential agar that promotes the growth and identification of *B. cereus* but inhibits the growth of background flora.
2. *Bacillus cereus* colonies turn pink-orange with an opaque halo.
3. The chromogenic agar has been suggested for the enumeration of the *B. cereus* group as a substitute for MYP.
4. Typical colonies will grow as pink-orange uniform colonies surrounded by a zone of precipitation.

BIOCHEMICAL PROPERTIES



1. Catalase: positive
2. Oxidase: negative
3. OF test: fermentative
4. Indole: negative
5. Methyl red: positive
6. Vogues proskauer: positive
7. Glucose: fermentative, production of acid
8. Sucrose: fermentative, production of acid
9. Lactose: no fermentation
10. Starch hydrolysis: positive
11. Nitrate reduction: positive
12. Gelatin hydrolysis: positive
13. Spore staining: endospore-forming bacteria
14. Motility: motile

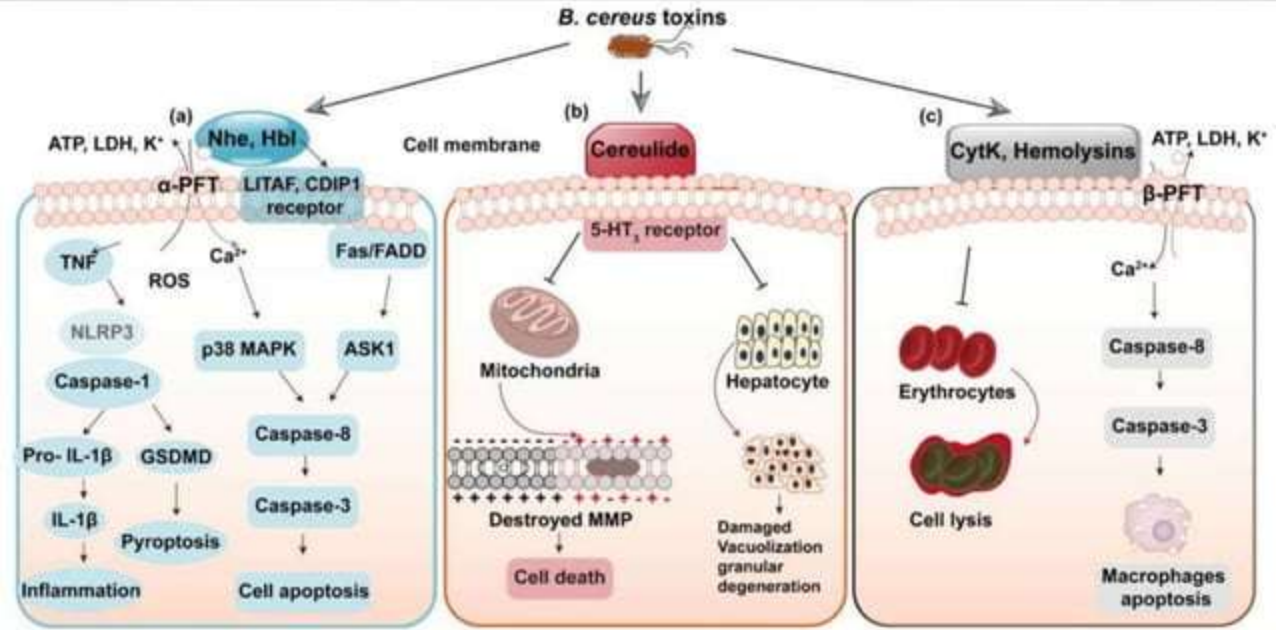
Basic Characteristics	Properties (Bacillus cereus)
Catalase	Positive (+ve)
Citrate	Positive (+ve)
Gelatin Hydrolysis	Negative (-ve)
Gram Staining	Positive (+ve)
Growth in KCN	Positive (+ve)
Hemolysis	Positive (+ve)
Indole	Negative (-ve)
Motility	Positive (+ve)
MR (Methyl Red)	Negative (-ve)
Nitrate Reduction	Variable
Oxidase	Negative (-ve)
Pigment	Negative (-ve)
Shape	Rods
Spore	Positive (+ve)
VP (Voges Proskauer)	Positive (+ve)

Fermentation of	
Adonitol	Negative (-ve)
Arabinose	Negative (-ve)
Arabitol	Negative (-ve)
Cellobiose	Variable
Fructose	Positive (+ve)
Galactose	Negative (-ve)
Glucose	Positive (+ve)
Glycerol	Positive (+ve)
Glycogen	Positive (+ve)
Inositol	Negative (-ve)
Inulin	Negative (-ve)
Lactose	Negative (-ve)
Maltose	Positive (+ve)
Mannitol	Negative (-ve)
Mannose	Negative (-ve)
Melibiose	Negative (-ve)
Raffinose	Negative (-ve)
Rhamnose	Negative (-ve)
Ribose	Positive (+ve)
Salicin	Variable
Sorbitol	Negative (-ve)
Starch	Positive (+ve)
Sucrose	Variable
Trehalose	Positive (+ve)
Xylose	Negative (-ve)

Enzymatic Reactions	
Acetate Utilization	Variable
Arginine Dehydrolase	Variable
Casein Hydrolysis	Positive (+ve)
Esculin Hydrolysis	Positive (+ve)
Lecithinase	Positive (+ve)
Lysine	Negative (-ve)
Ornithine Decarboxylase	Negative (-ve)
Phenylalanine Deaminase	Negative (-ve)
Tyrosine Hydrolysis	Positive (+ve)

TOXINS

- Food poisoning caused by B. cereus is an acute intoxication that occurs when this microorganism produces toxins, causing two types of gastrointestinal illness: an emetic (vomiting) syndrome or a diarrhoeal syndrome.
- B. cereus produces one emetic toxin (ETE) and
- Three different enterotoxins. Three pore-forming enterotoxins, responsible for the diarrhoeal type of food poisoning are
- Hemolysin BL (Hbl),
- Non-haemolytic enterotoxin (Nhe), and
- Cytotoxin K (CytK).


B. cereus toxins
(a)
ATP, LDH, K+
Nhe, Hbl
α-PFT
LITAF, CDIP1 receptor
TNF
ROS
Ca2+
Fas/FADD
NLRP3
p38 MAPK
ASK1
Caspase-1
Caspase-8
Pro- IL-1β
GSDMD
Caspase-3
IL-1β
Pyroptosis
Inflammation
Cell apoptosis
(b)
Cell membrane
Cereulide
5-HT3 receptor
Mitochondria
Hepatocyte
Destroyed MMP
Damaged Vacuolization granular degeneration
Cell death
(c)
CytK, Hemolysins
ATP, LDH, K+
β-PFT
Ca2+
Erythrocytes
Caspase-8
Caspase-3
Cell lysis
Macrophages apoptosis

VIRULENCE FACTORS

- *Bacillus cereus* is mainly known for causing food poisoning and severe non-gastrointestinal tract infections.
- The intestinal and non intestinal pathogenicity of this microorganism is mainly due to the synergistic effects of a number of virulence products that promote intestinal cell destruction and/or resistance to the host immune system.
- The various substances produced by *B. cereus* are mainly enterotoxins, hemolysins, phospholipases, and emetic toxin, whose activity may overlap in causing human disease.

- Virulence factors of B. cereus include
- Cytotoxin K,
- Cereulide,
- Metalloproteases,
- Sphingomyelinase, and
- Phospholipases

EPIDEMIOLOGY

- The natural environmental reservoir for *B. cereus* consists of decaying organic matter, fresh and marine waters, vegetables and fomites, and the intestinal tract of invertebrates, from which soil and food products may become contaminated, leading to the transient colonization of the human intestine.
- Spores germinate when they come into contact with organic matter or within an insect or animal host.
- A multicellular filamentous growth pattern containing refractile inclusions, termed arthromitus (rooted), has been observed in the guts of certain arthropods, which is regarded as the normal intestinal stage in soil-dwelling insects.

- In this setting, as long rod-shaped bacteria, the bacilli lose their flagella, attach to the arthropod intestinal epithelium, and sporulate .
- *B. cereus* also has a saprophytic life cycle in which spores germinate in soil, with the production of a vegetative bacillus, which could then sporulate, maintaining the life cycle .
- Defecation by or death of the host releases cells and spores into the soil, where vegetative cells may sporulate and survive until their uptake by another host .
- Furthermore, when *B. cereus* grows in soil, it undergoes a switching from a single-cell to a multicellular phenotype, which allows it to translocate through the soil .

CLINICAL SYMPTOMS

- *B. cereus* causes self-limiting (24-48 hours) food-poisoning syndromes (a diarrheal type and an emetic type), opportunistic infections and is associated with clinical infections such as endophthalmitis and other ocular infections.
- The diarrheal form of *B. cereus* food poisoning is characterized by abdominal cramps, profuse watery diarrhea, and rectal tenesmus, and, occasionally, fever and vomiting.
- The emetic form of B. *cereus* food poisoning is characterized by nausea, vomiting, and malaise, occasionally with diarrhea.
- *B. cereus* can cause wound infections, bacteremia, septicaemia, meningitis, pneumonia, central nervous system infections, endocarditis, pericarditis, respiratory infections, and peripheral infections.
- Infection in immunocompromised individuals can be life-threatening.

LABORATORY DIAGNOSTICS

- **SPECIMENS:** Faeces, vomitus, remaining food (if any), eye specimen (corneal swab)
- **DIRECT DETECTION METHODS**

1. Microscopically the organisms appear as large gram-positive rods in singles, pairs, or serpentine with square ends after Gram staining.
2. Endospores formation are seen as an unstained oval or round region within the center of the cell. Spores are oval (ellipsoidal) and not swelling of the mother cell.

- **SERODIAGNOSIS**

1. Serologic methods are available for the detection of *B. cereus* toxin in food and feces.
2. Microslide gel diffusion test is generally used as a toxin detection system.

- **MOLECULAR METHOD**

1. The toxigenic potential of *B. cereus* isolates, genes encoding emetic-toxin cereulide (ces), and enterotoxins (nhe, hbl and cytK) can be analyzed by multiplex PCR.

TREATMENT

1. Persons with *B. cereus* food poisoning require only supportive treatment.
2. Oral rehydration or, occasionally, intravenous fluid and electrolyte replacement for patients with severe dehydration is indicated. Antibiotics are not indicated.
3. Patients with the invasive disease require antibiotic therapy. *Bacillus cereus* is susceptible to clindamycin, erythromycin, vancomycin, aminoglycosides, and tetracycline. It is resistant to penicillin and trimethoprim.

PREVENTION AND CONTROL

1. Diarrheal and vomiting intoxications by this organism are readily preventable by appropriate food-handling procedures.
2. Meat and vegetables should not be held at temperatures between 10 and 45 °C for long periods, and rice held overnight after cooking should be refrigerated and not held at room temperature.
3. Prevention of infection in patients following surgery or in those who are immunocompromised or who are otherwise predisposed to infection depends on good practice.

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THANK YOU !!!