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Bacillus cereus

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History

- In 1887, *Bacillus cereus* isolated from air in a cowshed by Frankland and Frankland.
- In 1906, *B. cereus* was first associated with food poisoning in Europe. Outbreaks of food poisoning caused by aerobic, sporeforming bacilli termed "anthracoid" or "pseudoanthrax" were reported.
- In 1950, Steinar Hauge in Norway provided the first complete account of *B. cereus* poisoning, and proved that this microorganism is a human pathogen. From 1947–1949, Hauge investigated four outbreaks of food poisoning with a total of 600 persons affected. The food vehicle in all four outbreaks was vanilla sauce prepared from corn starch, rich in *B. cereus* spores. Hauge found that the corn starch used in this case had ~10^4 spores of *B. cereus* per gram. The dessert was prepared and stored at room temperature until it was served and eaten the next day. All individuals who ate the dessert had clinical symptoms of food poisoning. To provide evidence that *B. cereus* was the cause of food poisoning, Hauge demonstrated Koch's postulates by consuming a culture of the isolated *B. cereus* strain. He grew *B. cereus* to a level of 4×10^6 cells per ml, and drank 200 ml of bacterial suspension. After 13 hrs, the symptoms of food poisoning started.
- Since 1950, many outbreaks from a variety of foods including meat and vegetable soups, cooked meat and poultry, fish, milk and ice cream were described in Europe.
- In 1954, experiments with volunteers in USA failed to confirm Hauge's observations.
- In 1969, the first well-characterized *B. cereus* outbreak in the USA was documented.
- Since 1971, a number of *B. cereus* poisonings of a different type, called the vomiting type, were reported. This type of poisoning was characterized by an acute attack of nausea and vomiting 1–5 hrs after consumption of the incriminated meal. Sometimes, the incubation time was as short as 15–30 min or as long as 6–12 hrs. Almost all the vomiting type outbreaks were associated with consumption of cooked rice. This type of poisoning resembled staphylococcal food poisoning.

***B. cereus* in the US**

B. cereus food poisoning is underestimated probably because of the short duration of the illness (<24hrs).

Table 1: Best estimates of the annual cases and deaths caused by *B. cereus* in the US.

Agent	Cases	Percent	Deaths	Percent
B. cereus	27,360	0.2	0	0
Total bacterial	4,175,565	30.2	1,297	71.7
Total foodborne	13,814,924	100	1,809	100

Recent estimates indicate there are ~84,000 cases of *B. cereus* illness annually in the US with an estimate cost of $430/case — a total of $36 million.

Characteristics of *B. cereus*

- A gram-positive microorganism, but cells may become gram-variable in late log or stationary phase.
- Aerobic, facultative anaerobe, catalase positive, endosporeformer
- Cells are rod-shaped and straight, and can be found in pairs or chains with rounded or squared ends.
- It is normally motile by peritrichous flagella, but non-motile strains have been found.
- It grows well on nutrient agar, and has a requirement for some amino acids but not for vitamins.
- It has the ability to synthesize a variety of extracellular enzymes, toxins and antibiotics. Examples include proteases, amylases, phospholipases, and hemolysins.
- It is an opportunistic pathogen that can cause a variety of more or less severe infections in man and animals. Sporulation is not repressed by exposure to aerobic conditions (i.e., air).

Spores of *B. cereus*

- *B. cereus* endospores (spores formed within the cell) are oval, and sometimes round or cylindrical.
- Usually there is no more than one spore per cell.

- *B. cereus* spores are more hydrophobic than any other *Bacillus* spp. spores, and possess appendages and/or pili. These properties might be an important factor in foodborne illness because they enable the spores to adhere to several types of surfaces such as food processing equipment, and resist the cleaning or sanitation procedures.
- *B. cereus* spores also can adhere to epithelial cells. Later, they can germinate and produce enterotoxins.

Classification of *B. cereus*

- The genus *Bacillus* is very diverse, it is presently divided into six subgroups based on spore morphology.
- *B. cereus* falls in the *Bacillus subtilis* group, and it is closely related to *B. anthracis*, *B. mycoides* and *B. thuringiensis*. Some argue, because of the close relatedness, that the three later species should be classified as sub-species of *B. cereus*.
- *B. cereus* and *B. anthracis* are both recognized as pathogens, but the former is implicated with foodborne disease. *B. anthracis* can infect perorally, but is inefficient.

Table 2: Criteria to differentiate among four closely related *Bacillus* spp..

Species	Colony	Motile	Hemolysis	Susceptibility to Penicillin	Parasporal Body	Virulent to Mice
B. cereus	White	Yes	Yes	No	No	No
B. anthracis	White	No	No	Yes	No	Yes
B. mycoides	Rhizoid	No	No	No	No	No
B. thuringiensis	White/ Grey	Yes	Yes	No	Yes	No

Factors Affecting Growth of *B. cereus*

- Growth temperature range from 7–49˚C (44.6–120.2˚F) with a minimum of 4–5˚C (39.2–41˚F) and a maximum around 48–50˚C (118.4–122˚F).
- Generally, spore germination temperature range from 8-30˚C (46.4–86˚F)
- pH range for growth pH 4.9-9.3

- Minimum water activity 0.91–0.93
- Salt concentration as high as 7.5% NaCl, some tolerate 10%
- Eh no effect
- Thermal D value for spores at 100˚C around 3 min, but some spores much more resistant
- Spores are more resistant to irradiation than vegetative cells. The dose for 90% reduction of spores is 1.25–4kGy, and 0.17–0.65 kGy of vegetative cells.

Name of Illness Caused by *B. cereus*

B. cereus food poisoning is the general name used for the illness. However, *B. cereus* has two recognized types of foodborne illness: diarrheal and emetic. These two types are caused by two different metabolites or toxins. Other, non-foodborne illnesses can be caused by this pathogen, including respiratory tract and wound infections.

The diarrheal illness (more common in North America and Europe) is caused by a high molecular weight protein while the emetic or vomiting type (more common in Japan than the diarrheal type) of food poisoning is caused by a low molecular weight and heat-stable protein. In some outbreaks there seems to be an overlap between the diarrheal and the emetic types of illness.

Mechanisms of Pathogenicity

- To date, scientists have differences of opinion regarding the number *B. cereus* enterotoxins and the properties of these toxins.
- Literature information indicates that *B. cereus* produces several compounds including phospholipase and hemolysin, both found toxic to mice.
- *B. cereus* food poisoning does not confer immunity, although a population may be protected through immunity acquired by continuous exposure.
- Enterotoxin mode of action is thought to be by possible reverse of absorption of fluids, Na^+ and Cl^-, by epithelial cells leading to malabsorption of glucose and amino acids, necrosis, and mucosal damage.

Table 3: Properties of *Bacillus cereus* toxins.

Toxin	*Characteristics*	*Molecular mass*
Enterotoxins	Heat-labile protein(s) inactivated by trypsin, pepsin, and pronase, stable at pH 4-11, and stable at 45˚C (113˚F), 30 min	~41 kDa
Hemolysin BL (HBL)*	Three-component protein	~38-43.2 kDa
Non-hemolysin enterotoxin (NHE)	Three-component protein	~36.5-41.5 kDa
B. cereus enterotoxin (bceT)	Single protein	41 kDa
B. cereus enterotoxin (entFM)	Single protein	45 kDa
B. cereus cytotoxin (CytK)	Single protein	34 kDa
Cereulide (emetic toxin)	Cyclic dodecadepsipeptide, not inactivated by proteolytic enzymes (pepsin & trypsin), stable at pH 2-11, stable at 126˚C (258.8), 90 min	~1.2 kDa

* The best characterized *B. cereus* enterotoxin to date. It seems to be the most likely cause of *B. cereus* foodborne illness although some *B. cereus* strains don't produce this protein.

Nature of Illness

Generally, symptoms are transient and mild — possibly a contributor to under-reporting.

Large numbers of cells are needed to cause illness. Food could be spoiled by the time the microbial load reached the level required to cause illness.

The symptoms of *B. cereus* **diarrheal type** food poisoning include abdominal pain, watery diarrhea, rectal tenesmus, moderate nausea that may accompany diarrhea, seldom vomiting and no fever. Symptoms develop within 6-15 hrs and can persist for 24 hrs. This syndrome is rather mild, and tends to mimic the symptoms of *Clostridium perfringens* food poisoning.

The signs of *B. cereus* **emetic type** food poisoning include nausea and vomiting. Also, abdominal cramps and/or diarrhea may occur. The incubation period is about 1-5 hrs. The symptoms of this

illness mimic those of *Staphylococcus aureus* food poisoning.

Other observed complications of *B. cereus* infection include gangrene, septic meningitis, cellulitis, lung abscesses, infant death, endocarditis, and severe systemic and pyogenic infections.

A comparison of diarrheal and emetic types of *B. cereus* food poisoning is included in Table 4. Also, a comparison of food poisoning caused by *B. cereus*, *C. perfringens*, and *S. aureus* is provided in Table 5.

Table 4: Comparison of diarrheal and emetic types of *B. cereus* food poisoning[†].

Syndrome	Incubation	Duration	Dose	Foods
Diarrheal	8-16 hrs	12-24 hrs	10^3-10^7 CFU, ingested	milk, soup, meat products, puddings
Emetic	1-5 hrs	12-24 hrs	10^5-0^8 per g of food	rice, pasta, noodles, pastries

[†] Granum, P.E. (1994). *Bacillus cereus* and its toxins. *J. Appl. Bacteriol. Symp. Suppl*. 76: 61S-66S.

Table 5: Comparison of food poisoning caused by different bacterial agents[†].

Pathogen	Incubation (hrs)	Duration of Illness, hrs	Dominating Signs	Type of Disease	Frequently Implicated Food
***B. cereus*[*], diarrheal**	8–16	12–24	diarrhea	toxico-infection	Meat products, soups, vegetables, puddings and sauces
C. perfringens	8–16	12–24	diarrhea	toxico-infection	Meats, meat products, and gravy
***B. cereus*[**], emetic**	1–5	12–24	diarrhea (fairly common) vomiting	intoxication	Fried rice from Chinese restaurants and "take out" shops
S. aureus	1–5	12–24	diarrhea vomiting	intoxication	Cooked meats and poultry and dairy products

[†] Source: Gilbert, R.J. and Kramer, J.M. (1987). In "Progress in Food Safety" (Cliver, D.O. and Cochrane, B.A. eds.), pp. 85-93. Food Research Institute, University of Wisconsin, Madison.

• Outbreaks reported since 1950 in several countries including Norway, Denmark, Italy, the Netherlands, Hungary, Sweden, Poland, Rumania, the USSR, the United States, Germany, and Canada.

** Outbreaks reported since 1971 in Great Britain, Canada, Australia, the Netherlands, Finland, the United States and Japan.

Reservoirs

B. cereus is ubiquitous in nature. It is believed that the primary habitat of *B. cereus* is soil. It is found rather frequently in foods such as meats, poultry, milk, cereals, starches, herbs, and spices.

Products Associated with *B. cereus* Food Poisoning

Foods implicated with the diarrheal type of *B. cereus* poisoning include milk, vegetables, meat, and fish. Foods associated with the emetic type of poisoning include rice products, potato, pasta, and cheese products. Other foods such as sauces, pastries, soups, puddings, and salads were identified as vehicles in food poisoning outbreaks.

Table 6: Food samples positive for *B. cereus.*

Food Sample	Percent Positive
Pork	4–7
Beef	11–63
Chicken	0–7
Meat additives	39
Raw milk	9
Pasteurized milk	35
Dairy products	0–63
Raw rice	100
Pasta and flour	0
Seafood	1

Infective Dose

The infective dose of *B. cereus* ranges from 10^4 to 10^{11} cells per gram of food. This is dependent on a number of factors including the presence of viable cells or spores in the food, the amounts of enterotoxin(s) produced, and the susceptibility of target population.

Target Populations

It is believed that all individuals are susceptible to *B. cereus* food poisoning, but more severe symptoms have been associated with young adults and the elderly.

Detection of *B. cereus*

- Refer to the Compendium of Methods for the Microbiological Examination of Foods
- Blood agar can be used as a plating medium.
- Nutrient broth followed by blood agar useful for most probable number count.

Prevention

Since *B. cereus* is ubiquitous in the environment, preventing contamination of food with its spores is almost impossible. Thus, measures to inhibit spore germination and prevent the growth of vegetative cells in cooked, ready-to-eat foods might be the approach to effectively prevent and

control the spread of this pathogen. Thorough cooking is most likely to destroy the vegetative cells and spores. However, temperatures under 100˚C (212˚F) might allow spore survival. Non-refrigerated storage of foods and especially rice should be avoided. Also, foods that require heating or cooling should undergo that process rapidly.

References:

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