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## Spores

Some rod-shaped pathogenic vegetative bacterial cells, such as *Clostridium perfringens* and *Clostridium botulinum*, and *Bacillus cereus*, have the ability to form spores. Spores are formed when environmental conditions around the vegetative cells become poor. When a spore is formed, the reproductive system of vegetative cells is surrounded by a tough shell, and the outer part of the cell falls off. Spores are much more resistant to heat, chemicals, etc. than are vegetative cells. The spore state is a period of no growth, similar to hibernation, in which the cell dries up. Spores are found in soil, water, and the environment. Any food that comes in contact with the soil becomes contaminated with spores. This includes fish (which ingest mud), vegetables, potatoes, rice, flour, milk, eggs, meat, and poultry.

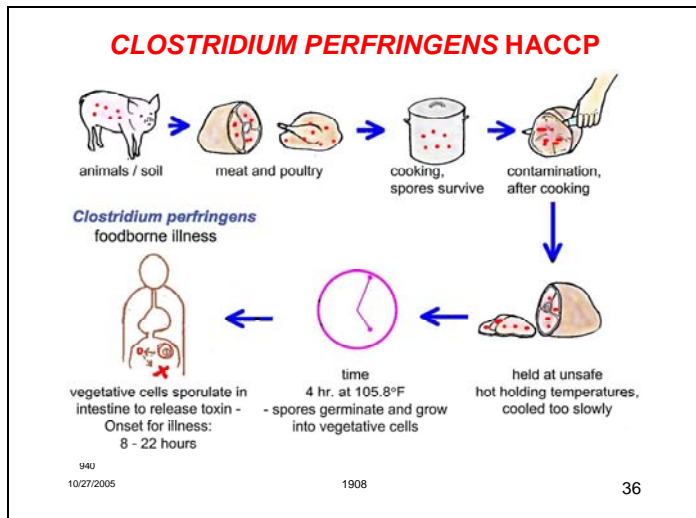
## The Spore Cycle

The spore cycle begins when foods such as vegetables and cereals are harvested, and meat and poultry are slaughtered. The cycle continues when the food is received and heated at the foodservice. Raw food at 41°F has vegetative cells and some *Clostridia* and *Bacillus* spores. During initial heating, the vegetative cells of *Clostridia* and *Bacillus* can multiply between 38 to 125°F. Rapid multiplication (less than 1 hour) can take place between 85 to 120°F. Therefore, it is necessary to cook food from 41°F to the safe temperature of 130°F in less than 6 hours. When the food is cooked uniformly to temperatures of 145 to 165°F, sufficient heat has been applied to inactivate the vegetative cells to a safe level. The food becomes pasteurized. The higher the temperature, the faster vegetative cell destruction occurs.

The spores, however, survive. (It takes hours of cooking at 212°F and 3 minutes of the canning temperature of 250°F to assure inactivation of *C. botulinum* spores.) When the product is cooled to below 125°F, the spores can germinate, given more than 4 hours, into vegetative cells that multiply in the food product(s). Holding food at temperatures of 85 to 120°F for as little as 2 hours will allow enough bacterial multiplication of *C. perfringens* to cause foodborne illness. To control spore outgrowth, food must be kept at temperatures greater than 124°F (greater than 150°F for quality and some government regulations). Roast beef should be held 130°F or above, or

cooled from 130 to 41°F in less than 6 hours according to FDA Food Code recommendations. USDA Guidelines recommend continuously cooling food, within 90 minutes after cooking, from 120 to 55°F within 6 hours, followed by further cooling to 40°F (no time limit) before boxing. **Note**, 130°F is a safe holding temperature that is only a few degrees above the multiplication temperature of *C. perfringens*. It is important to note that a small mistake in maintaining holding temperature can cause roast beef to become hazardous in 4 hours if held at 110°F. FDA regulations state that food should be held at 135°F. Some states require 140°F or 150°F. These temperature requirements allow a margin of safety.

During multiplication, *C. botulinum* and one form of *B. cereus* produce toxins that probably will not be inactivated when the food is reheated. People who eat food containing these toxins will become ill and may die. In other foodborne illness cases, it is the large number of vegetative cells of *C. perfringens* and another form of *B. cereus* produced in the food before it is consumed that makes people ill. To complete the cycle, bacteria and spores are passed from the body and returned to the environment through sewage waste to cycle again.



Foods that are allowed to remain between 80 and 120°F, held in warming tables or cabinets at less than 130°F, or cooled in large containers in refrigerators allow the spores to germinate and the vegetative cells to multiply to large numbers. But, in this one case, reheating will make the food safe. Reheating food to above 140°F according to the *Salmonella* time-temperature kill values will destroy the vegetative cells of *C. perfringens* that contaminate the food and make the food safe to eat.

This organism can also multiply during slow heating. Food must be heated from 41 to above 130°F in less than 6 hours to assure the control of this bacteria.

### Illness Characteristics

To cause *C. perfringens* foodborne illness, the vegetative cells must multiply to greater than 100,000 per gram in food. After a person eats the food containing the cells of *C. perfringens*, the cells enter the intestine where they release toxin as they sporulate. This release of toxin in the gut causes the person to become ill. The illness (characterized by abdominal cramps and intense, putrid smelling diarrhea) develops in about eight to twenty hours. People recover quickly and are usually able return to work the next day.

Because *C. perfringens* produces no spoilage characteristics when it multiplies in food, its victims often note that the offending food was the "best chili, turkey hamburger, or roast beef they had ever tasted."

### *Clostridium perfringens* Food Intoxication

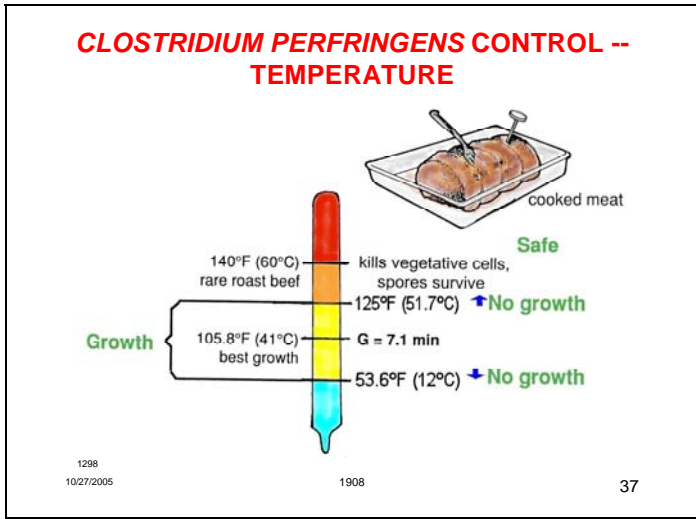
*Clostridium perfringens* is found in soil and dust and in the feces of man and animals. It has two forms: a **spore** form, which survives the cooking process but does not cause illness directly; and a **vegetative cell** form, which the spore becomes when environmental conditions permit growth. The presence of 100,000 vegetative cells per gram in food will cause illness. (The same number of spores will have no effect since the presence of spores of *C. perfringens* in food does not cause the illness.) *Clostridium perfringens* grows only in the absence of air. It can grow in sealed packages of food, in the internal mass of food (e.g., in large pieces of meat or poultry and large containers of casseroles), or in cooked foods as shallow as 1 inch deep.

*Clostridium perfringens* requires protein for growth. This microorganism is usually present in foods of animal origin such as meat and poultry, and in other protein-containing foods such as gravies, sauces, and soups. It grows well in mashed potatoes, beans, and lima beans. It can survive the curing of meat because the organism is moderately salt tolerant and prefers an anaerobic environment. It can grow in the liquid portion of unsalted butter.

Active vegetative forms of *C. perfringens* can change to dormant, hardy, heat-resistant spores when the environment becomes hostile. The spores can survive indefinitely and are also resistant to dehydration and cold temperatures. Heat at 180°F does not destroy the spores but instead stimulates the spores to germinate and develop into vegetative cells when adequate environmental conditions are met. *Clostridium perfringens* cells multiply very rapidly in cooked food held at room temperature. At 105.8°F, they double every 7 to 8 minutes in ground beef. It takes only a couple of hours for take-out food, held at 90 to 115°F to become hazardous.

### *Clostridium perfringens* Transmission

*Clostridium perfringens* is transferred to meat and poultry from animal feces during processing, from workers who have not washed their hands after using the toilet, from soil and dust on equipment, and from very small lesions in the animals' intestinal walls. The insides of meat can also become contaminated when it is sliced, chopped, stuck with a dirty fork, or put onto a spit.



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### **Clostridium perfringens Control**

*Clostridium perfringens* foodborne illness outbreaks result when this microorganism is allowed to multiply to large numbers (more than 100,000) during slow cooking, improper hot holding and inadequate cooling (i.e., the failure to keep hazardous foods contaminated with spores and viable cells of *C. perfringens* out of critical growth range temperatures of 53.6 to 125°F for extended periods of time).

#### **Control Factors**

Personnel in food production and foodservice establishments must:

- a. Assume that most raw and cooked meat and poultry products are contaminated with 10 to 100 cells of *C. perfringens* per gram. If these products are kept below 53.6°F, *C. perfringens* will not multiply. The spores will survive cooking and will grow out into vegetative cells when foods are cooled too slowly, or are not held above 130°F.
- b. Use cooking methods that ensure that food products, particularly large roasts, poultry items, and high protein casseroles, pass a center temperature between 41 to 130°F in 6 hours or less. Food should be pasteurized according to *Salmonella* reduction standards. (Remember, this only inactivates the *C. perfringens* vegetative cells, not the spores.)
- c. Use rapid cooling methods for high-protein items. The FDA Food Code recommends cooling food from 135 to 70°F within 2 hours followed by further cooling to 41°F (6 hours or less, total time). USDA Guidelines for cooling recommend continuously cooling food, within 90 minutes after cooking, from 120°F to 55°F within 6 hours, followed by further cooling to 40°F (no time limit) before boxing.

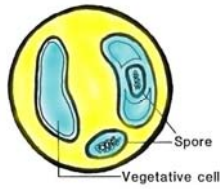
For example, when cooling products:

- 1) Large roasts should be sliced and placed in layers not thicker than 2 inches in depth if cooled in an ordinary refrigerator.
- 2) Stews, soups, casserole items, gravies, and sauces should be placed in pans that are not thicker than 2 inches in depth for cooling in an ordinary refrigerator.
- 3) High-velocity fans must be used within 4 inches of the pans of food cooling in the refrigerator to ensure rapid circulation of air at less than 35°F over the food if cooling in 6 hours is required.

- d. Government regulations for holding food above 135°F provide an extra measure of safety because of the unreliability of thermostats on hot holding equipment.) Customers prefer most hot food to be above 150°F and soup to be at 165°F when they put it in their mouths.
- e. Use good personal hygiene and proper methods of hand washing when handling food products. People carry the organism in their intestines. People can be carriers or shedders.
- f. Use sanitized equipment such as cook's forks, thermometers, and metal spits to penetrate or serve meat, poultry, stews, soups, and casseroles. Unsanitized equipment can inject *C. perfringens* into the center of food products where they will grow well in the anaerobic conditions.

## CHARACTERISTICS OF *BACILLUS CEREUS*

- Grows with and without air.
- Grows between 39.2°F and 122°F.
- Source is soil. Found in rice, spices, cereals, milk.
- Forms spores that are resistant to cooking / baking temperatures. Spores grow out as vegetative cells when food cools.
- Two types of illness:  
Emetic illness (vomiting) – ½ to 6 hours after ingestion.  
Diarrheal illness – 10 to 12 hours after ingestion.
- Heat-resistant toxin(s) – can withstand temperature (250°F) 90 minutes.



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### *Bacillus cereus* - Characteristics

#### Bacterial Characteristics

Cells of *Bacillus cereus* are large, gram-positive rods that are motile by means of flagella. Cells are aerobic spore formers that are also capable of growing under anaerobic conditions. Spores are formed when conditions for growth of vegetative cells are not present.

*Bacillus cereus* is responsible for two types of foodborne illness: the emetic (vomiting) illness and diarrheal illness, which are caused by two distinct enterotoxins produced by different strains of this microorganism

Spores of this microorganism are present in many foods from harvest through processing. The organism, normally present in most food, is not a hazard at numbers below 1,000 CFU (colony forming units) per gram. Hazardous levels of this pathogen can develop when food (especially cooked foods in which most competitive microorganisms have been destroyed) is held in the range of 85 to 120°F for a long period of time (e.g., the number of hours typical of cooling a 6 inch, covered pan of cooked rice in an ordinary refrigerator). Under these conditions, the organism can grow to large numbers, releasing toxin during growth in the food and/or in the intestinal tract after the food is consumed.

Recently, psychrotrophic strains of this pathogen were shown to grow at a temperature range of 39.2 to 98.6°F in pasteurized milk, mousses, and cook/chill meals (van Netten et al., 1990).

#### Source

*Bacillus cereus* is common in the soil and dust throughout the world. It is frequently isolated in grains, flour, starch, and other cereal products. Prepared foods implicated in the outbreaks of foodborne illness due to *B. cereus* include: mashed potatoes, pasta, macaroni and cheese, feta cheese, stuffing, rice and rice dishes (fried rice), malted milk powder, meat and items made with meat and poultry, soups, instant breakfast products, vanilla puddings and cream sauce, and other products that incorporate cereal products.

This microorganism has been found in a variety of food stuffs. For example, *B. cereus* is a contaminant of many spices. In the

United States, 25% of 175 samples of dry food, distributed nationally, yielded the organism (Kim and Geopfert, 1971).

#### Growth Conditions

**Temperature.** Recent research, as reported by van Netten et al. (1990), has shown that psychrotrophic strains of *B. cereus* are capable of growth at 39.2°F. The maximum temperature for vegetative cell growth and spore outgrowth is 122°F (Kramer and Gilbert, 1989). Optimum growth occurs at temperatures of 82 to 95°F (Adams and Moss, 1995). Wong et al. (1988) reported a generation time of 27 minutes in pasteurized milk at 86°F. Johnson et al. (1983), reported a generation time of 26 to 57 minutes at 86°F in cooked rice. Beuchat et al. (1980), reported a generation time of 18-27 minutes in laboratory media. The variability in growth and generation time is dependent on strain, temperature, and nutrient supply of the media. The organism will grow significantly better in dishes containing beef, chicken, or egg in combination with rice, (products containing various nutrients) than in plain rice.

Plain rice often contains 100 vegetative cells per gram as well as spores of *B. cereus*. This population is capable of multiplying to 10<sup>5</sup> microorganisms in as little as 300 minutes (5 hours) at 86°F.

**Nutrients.** *Bacillus cereus* produces enzymes than can hydrolyze (split) starch and protein. It thus uses these products of hydrolysis (i.e., sucrose, maltose, lactose, mannose, acids) for growth and reproduction.

**pH.** The pH range for the organism's growth is 4.3 to 9.3. In meat, *B. cereus* grows at a pH as low as 4.35.

**Atmosphere.** *Bacillus cereus* is aerobic but can be facultatively anaerobic. Emetic strains can produce sufficient numbers of microorganisms and enterotoxin (in 12 hours at room temperature) in an aerobic environment to cause illness, long before spoilage is evident.

**Water activity.** The minimum a<sub>w</sub> reported for the growth of *B. cereus* is 0.91 to 0.96 in fried rice (Bryan et al., 1981)

#### Spores

Like all spores, the spores of *B. cereus* are resistant to heat and will survive both cooking and baking temperatures. The time required to inactivate spores is dependent on temperature, strain, media, and food. The thermal destruction time for spores suspended in skim milk at 212°F is 2.7 to 3.1 minutes (Kramer et al. 1989). Time required for destruction of spores in rice varies from 22 to 36 minutes. Fat has a protective effect on the spores of *B. cereus*. If oil is present, spore survival may exceed 30 minutes.

Individual spores may possess an unusually high heat resistance. Hence, in samples of 10<sup>5</sup> to 10<sup>6</sup> spores, 1 may survive after 4 hours at 275°F (Franklin, 1970). Spores of *B. cereus* have been isolated from commercially canned foods (Bradshaw et al., 1975). Spore germination is inhibited by carbon dioxide and bicarbonate.

Spores survive in slowly heated rice dishes, casseroles, and meat loaves. If slow cooling follows, spores will outgrow and produce vegetative cells. Once the vegetative cells have been produced from spores of either emetic or diarrheal strains of *B. cereus*, the generation time is approximately 26 to 57 minutes

(Wong et al., 1988, Johnson, 1984) depending on the food supply and temperature.

Vegetative cells sporulate (form spores) when growth conditions are less than optimum (during slow heating, cooling, and changes in other environmental conditions such as pH or acidity). In this manner, the microorganisms are able to survive.

### Toxin Production

*Bacillus cereus* forms enterotoxins, which produce two different types of illness. Certain strains produce enterotoxins that cause diarrheal-type illness and other strains produce enterotoxins that cause the emetic (vomiting)-type illness.

Diarrheal illness due to *B. cereus* is often associated with meat products, soups, potatoes, starchy vegetables, pudding, and sauces. The emetic syndrome is most often associated with rice and pasta products that were held at improper holding temperatures or cooled too slowly.

The enterotoxin associated with diarrheal illness is easily destroyed by heat: (D 132.8°F = 5 minutes. The enterotoxin associated with the emetic illness is quite heat resistant and is stable to 250°F for 90 minutes (Johnson, 1984).

### Infective Dose

In order to produce sufficient amount of toxin or sufficient number of cells to cause illness when food is ingested there must be more than  $5 \times 10^5$  cells per gram of food (Doyle, 1988). The FDA (1993) states that "The presence of large numbers of *B. cereus* in food is indicative of active growth and proliferation of the organism and is consistent with a potential hazard to health."

### Symptoms

Symptoms of the diarrheal illness include abdominal pain and profuse watery diarrhea with little vomiting and no fever. Symptoms appear after 10 to 12 hours and usually subside within another 12 hours. The diarrheal illness is often confused with illness caused by *Clostridium perfringens*.

Symptoms of the emetic illness mimic illness symptoms of *Staphylococcus aureus*. They include nausea, vomiting, and possible diarrhea, usually within 1/2 hour to 6 hours after ingestion. There is no fever and recovery occurs within 6 to 24 hours.

### Incidence

As a cause of foodborne illness, *B. cereus* was first reported from Norway in 1950. The first well-documented incident in the U.S. occurred in 1969. Meat loaf contaminated with  $7 \times 10^6$  *B. cereus*/g was the vehicle for transmission of the diarrheal illness involving 15 people.

**Outbreak Example.** The following example appeared in MMWR 35(25):408-410, 1986.

*Bacillus cereus* -- Maine. On September 22, 1985, the Maine Bureau of Health was notified of a gastrointestinal illness among patrons of a Japanese restaurant. Because the customers were exhibiting symptoms of illness while still on the restaurant premises, and because uncertainty existed as to the etiology of the problem, the local health department, in concurrence with the restaurant owner, closed the restaurant at 7:30 p.m. that same day.

Eleven (31%) of the approximately 36 patrons reportedly served on the evening of September 22 were contacted in an effort to determine the etiology of the outbreak. Those 11 comprised the last 3 dining parties served on September 22. Despite extensive publicity, no additional cases were reported.

A case was defined as anyone who had vomiting or diarrhea within 6 hours of dining at the restaurant. All 11 individuals were interviewed for symptoms, time of onset of illness, illness duration, and foods ingested. All 11 reported nausea and vomiting; 9 reported diarrhea; 1 reported headache; and 1 reported abdominal cramps. Onset of illness ranged from 30 minutes to 5 hours (mean 1 hour, 23 minutes) after eating at the restaurant. Duration of illness ranged from 5 hours to several days, except for 2 individuals still symptomatic with diarrhea 2 weeks after dining at the restaurant. Ten persons sought medical treatment at local emergency rooms on September 22. Two ultimately required hospitalization for rehydration.

Analysis of the association of food consumption with illness was not instructive, since all persons consumed the same food items: chicken soup; fried shrimp; fried zucchini, onions, and bean sprouts; cucumber, cabbage, and lettuce salad; ginger salad dressing; hibachi chicken and steak; and tea. Five people ordered hibachi scallops, and 1 person ordered hibachi swordfish. However, most individuals sampled each other's entrees.

One vomitus specimen and 2 stool specimens from 3 separate individuals yielded an overgrowth of *B. cereus* organisms. The hibachi steak was also culture-positive for *B. cereus*, although an accurate bacterial count could not be made because an adequate amount of the steak remained for laboratory analysis. No growth of *B. cereus* was reported from the fried rice, mixed fried vegetables, or hibachi chicken.

According to the owner, all meat was delivered 2 to 3 times a week from a local meat supplier and refrigerated until ordered by restaurant patrons. Appropriate-sized portions for a dining group were taken from the kitchen to the dining area and diced or sliced, then sautéed at the table directly in front of restaurant patrons. The meat was seasoned with soy sauce, salt, and white pepper, open containers of which had been used for at least 2 months by the restaurant. The hibachi steak was served immediately after cooking.

The fried rice with the meal was reportedly customarily made from leftover boiled rice. It could not be established whether the boiled rice had been stored refrigerated or at room temperature.

The emetic syndrome has almost always been associated with fried rice served in Oriental restaurants. The common practice of storing boiled rice at room temperature for subsequent preparation of fried rice has generally been implicated in such outbreaks. However, a recent, well-documented outbreak of the emetic syndrome of *B. cereus* in a British prison implicated beef stew. This was thought to be caused by adding to the stew vegetables that were cooked a day earlier.

Fresh meat cooked rapidly, then eaten immediately, seems an unlikely vehicle for *B. cereus* food poisoning. The laboratory finding of *B. cereus* in a food item without quantitative cultures and without accompanying epidemiological data is insufficient to establish its role in the outbreak. A negative culture of fried

rice eaten with the meal does not exclude the obvious vehicle; reheating during preparation may eliminate the bacteria in the food without decreasing the activity of the heat-stable toxin. While the question of the specific vehicle remains incompletely resolved, the clinical and laboratory findings substantially support *B. cereus* as the cause of the outbreak.

Most episodes of food poisoning undoubtedly go unreported, and in most of those reported, the specific pathogens are never identified. Alert recognition of the clinical syndrome and appropriate laboratory work permitted identification of the role of *B. cereus* in this outbreak.

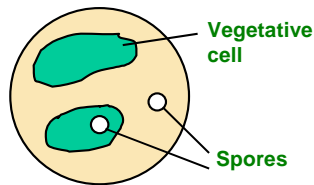
#### References

- Adams, M.R., and Moss, M.O. 1995. Food Microbiology. The Royal Society of Chemistry. University of Surrey, Guildford. UK. pp. 160-164.
- Beuchat, L.R., Ma-Lin, R.C.F.A. and Carpenter, J.A. 1980. Growth of *Bacillus cereus* in media containing plant seed materials and ingredients used in Chinese cookery. J. Appl. Bacteriol. 48:397-407.
- Bradshaw, J.G., Peeler, J.T., and Twedt, R.M. 1975. Heat resistance of ileal loop reactive *Bacillus cereus* strains isolated from commercially canned food. Appl. Microbiol. 30(6):943-945.
- Bryan, F.L., Bartleson, C.A. and Christopherson, N. 1981. Hazard analysis, in reference to *Bacillus cereus*, of boiled and fried rice in Cantonese-style restaurants. J. Food Protect. 44(7):500-512.
- Doyle, M.P. 1988. *Bacillus cereus*. Food Technol. 42(4):199.
- FDA. 1993. HACCP. Regulatory Food Applications in Retail Food Establishments. Dept. of Health and Human Services. Division of Human Resource Development. HFC-60. Rockville, MD.
- Fermanian, C., Fremy, J., and Lahellec, C. 1993. *Bacillus cereus* Pathogenicity: A review. 1993. J. Rapid Methods and Autom. Microbiol. 2:83-134.
- Franklin, J.G. Spores in milk: Problems associated with UHT processing. J. Appl. Bacteriol. 33:180-191.
- Granum, P.E. 1997. In Doyle, M. P., Beuchat, L. R., and Montville, T. J. eds. Food Microbiology. Fundamentals and Frontiers. American Society of Microbiology. Washington, D.C. pp, 327-336.
- Johnson, K.M. 1984. *Bacillus cereus* foodborne illness - an update. J. Food Protect. 47:145-153.
- Johnson, K.M. Nelson., C.L., and Busta, F.F. 1983. Influence of temperature on germination and growth of spores of emetic and diarrheal strains of *Bacillus cereus* in a growth medium and in rice. J. Food Sci. 48:286-287.
- Kim, H.V. and Goepfert, J.M. 1971. Occurrence of *Bacillus cereus* in selected dry food products. J. Milk Food Technol. 34:12-15.
- Kramer, J.M. and Gilbert, R.J. 1989. *Bacillus cereus* and other *Bacillus* species. In Foodborne Bacterial Pathogens. Doyle, M.P., Ed. Marcel Dekker, Inc. New York, NY. pp. 22-70.
- Mossel, D.A.A., Corry, J.E., Struijk, C.B., and Baird, R. 1995. Essentials of the Microbiology of Foods. John Wiley and Sons, New York, NY. pp. 137-138.
- National Research Council. 1985. An Evaluation of the Role of Microbiological Criteria for Foods and Food Ingredients. National Academy Press. Washington, D.C.
- Turnbull, P.C.B. 1976. Studies on the production of enterotoxins by *Bacillus cereus*. J. Clin. Pathol. 29:941-948.
- van Netten, P., van de Moosdijk, A., van Hoensel, Mossel, D.A.A., and Perales, I. 1990. Psychrotrophic strains of *Bacillus cereus* producing enterotoxin. J. of Appl. Bacteriol. 69:73-79.
- Wong, H.C., Chen, Y.L., and Chen, C.L.F. 1988. Growth, germination and toxigenic activity of *Bacillus cereus* in milk products. J. Food Protect. 51(9):707.



## CHARACTERISTICS OF *CLOSTRIDIUM BOTULINUM*

- Grows without air (oxygen) in canned foods and vacuum packages.
- Common in soil.
- Proteolytic types A and B growth: 50 to 118°F.
- Non-proteolytic types B and E growth: 38 to 113°F.
- Spores survive boiling temperatures to outgrow as vegetative cells that grow and produce toxin in little or no air (oxygen).
- Neurotoxins are deadly.
- Toxin(s) destroyed: 185°F for 5 minutes.



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### *Clostridium botulinum* - Characteristics Bacterial Characteristics

*Clostridium botulinum* microorganisms are anaerobic, spore-forming, gram-positive rods that are motile by means of flagella. There are seven types of *C. botulinum*, A, B, C, D, E, F, and G. Human botulism is principally caused by types A, B, and E. Types F and G have caused extremely few, rare cases of human botulism. Types C and D cause botulism in birds and animals.

Botulism is currently classified into four categories: (Pierson and Reddy, 1988; FDA, 1993)

1. Classical foodborne botulism intoxication caused by the ingestion of small amount of preformed botulinum toxin in contaminated food.
2. Wound botulism (a rare occurrence), which results from the growth of *C. botulinum* and production of toxin in infected wounds.
3. Infant botulism, is thought to be caused by the ingestion of *C. botulinum* spores that colonize and produce toxin in the intestinal tract of infants. At this time, honey is the only implicated food source of *C. botulinum* spores. (Parents of newborn infants are now warned not to give honey to babies.) Only infants under 1 year of age are affected.
4. Undetermined cause of botulism that involves individuals older than 12 months of age. It has been suggested that some cases of botulism in adults assigned to this category might be the result of intestinal colonization and resultant toxin production within the gut. In these cases, patients had surgical alteration of the gastrointestinal tract and/or antibiotic therapy that may have altered the normal gut microflora and allowed *C. botulinum* to colonize the intestinal tract.

Most cases of human botulism are due to the growth of types A, B, and E *C. botulinum* in food and subsequent production of neurotoxins. Ingestion of food containing the neurotoxins causes severe illness and possibly death, if antitoxins are not administered promptly.

Type A *C. botulinum* and some strains of type B are proteolytic (capable of splitting proteins into their constituent amino acids). The growth of proteolytic strains of *C. botulinum* produces off flavors and odors in food and food products. Type A and

proteolytic type B may be present on meat and vegetables, particularly those growing in or near the ground.

Type E *C. botulinum* is most often associated with fish and seafood products. Type E strains are found in the water and sludge near bodies of water (i.e., oceans, lakes, and rivers.) Type E *C. botulinum* and non-proteolytic strains of type B *C. botulinum* are capable of growing at refrigeration temperatures. Because both of these strains are non-proteolytic, they can grow and produce toxin in food without changing its flavor and odor.

The vegetative cells of these microorganisms are destroyed when products are heated during most cooking procedures. However, the spores can survive most cooking procedures and will germinate to form vegetative cells if these products are kept in anaerobic conditions at temperatures that support growth. When vegetative cells grow in these anaerobic conditions, a lethal neurotoxin is produced.

### Source

Spores and vegetative cells of *Clostridium botulinum* are present in soil, water, and sludge near bodies of water. The number of foods in which types of *C. botulinum* have found is limitless.

### Growth Conditions

**Temperature.** Type A and proteolytic type B strains of *C. botulinum* grow at temperatures between 50 to 118°F (Hauschild, 1989). Ohye and Scott (1953) found the optimal temperature range for growth to be 98.6 to 104°F. At 98.6°F, the generation time is 0.7 hours (42 minutes). The following table indicates the generation times for these strains at various temperatures.

Gibson et al. (1987) reported a generation time for *C. botulinum* type A in pasteurized pork slurry of 1.2 hours at 68°F.

The temperature range for growth of type E and non-proteolytic type B strains is 38 to 113°F. This means that botulism can occur from ingestion of refrigerated foods, if they are stored for long periods of time (14 to 21 days) in anaerobic conditions.

INFLUENCE OF TEMPERATURE ON THE GROWTH OF *CLOSTRIDIUM BOTULINUM* TYPES A AND B\*

Temperature °F	Approx. lag time (hour)	Approx. generation time (hour)
54	**	89
59	160	28
64	63	8
68	32	4
77	20	2
86	8	1
98.6	5	0.7
108.5	8	1
113	**	2

\* Adapted from data of Ohye and Scott (1953) as reported by Sperber (1982). (The cultures used in this study were grown in laboratory media of neopeptone, yeast extract, glucose, and water.)

\*\* Not reported.

Freezing decreases the number of vegetative cells in foods but has no effect on spores or preformed toxins.

**Nutrients.** These organisms require a supply of amino acids (protein), purines, polyalcohols, and sugars (glucose or maltose)

is known to be needed for toxin production), (Frazer, 1978 and Ketchum, 1984).

**pH.** The pH range for growth of type A and proteolytic types of *Clostridium botulinum* is 4.6 to 9.0. It has been reported that these strains will grow in at pH as low as 4.0. In these instances, either yeasts or molds grew in areas of the food, increasing the pH in these areas to a level that allowed spore germination and vegetative cell growth. Tanaka (1982) reported that toxin was produced in a pork slurry at a pH of 4.30 to 4.36. He postulated that *C. botulinum* was able to grow and produced toxin at a higher pH within precipitated protein matrices.

Types E and nonproteolytic strains of type B. *C. botulinum* grow in a pH range of 5.0 to 9.0.

**Atmosphere.** *Clostridium botulinum* is an anaerobe, but may grow under certain conditions of reduced oxidation-reduction potential. It is capable of growth in the absence of air in canned products, vacuum packaged products, and in the interior portions of food products from which oxygen has been eliminated by cooking. Exclusion of oxygen inhibits aerobic microorganisms such as the lactic acid bacteria, which, when air is available, tend to suppress growth of *C. botulinum*.

**Water Activity.** The water activities, effective in inhibiting the growth of vegetative cells, are about 0.94 for type A and nonproteolytic strains of type B, *C. botulinum*, and 0.97 for type E and nonproteolytic strains of type B, *C. botulinum*. A salt (sodium chloride) concentration of 10% in brine is effective in lowering the water activity to 0.94, a brine concentration of 5% salt is effective in reducing the water activity to 0.97 (Hauschild, 1989).

**Other Factors.** Competitive microorganisms have a protective effect in foods by inhibiting the growth of *C. botulinum* and by causing spoilage in food products before a significant population of *C. botulinum* can grow and produce toxin.

Nitrites added to cured meat, poultry, and fish products inhibit the growth of *C. botulinum*. The effectiveness of nitrites is enhanced by the addition of acidity, salt, and isoascorbate.

Liquid smoke products, which are commonly applied to the surface of many cured meat and fish products, also inhibit the growth of this microorganism. Smoking of products in a smoke house has little prohibitive effect.

Nisin, an antibiotic, is used to inhibit the growth of *C. botulinum* in cheese spreads.

### Heat Resistance of Vegetative Cells and Spores

The vegetative cells of *C. botulinum* are readily inactivated by most cooking methods. However, both spores and/or toxins may survive if heating is not adequate. The ingestion of vegetative cells and spores does not cause illness (except in infants and certain individuals whose intestinal microflora does not inhibit the growth and toxin production of *C. botulinum*).

The primary consideration for safety in food preservation is the destruction of *C. botulinum* spores in the processing of food products. The heat resistance of spores varies among types. Spores of proteolytic types or strains (type A, proteolytic types B and F) require 0.3 to 0.23 minutes for 90% reduction of spores at 250°F [D<sub>250</sub>]. Spores of nonproteolytic types E, B, and F are less

heat resistant and are destroyed at 180°F [D<sub>180</sub>] in 0.8 to 6.6 minutes in various foods (Simunovic et al., 1985).

The following table summarizes some growth conditions for *C. botulinum*.

**MINIMAL REQUIREMENT FOR GROWTH AND HEAT RESISTANCE OF CLOSTRIDIUM BOTULINUM TYPES A, B, E, AND F\***

Properties	Group	
	I (Proteolytic)	II (Non-proteolytic)
Toxin types	A, B, F	B, E, F
Inhibitory pH	4.6	5.0
Inhibitory salt (NaCl) concentration	10%	5%
Minimal water activity	0.94	0.97
Temperature range for growth	50-118°F	38-113°F

\* Adapted from Hauschild, 1989.

Commercial canning procedures are designed to destroy *C. botulinum* spores and make the survival of any spores extremely rare. The canning industry has adopted the 12D concept for heat processing low-acid canned foods (meats, vegetables, and any other products with a pH above 4.4 to 4.6). The 12D process is intended to reduce a bacterial spore population from 1,000 spores in each billion cans of food to 1 spore in 1 billion cans. This heat processing method has been quite effective in providing a safe supply of canned food. Incidents of botulism due to the consumption of commercially canned foods has been due to inadequate heat processing (inaccurate retort temperatures and timing) and/or recontamination after processing through leaks of cooling water through side seams in cans of food.

### Toxin Destruction

The neurotoxins produced by all types of *C. botulinum* are less heat resistant than the enterotoxins produced by *Staphylococcus aureus*. Woodburn et al. (1979) found that any botulinum toxin was inactivated if it was heated to 174°F for 20 minutes [D<sub>174</sub> = 20 minutes] or 185°F for 5 minutes [D<sub>185</sub> = 5 minutes].

Many food products involved in documented botulism incidents were not heated or were heated insufficiently after toxins have been produced to inactivate the toxins.

### Symptoms

Symptoms of this foodborne intoxication develop within 12 to 72 hours after consumption of the toxin-containing food. Symptoms include: nausea, vomiting, fatigue, dizziness, headache, skin dryness, dryness of the mouth and throat, constipation, paralysis of muscles, double vision and difficulty in breathing. Duration and severity of the illness is dependent upon the amount of toxin ingested and the overall health of individuals. Treatment involved administration of anti-toxin and respiratory therapy. Death results in 10% of diagnosed cases.

### Toxic Dose

The botulinum toxins are the most toxic substances known. A very small amount (a few nanograms) in food can cause illness. Botulinum toxin is produced when cells grow in suitable environmental conditions. Type A toxin has been reported to be

more lethal than types B or E toxins (Jay, 1996). A bacterial cell population of  $10^4$  to  $10^5$  cells/gram of food is required to produce sufficient toxin.

### **Incidence**

The word botulism is derived from the word botulus (Latin for sausage). A German scientist, E. P. M. van Ermengen in 1896, isolated the microorganism in inadequately cured ham. The ham had caused illness in 34 people, which resulted in 3 deaths. He named the microorganism *Bacillus botulinus*, because this illness had the same disease symptoms as blood sausage poisoning. The organism was later renamed *Clostridium botulinum*.

Most botulism outbreaks in the United States have been associated with vegetables. However, fish and seafood products, meat products (beef, pork, and poultry), condiments (chili sauce, tomato relish, and salad dressing), and dairy products have also been causes of incidents. Between 1899 and 1976, home-processed foods were responsible for the majority of the incidents (72%), while commercially processed foods were involved in about 8.6% outbreaks. Unknown vehicles were responsible for the other 20% of outbreaks.

In 1960 and 1963, 4 outbreaks of type E botulism occurred in the United States involving 23 cases resulting in 9 deaths. These outbreaks reactivated the interest in botulism. Three outbreaks of type E botulism were traced to fish or fish products; the other 1 was traced to canned mushroom sauce. (Canned food has seldom been involved in type E outbreaks, but canned sprats, mushroom sauce, and tuna fish have been involved.) In the canned tuna fish outbreak in Detroit, Michigan, 1963, the product was apparently contaminated after retorting because of faulty can seams. In 1963, 2 other outbreaks of type E botulism occurred from commercially prepared smoked whitefish chubs originating from the Great Lakes.

After 1970, a variety of commercially canned foods (vichyssoise, peppers, marinated mushrooms, and beef stew) were found to be contaminated with types A or B, *C. botulinum*. Consumption of these products caused 13 clinical cases of botulism, including 2 deaths in the United States and Canada. During 1973 and 1974, a total of 30 cans of mushrooms were found to contain type A *C. botulinum* toxin. Each year from 1980 to 1988 the number of cases of foodborne botulism reported to the CDC in Atlanta, Georgia, ranged between 17 to 50 cases a year. During that same period of time, there were 50 to 100 cases of infant botulism per year.

### **Food Analysis**

Botulism is foodborne and usually results from ingestion of the preformed toxin. Therefore the source of an outbreak is based on detection and identification in the food involved. The most widely accepted method is the injection of extracts of the food into passively immunized mice (mouse neutralization test). The test takes 48 hours. This analysis is followed by culturing all suspect food in an enrichment medium for the detection and isolation of the microorganism. This latter test takes 1 week.

**Outbreak Examples.** The following outbreak example appeared in MMWR 32:39-45, 1983.

Botulism and Commercial Pot Pie -- California. On August 3, 1982, a 56-year-old woman residing in Los Angeles County,

California, developed diplopia, weakness, difficulty breathing, and chest pain. She had respiratory arrest on admission to the hospital but was intubated, resuscitated, and placed in intensive care. Examination showed complete bilateral ptosis, ophthalmoplegia, facial muscle weakness, and areflexia. Cerebrospinal fluid was normal except for increased glucose; tensilon test was negative. She had a history of seizure disorder, diabetes mellitus, and organic brain syndrome. An infectious disease consultant thought her subsequent fever was due to pneumonia secondary to aspiration, and he suspected botulism as the underlying cause of her illness.

The patient lives with her husband and grown son who both prepare meals for her and attempt a strict diet in consideration of her diabetes. When asked about the patient's food history before onset of illness, the husband and son named no likely suspects for botulism. No home-preserved foods had been served, and, with one exception, she had not eaten other foods that were not freshly prepared for her or were not also consumed by her husband and son. The exception was commercial beef pot pie, which was accidentally mishandled, then consumed by the patient one day before illness began.

The son had prepared the pot pie for an earlier evening meal. The frozen pie was baked in an oven for 40-45 minutes. As he was about to serve it to his mother, his father came home with some freshly cooked hamburgers just purchased at a take-out restaurant. The pot pie was put aside on an unrefrigerated shelf. Two and one-half days later, the son came home and found his mother had just consumed this pot pie without reheating it.

An uneaten portion of the pot pie, still in its metal plate, was retrieved by the family members. Type A botulism toxin was found in this pie by a mouse-inoculation test performed at a U.S. Department of Agriculture laboratory in Beltsville, Maryland, and type A toxin was also demonstrated in the patient's serum by the state's Microbial Disease Laboratory.

This is the third case of botulism associated with commercial pot pies reported from California; 1 other episode involving 2 clinically diagnosed patients was reported from Minnesota in 1960. Mishandling of the pot pies occurred in three of these episodes, and mishandling was also suspected in the fourth. The known mishandlings consisted of leaving the baked pot pie in the oven with the pilot light on, thereby maintaining "incubatory" temperatures overnight. The pies were then eaten with no reheating to destroy toxin. Or, as in the present case, the baked pie sat out at room temperature for over 2 days during hot weather -- conditions that also could simulate an incubator.

In these situations, it is suspected that the original baking killed competing organisms in the pies and eliminated much of the oxygen. The heat-resistant, anaerobic *C. botulinum*, which was evidently present and can be found in many fresh, frozen, and other food products, was then presumably able to germinate and produce toxin under the crust during storage at warm, incubator-like temperatures. Products such as pot pies should be kept frozen before heating and ideally should be served hot after the first cooking. If any such products are to be saved, they should be quickly refrigerated, then reheated to hot temperatures. This would minimize any risk of botulinal poisoning.

Other Examples. Foodservice establishments have also been involved in botulism incidents. In November 1978, 7 cases of

type A botulism occurred in persons who had eaten in a restaurant in Colorado (Seals et al., 1981). The outbreak was recognized when 2 persons who had eaten at the restaurant were hospitalized with botulism; 5 additional cases were reported. Potato salad made at the restaurant and served during an 11-day period was incriminated as the vehicle of transmission. The potato salad had been prepared from potatoes baked for service in aluminum foil. The potatoes were "left-over" and were allowed to remain in the foil-wrapping at room temperature before being used to prepare potato salad. Laboratory studies confirmed that *C. botulinum* spores on the surface of the potatoes could survive baking and that botulinum toxin could be produced in potatoes contaminated with *C. botulinum* spores in sealed aluminum foil wrappers if these products were held at ambient temperatures for 1 day or less (Sugiyama et al., 1981).

In October of 1983, 28 people were hospitalized in Illinois with neurological signs and symptoms of botulism. Twelve patients required ventilatory support, and 20 patients were treated with antitoxin. One patient died 6 months after the onset of the illness. Type A toxin and/or type A *C. botulinum* was identified from specimens of 18 patients. Case control studies implicated sautéed onions made from fresh raw onions, served on a patty melt sandwich in a local restaurant as the vehicle of transmission. Type A toxin was detected in washings from a wrapper in which a patty-melt sandwich was taken home by one of the ill persons. Type A *C. botulinum* was also cultured from 5 of 75 raw onions taken from the restaurant. Onions used to prepare the patty-melt sandwiches had been partially cooked in butter a day previous to making the sandwiches. They were placed in a pan, covered with a layer of melted butter, and were stored on a warm counter on the back of the grill for 12 to 24 hours before the sandwiches were prepared. These storage conditions were optimal for growth of *C. botulinum* and subsequent toxin production (MacDonald et al., 1985).

Between July 26 and September 5, 1985, 37 cases of type B botulism were caused by food served at a restaurant in Vancouver, British Columbia. Seven persons required mechanical ventilation. *C. botulinum* type B toxin was found in the serum of 3 patients, and type B spores were found in cultured feces of 1 patient a month after the outbreak. Commercially bottled chopped garlic in soybean oil was implicated by the Centers for Disease Control as the food vehicle in this outbreak. Although the product involved was labeled "Keep Refrigerated" in very small print, the garlic jar at the restaurant was kept at room temperature (Solomon and Kauter, 1988).

An international outbreak of type E botulism associated with ungutted, salted whitefish occurred in the fall of 1987 (Centers for Disease Control, 1987). In November of 1987, a Russian immigrant and his 9-year-old son were admitted to a suburban New York hospital with symptoms indicative of botulism. The father's stool specimen contained type E botulinum toxin. The father had purchased a whole, ungutted, salted, air-dried whitefish known as kapchunka from a delicatessen in Queens, New York City. He and his son ate the fish a week later and both became ill within the next day. At the same time, the Centers for Disease Control in Atlanta, Georgia, received a report from the Ministry of Health, Jerusalem, Israel, of 5 additional cases suspected to be botulism. One case was fatal. The patients had eaten whitefish purchased at a grocery in Brooklyn, New York City in the middle of October 1987 and

then taken to Israel. The fish as well as a serum sample from 1 surviving patient yielded type E botulinum toxin.

Kapchunka is an ethnic food consumed in this country primarily by Russian immigrants. In 1981, a California man became ill, and, in 1985 2 Russian immigrants died in New York City after consuming this fish product.

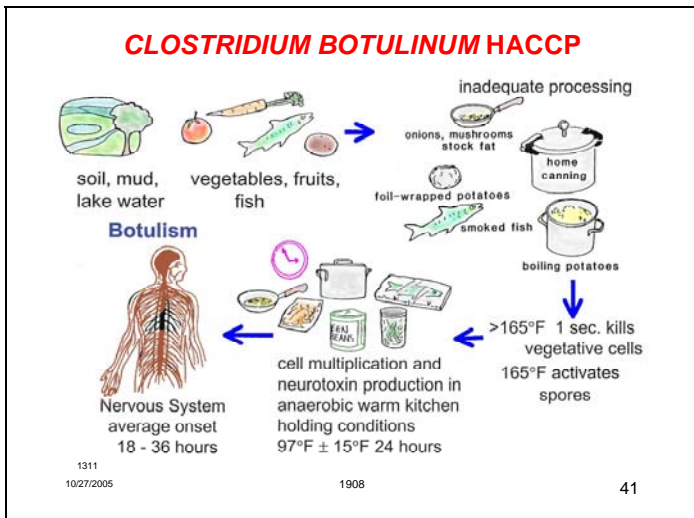
In the fall of 1988, 3 carnival workers in Louisiana became ill with botulism after consuming cole slaw, purchased at a delicatessen, that was allowed to remain unrefrigerated for 3 days.

These incidents are examples of improper processing and/or storage of food products that allowed spores of *C. botulinum* to germinate into vegetative cells that multiplied and produced toxin in foods. Botulism resulted when these foods were consumed and fatalities occurred.

#### References

- Adams, M.R., and Moss, M.O. 1995. Food Microbiology. The Royal Society of Chemistry. University of Surrey. Guildford, UK. pp. 168-177.
- Centers for Disease Control. 1985. Botulism from fresh foods-California. MMWR. 34:156-157.
- Centers for Disease Control. 1987. International outbreak associated with ungutted, salted whitefish. MMWR. 36:812-813.
- Dodds, K.L. and Austin, J.W. 1997. In Doyle, M. P., Beuchat, L. R., and Montville, T.J., Eds. Food Microbiology. Fundamentals and Frontiers. American Society of Microbiology. Washington, D.C. pp. 288-304.
- FDA. 1993. HACCP. Regulatory Food Applications in Retail Food Establishments. Dept. of Health and Human Services. Division of Human Resource Development, HFC-60. Rockville, MD.
- Jay, J.M. 1996. Modern Food Microbiology. Fifth Ed. Chapman & Hall Inc. New York, NY.
- Gibson, A.M., Bratchell N., and Roberts, T.A. 1987. The effect of sodium chloride and temperature on the rate and extent of growth of *Clostridium botulinum* type A in pasteurized pork slurry. J. Appl. Bacteriol. 62:479-490.
- Hauschild, A.H.W. 1989. *Clostridium botulinum*. In Foodborne Bacterial Pathogens. Doyle, M. P., Ed. Marcel Dekker. New York, NY.
- Ketchum, P.A. 1984. Microbiology. John Wiley and Sons, Inc. New York, NY.
- Lynt, R.K. and Kauter, D.A. 1982. *Clostridium botulinum* ABMPS Report No. 125. Microbiological Safety of Foods in Feeding Systems. Nat'l. Academy Press. Washington, D.C.
- MacDonald, K.L., Spengler, R.F., Hatheway, C.L., Hargrett, N.T., and Cohen, M.L. 1985. Type A botulism from sautéed onions. J. Am. Med. Assoc. 283(9):1275-1278.
- Mossel, D.A.A., Corry, J.E., Struijk, C.B., and Baird, R. 1995. Essentials of the Microbiology of Foods. John Wiley and Sons, New York, NY. pp.143-146.
- Pierson, M.D., and Reddy, N.R. 1988. *Clostridium botulinum*. Food Technol. 42(4):196-198.
- Seals, J.E., Snyder, J.D., Edell, T.A., Hatheway, C.L., Johnson, C.J., Swanson, R.C., and Hughes, J.M. 1981. Restaurant associated botulism: transmission by potato salad. Am. J. Epidemiol. 113:436-44.
- Simunovic, J., Oblinger, J.L. and Adams, J.P. 1985. Potential for growth of nonproteolytic types of *Clostridium botulinum* in pasteurized restructured meat products: A review. J. Food Protect. 48 (3):265-276.
- Solomon, H.M. and Kauter, D.A. 1988. Outgrowth and toxin production by *Clostridium botulinum* in bottles chopped garlic. J. Food Protect. 51(11):862-865.

- Sperber, W.H. 1982. Requirements of *Clostridium botulinum* for growth and toxin production. Food Technol. 36(12):89-94.
- Sugiyama, H., Woodburn, M., and Yang, K.H. 1981. Production of botulinum toxin in inoculated pack studies of foil-wrapped baked potatoes. J. Food Protect. 44:896-898.
- Tanaka, N. 1982. Toxin Production by *Clostridium botulinum* in media at pH lower than 4.6. J. Food Protect. 45(3):234-237
- Woodburn, M.J., Somers, E., Rodriguez, J., and Schantz, E.J. 1979. Heat inactivation rates of botulinum toxin A, B, E, and F in some foods and buffers. J. Food Sci. 44:1658-1661.



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## **Clostridium botulinum - Process Hazard Analysis and Critical Controls**

### **Clostridium botulinum Transmission**

Spores and vegetative cells of *Clostridium botulinum* are present in the soil and sediment from rivers, lakes and oceans and are therefore present on many of the food products harvested from these sources. These foods include: fresh and canned vegetables; foil-wrapped, unrefrigerated baked potatoes; unrefrigerated, wrapped, or vacuum-packed fish; keep-refrigerated, cooked or partially cooked convenience food items; and imported canned products (e.g., antipasto, smoked salmon and other seafoods). Liver pate and ham have also been implicated in botulism outbreaks because meat is slightly contaminated with types A and B *C. botulinum* spores.

In general, the presence of *C. botulinum* and/or its toxins in canned foods indicates faulty processing. Inadequately processed foods such as meats and vegetables (green beans, corn, beets, asparagus, and spinach), particularly home-canned items, have often been associated with botulism.

Frozen foods have not been associated with outbreaks of botulism. The vegetative cells do not grow at freezing temperatures. However, spores of *C. botulinum* may survive in foods during long freezer storage periods. After the food has been thawed, these spores can form vegetative cells. The cells will grow and produce toxin in foods if environmental conditions of temperature, pH, and atmosphere provide favorable growth conditions.

Spores of *C. botulinum* cannot germinate in foods with a pH lower than 4.6. Botulinal toxin can be produced in food in 1 to 10 days. Growth and subsequent toxin production is based on temperature, atmosphere, and other environmental conditions.

### **Commercial Sterilization**

Commercial sterilization refers to the heat treatment given various processed foods. This "sterilization" process does not mean that these foods are free of bacterial spores. It does mean that a sufficient heat treatment has been given to these products to reduce a hypothetical population of 1 billion spores in each of 1,000 cans to only 1 spore per 1,000 cans. Low-acid foods (vegetables, meat, fish, and poultry items) require processing at

elevated temperatures for sufficient periods of time in order to ensure the destruction of botulinal spores.

Some products (e.g., beets, cucumbers) are acidified with acetic acid (vinegar) prior to processing. This acidification permits the use of milder preservation heat treatments. It is essential that the pH of such acidified foods be permitted to equilibrate thoroughly before the heat process is applied. This requires sufficient acid, stirring and time for the pH to decrease to 4.6 or below at the center of solid materials. Most fruits are naturally acid, and are processed at temperatures that do not exceed 212°F. Since spores of *C. botulinum* do not germinate in acid foods (pH below 4.4-4.6), processing times and temperatures are based on the destruction of vegetative cells of microorganisms that cause spoilage in these products.

### **Detection**

Food containing botulinal toxin in any amount is unacceptable. Excellent methods are available for detection of *C. botulinum* and its toxins. They are invaluable for the examination of foods implicated in botulism outbreaks and for other investigational purposes. However, the expertise required in application of the methods and in the interpretation of results precludes their use in most laboratories that routinely analyze food. The probability that the examination of a reasonably sized sample of low-acid canned food contaminated with *C. botulinum* would result in detection of the organism is too low to assure the level of safety necessary. The safety of low-acid canned foods depends primarily on instrumentation and adequate process mechanisms to provide assurance that processing is adequately accomplished and that container integrity is maintained.

### **Control**

Control of the botulism hazard in perishable foods must be based on adherence to food handling practices that prevent the growth of *C. botulinum*. In order to prevent outbreaks of botulism in foodservice:

1. Canned foods should be rejected if they exhibit defects such as swelling, rust, and/or leakage. These types of food must not be used to prepare any food items for retail food use.
2. Canned goods should be stored under conditions recommended for these items.
3. Any prepared food products that have received a light heat treatment and are labeled "keep refrigerated" and perishable fish items (e.g., smoked fish products) must be stored at 37°F or below.
4. Fresh vegetables (e.g., mushrooms) must be packaged in containers or bags that allow air (oxygen) to enter the packages when they are stored at temperatures of 50°F or above.

### **References**

- Adams, M.R., and Moss, M.O. 1995. Food Microbiology. The Royal Society of Chemistry. University of Surrey, Guildford. UK. pp.168-177.
- Dodds, K.L. and Austin, J.W. 1997. In Doyle, M.P., Beuchat, L.R., and Montville, T.J., Eds. Food Microbiology. Fundamentals and Frontiers. American Society of Microbiology. Washington, D.C. pp. 288-304.
- FDA. 1993. HACCP. Regulatory Food Applications in Retail Food Establishments. Dept. of Health and Human Services. Division of Human Resource Development. HFC-60. Rockville, MD.

- Hauschild, A.H.W. 1989. *Clostridium botulinum*. In Foodborne Bacterial Pathogens. Doyle, M. P., Ed. Marcel Dekker. New York, NY.
- Jay, J.M. 1996. Modern Food Microbiology. Van Nostrand Reinhold Company. New York, NY.
- Lynt, R.K. and Kautler, D.A. 1982. *Clostridium botulinum*. In Microbiological Safety of Foods in Feeding Systems. Abmps Report No. 125. National Academy of Sciences Press. Washington, D.C.
- Mossel, D.A.A., Corry, J.E., Struijk, C.B., and Baird, R. 1995. Essentials of the Microbiology of Foods. John Wiley and Sons, New York, NY. pp.143-146.
- NAS. 1975 Prevention of Microbial and Parasitic Hazards Associated with Processed Foods. Food Protection Committee. National Academy of Sciences Press. Washington, D.C.
- Sakaguchi, G. 1979. Botulism. In Food-borne Infections and Intoxications, 2nd Ed. H. Riemann and F.L. Bryan, Eds. Academic Press. New York, NY.

