

***Bacillus*: Recent Advances in *Bacillus cereus* Food Poisoning Research**

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I. INTRODUCTION

Members of the genus *Bacillus* are found over a wide range of environmental conditions. This environmental diversity is due largely to the production of endospores that are highly resistant to extreme conditions (i.e., heat, cold, desiccation, salinity, etc.), allowing survival through adverse periods. Norris et al. have provided a thorough review of the environmental ranges from which *Bacillus* species have been isolated (1). The ubiquitous nature of the bacilli is conducive for the natural contamination of food products through such vehicles as dust, soil, and materials of animal and plant origin. Although various *Bacillus* species have been isolated from food products, *Bacillus cereus* is the species most commonly associated with cases of food poisoning.

Bacillus cereus is a facultatively anaerobic, rod-shaped spore-former that is widespread in the environment and has been implicated in foodborne disease. Examination of food poisoning statistics suggests that the organism is a relatively minor causative agent of food poisoning. For the period 1973-1987, 58 of 1869 (3.1%) outbreaks of bacterial food poisoning in the United States were attributed to *B. cereus*. There were 1123 cases (1.0%) of *B. cereus* illness out of a total of 108,906 cases attributed to bacteria (2). During 1976-1984, Canada had 64 outbreaks of *B. cereus* foodborne illness out of a total of 933 bacterial and viral outbreaks; there were 535 cases of *B. cereus* food poisoning out of a total of 26,176 cases (3-10). Thus, 6.9% of the outbreaks and 2% of the cases of bacterial and viral food poisoning in Canada were due to *B. cereus*. Todd has estimated that there are 84,000 cases of *B. cereus* foodborne illness each year in the United States with no deaths (11). The estimated annual cost to the people of the United States is \$36 million. Canada has an estimated 23,000 foodborne *B. cereus* cases each year with no deaths and an estimated cost of \$10 million (12).

Here, we summarize and evaluate some of the recent advances in *B. cereus* food poisoning research. Clinical aspects of foodborne and other human diseases caused by *Bacillus* species are discussed by Turnbull and Kramer (13). Previous reviews of *B. cereus* food poisoning include those by Goepfert et al. (14), Gilbert (15), Johnson (16), and Kramer and Gilbert (17).

II. OUTBREAKS

Earlier outbreaks of *B. cereus* food poisoning have been discussed by Goepfert et al. (14), Davies and Wilkinson (18), Gilbert (15), Johnson (16), and Shinagawa et al. (19). Certain aspects of selected outbreaks are presented below.

Example 1: The diarrheal toxin of *B. cereus* accounted for an outbreak of gastroenteritis in a Rhode Island nursing home involving 46 patients and staff members during 1985. Improper cooling and storage of cooked beef stew was considered to be responsible for the outbreak. The beef stew had been cooled uncovered on a stovetop at room temperature for 3-5 h (type and size of container was not given). The food was transferred to metal hotel pans (12 inches \times 18 inches, depth not given) and refrigerated uncovered overnight. The next day, the stew was placed in smaller hotel pans and steamed; the stew was not reboiled. The predominant symptoms of affected individuals were cramps and watery diarrhea with average duration of 16 h. There were no deaths. None of the beef stew was available for analysis; however *B. cereus* was recovered from 10 stool

specimens collected from 23 affected persons. Also tested were 21 randomly selected healthy persons, of which 1 was positive for *B. cereus*, but it was unknown if that person ate the stew. All isolates were the same bio- and serotype and had nearly identical plasmid profiles. The isolates gave positive vascular permeability reaction (VPR) in rabbits, indicating production of *B. cereus* diarrheal enterotoxin. The authors suggested that the single isolate recovered from the healthy person may have occurred naturally in the digestive tract or that the dosage ingested was too low to cause illness. There was no evidence for person-to-person or secondary spread of illness (20).

Example 2: In Scotland, 8 people became ill with vomiting and diarrhea within 3 h after eating in a Chinese restaurant. A common serotype of *B. cereus* was isolated from feces of patients, chicken in batter, cooked beef, and fried rice. A week later in the same city, an identical serotype of *B. cereus* was isolated from a stool specimen from one of two people who suffered acute vomiting after eating rice and curry in an Indian restaurant. The *B. cereus* isolates were positive for toxin in a HEP-2 test (a test producing semiquantitative vacuolation of tissue culture cells in response to the emetic toxin). While the specific food preparation error was not given, it is probable that improper storage of cooked rice was responsible for the outbreak (21).

Example 3: An unconfirmed total number of cases resulted from a *B. cereus* outbreak in 1985 at a Japanese restaurant in Maine. Approximately 36 people were believed to have eaten at the restaurant, of which 11 people were contacted. All 11 reported nausea and vomiting and 9 reported diarrhea. Onset of illness ranged from 30 min to 5 h after eating; duration of symptoms generally ranged from 5 h to several days, but 2 people had diarrhea for more than 2 weeks. Hospital emergency room treatment was sought by 10 people and 2 required rehydration therapy. *B. cereus* was isolated from vomit and stool specimens and from hibachi steak. *B. cereus* was not isolated from fried rice, mixed fried vegetables, or hibachi chicken. Fried rice was prepared from leftover boiled rice (which may have been stored at room temperature) and preparation of the fried rice may have destroyed viable organisms without destruction of the heat-stable emetic toxin. While it appears definite that the outbreak was due to *B. cereus* intoxication, the responsible food was not confirmed (22).

Example 4: While not an outbreak (at least two people must be affected), the following *B. cereus* illness is interesting. A woman complained of abdominal cramps and watery diarrhea after eating apples dried in a newly acquired electric home food dehydrator. The diarrhea lasted for approximately 30 h. Three weeks later, a similar episode happened to the same person after she ate newly dried apples. When a third episode oc-

curred, laboratory analysis revealed *B. cereus* in the dried apples and on the internal surfaces of the dehydrator. It is not clear from the article whether the woman ate the apples in the dry state or ate them after rehydrating. If the apples were hydrated for a long period and were unboiled before eating, it is possible that contaminating *B. cereus* present on the dried apples could grow to numbers high enough to lead to food poisoning (23).

Example 5: Beef stew containing *B. cereus* caused vomiting and nausea in at least 42 inmates of a British prison. Some of the affected inmates also had diarrhea and abdominal pain. Vomiting started 1.5 to 3 h after eating the meal and diarrhea started about 4 h after eating. Serotype H1 of *B. cereus* was isolated from the stew and from fecal specimens of patients. This serotype has been associated with both emetic and diarrheal *B. cereus* food poisoning. Extracts of the stew were cytopathic for several tissue culture cell lines, suggesting the presence of preformed toxin. The vegetables for the stew had been prepared several hours before the meal and left at room temperature. The previously prepared vegetables were cooked with raw minced beef in a steam-heated boiling pan just before serving. The improper storage of the cooked vegetables could have supported the growth of *B. cereus* with formation of the heat-stable toxin (24).

Example 6: A six-month-old child exhibited symptoms of food poisoning —diarrhea and occasionally vomiting—when fed his mother's milk. The symptoms did not appear to be an allergic reaction since the child could accept milk from other sources without difficulty. *B. cereus* was present in the baby's stools after ingesting his mother's milk. Milk from the mother, collected aseptically, contained approximately 5×10^4 colony-forming units per ml (CFU/ml) *B. cereus*. The organism was isolated from milk from both breasts. External breast abscesses or other abnormalities were not noted but the mother did complain of painful breasts. It was possible that the mother was suffering from a mastitis condition induced by *B. cereus*. The isolates gave positive VPR and ileal loop reactions indicative of the diarrheal toxin. Treatment of the mother with gentamicin cleared the infection and the infant no longer showed symptoms after ingesting his mother's milk (25).

Example 7: Symptoms of emetic food poisoning were present in individuals who had a macaroni and cheese lunch in a cafeteria in Alabama. Eight women experienced nausea and abdominal cramps 1–3 h after eating lunch; vomiting was present in four of the women. The symptoms resolved in a few hours, but four of the women subsequently had diarrhea. *B. cereus* was isolated from the remaining macaroni and cheese. Boiled macaroni

had been added to a mixture of powdered milk, cheese, and water and then baked for an unspecified length of time and left in the warm oven (ascertained to be 26.7°C) until needed on the cafeteria serving line. *B. cereus* was isolated from the powdered milk used in preparation of the dish. New food-handling procedures were instituted in the cafeteria that included refrigeration of the macaroni and cheese dish immediately after baking and then warming the dish just before serving (26).

Example 8: Turkey loaf from which *B. cereus* was isolated was involved in an outbreak of diarrhea in 28 patients in a hospital located in Kentucky. The average age of the patients (all male) was 72.7 with a range of 50–83 years, and most were bedridden with cerebrovascular disease. Abdominal cramps and watery diarrhea characterized the illness and *B. cereus* subsequently was isolated from stool specimens. Vomiting was noted in 1 patient and none had fever. The average length of diarrhea was 2.3 days, with diarrhea resolving in 20 patients within 24 h. The duration for 8 patients ranged from 2–10 days. Two patients required rehydration. Isolates from both turkey and stool specimens were shown to be toxin producers by use of rabbit ileal loop or suckling mouse assays and by cytotoxic response (cell lysis) in Y-1 adrenal cells. The particular error in food preparation that led to the outbreak was not determined (27).

Symptoms found in the above outbreaks included diarrhea without vomiting or vomiting that sometimes was followed by diarrhea. The diarrheal toxin does not appear to induce vomiting but, on occasion, the emetic toxin appears to cause both vomiting and diarrhea. It would appear that, under some conditions, emetic-toxin-producing strains also may produce a diarrheal-type toxin.

In the outbreaks listed above, the error in food preparation appeared to be improper holding of the food at temperatures high enough to allow growth of *B. cereus*. Prompt cooling of the food with refrigerated storage would have prevented the outbreaks.

In the 58 outbreaks of *B. cereus* food poisoning in the United States for the period 1973–1987, Chinese food accounted for 24 outbreaks, Mexican food for 5, beef for 3, fruits and vegetables for 3, shellfish for 2, and 1 each for chicken, finfish, ice cream, and turkey; 17 outbreaks were caused by other foods (unlisted) or causes were unknown (2). In 48 of the outbreaks, contributing factors to illness were demonstrated and, in most outbreaks, more than one error in food handling was involved. Improper holding temperatures were found in 94% of the outbreaks, contaminated equipment in 53%, inadequate cooking was involved in 32%, poor personal hygiene in 24%, but in only 5% of the outbreaks was an unsafe source of food used.

In recent years, the incidence of *B. cereus* in raw and processed foods has received considerable attention. The results of various studies indicate that few foods are free of the organism.

Kamat et al. studied the incidence of *B. cereus* in 124 different Indian foods purchased from local markets in the Bombay area (28). The organism was present in 28.5% of rice and rice products. Interestingly, they found that all samples of boiled rice were positive. *B. cereus* was contained in 40% of fish, 80% of chicken and meat products, and 30% of spices. The most heavily contaminated foods were milk, milk products, and ice cream, with counts ranging from 10^3 to 10^5 CFU/ml or g; 100% of milk products tested contained *B. cereus*. Chili powder and cumin sold in retail shops in Bombay showed high aerobic plate counts of 10^4 - 10^8 CFU/g. The *B. cereus* count for 18 samples of chili powder ranged from less than 10 to 10^3 CFU/g; the majority of cumin samples contained less than 10 *B. cereus* per g (29). Bachhil and Jaiswal found that 35% of 20 samples of buffalo meat obtained from an Indian slaughterhouse contained *B. cereus* at a mean level of 10^5 CFU/g (30). All 10 samples of kabob and 30% of 10 samples of curry obtained from restaurants contained the organism at a mean level of 10^3 CFU/g. Bachhil and Jaiswal found that 90% of cell-free filtrates of their *B. cereus* isolates were enterotoxigenic based on positive dermonecrotic reactions in guinea pigs.

Nigerian spices collected from markets in Port Harcourt were found to be contaminated heavily with microorganisms. Alligator pepper, red pepper, black pepper, thyme, and curry powder (230 samples in all) had total aerobic mesophilic counts of 10^4 to 10^8 CFU/g while the *B. cereus* counts ranged from 10^2 to 10^4 CFU/g (31). These spices are added routinely to food after cooking but, if the spiced food is temperature abused, growth of *B. cereus* is possible. The presence of *B. cereus* was determined in 35 different spices and herbs (523 samples) destined for use in Australia (32). All samples of spices or herbs were positive except for cloves, cayenne pepper, and chives. Counts generally were less than 3 CFU/g except for single samples of black peppercorns, tumeric, cumin seed (all 10^5 CFU/g), broken mace, and ginger (both 5×10^3 CFU/g).

A survey of 302 skim-milk powders from Japan done by Suzuki et al. indicated that 10.3% contained *B. cereus* at levels less than 300 CFU/g (33). Wong et al. tested 193 dairy products purchased in local markets in Taipei, Taiwan, for *B. cereus* and found that 27% of milk powder, 2% of pasteurized milk, 17% of fermented milk, 52% of ice cream, and 35% of soft ice cream contained the organism at levels ranging from 5-800 CFU/g or ml (34). Of tested isolates, 98% were toxic to Vero (African green mon-

key kidney) cells and 68% produced cytotoxic reactions (rounding) in Chinese hamster ovary (CHO) cells. All were hemolytic and of 11 of these, 3 were lethal to mice. *B. cereus* was detected in 11 out of 12 samples of uncooked polished and semipolished rice available for sale at various locations in Taipei and Taichung, Taiwan, at levels ranging from less than 40 to less than 200 CFU/g (35).

Of 29 samples of Venezuelan soft queso blanco cheese, 69% contained *B. cereus* at levels ranging from 10^5 to 10^8 CFU/g (36). When 51 samples of hard blanco cheese were tested for *B. cereus*, 63% were positive at levels ranging from 10^4 to 10^7 CFU/g. The authors suggested that antimicrobial products within the cheeses may have contributed to negative samples; however, their data also show wide variations of salt and fat content, pH, and moisture, which could effect the growth of *B. cereus*.

Mosso et al. determined the numbers of *B. cereus* spores in 102 samples of food (salad dressings, dried soups, sweet desserts, milk and milk products, rice dishes, pasta, and flour) collected from retail markets in Madrid, Spain (37). Only 14.7% of the foods contained *B. cereus* (at levels less than 10^4 CFU/g or ml); however, 82.4% of the foods contained *Bacillus* species spores. Approximately half of the *B. cereus* isolates demonstrated VPR, which is indicative of diarrheal enterotoxigenic activity (37).

Of a total of 102 retail samples of poultry products obtained from Bristol, England, supermarkets, 6.9% were positive for *B. cereus* at levels ranging from 10^1 to 10^4 CFU/g (38). The products included both raw and cooked items that were either fresh or frozen. The authors also mention that high numbers of other organisms (10^7 CFU/g) frequently were recovered, which may have reduced detection of *B. cereus*.

Ternstrom and Molin found that, of 45 samples each of pork, beef, and chicken carcasses sampled from slaughterhouses in Sweden, 7%, 11%, and 0%, respectively, contained *B. cereus* (39). Raw meats obtained from slaughterhouses, meat-processing plants, or retail meat shops in Japan were shown to contain *B. cereus* (40). Of 63 raw beef samples, 7.9% were positive for *B. cereus* at less than 10^1 to 10^3 CFU/g, 4.4% of 206 pork samples contained *B. cereus* at levels ranging from less than 10^1 to 10^3 CFU/g, and 7.2% of 194 raw chicken samples were positive at levels ranging from less than 10^1 to 10^4 CFU/g. *B. cereus* was present in 3.8% of 210 ham samples at 10^1 to 10^3 CFU/g, in 14.9% of 202 sausage samples at less than 10^1 to 10^3 CFU/g, in 45.5% of 112 uncooked hamburger samples at less than 10^1 to 10^4 CFU/g, and in 23.7% of 211 cooked hamburger samples at levels ranging from less than 10^1 to 10^4 CFU/g.

Konuma et al. also studied the incidence of *B. cereus* in 609 samples of meat-product additives, which included spices, seasonings, starch, vegetable proteins, animal proteins, sugar, and salt (40). The overall incidence

was 39.1%; sugar had an incidence of 6.0% and salt was negative for *B. cereus*. The other additives had a *B. cereus* incidence ranging from 37.7% for seasonings to 55.8% for starch.

Samson et al. conducted a survey of the microbiological quality of 110 commercial tempeh samples obtained from shops, tempeh manufacturers, and restaurants in the Netherlands and found that 98% of the samples had a high aerobic plate count of greater than 10^7 CFU/g (41). *B. cereus* was present in 16% of the samples with counts ranging from 10^2 to 10^7 CFU/g; 11% of the samples had levels greater than 10^5 CFU/g.

Seeds for use in home sprouting kits were shown to be contaminated with *B. cereus* (42). *B. cereus* was present at levels of 3 to greater than 100 CFU/g in 67.3% of 98 samples of seeds (alfalfa, mung beans, wheat, or seed mixtures). Growth of *B. cereus* from naturally contaminated alfalfa, mung bean, and wheat seeds reached mean \log_{10} values per g of 3.72, 4.52, and 5.39, respectively, after incubation.

Harmon and Kautter obtained 106 ready-to-serve moist foods from serving lines at different cafeterias in the Washington, D.C., area (43). *B. cereus* was contained in 83% of the foods. Of 8 samples of cooked noodles, 10 samples of mashed potatoes, 12 samples of cooked rice, 10 samples of succotash, 6 samples of lima beans, 24 samples of skim milk, and 4 samples of turkey gravy, 70% or more were positive for *B. cereus*. Only 1 of 4 samples of beef gravy or egg salad were positive, but all 4 samples of chicken salad were negative. They also obtained 12 samples of powdered infant formula and 18 samples of nonfat dry milk from various grocery stores and determined that 75% of the formula samples and all of the dry milk samples contained *B. cereus*.

Wood and Waites isolated *B. cereus* from various sites at an egg-laying facility and at an egg-breaking plant (44). Melange (a mixture of cracked, broken, and substandard eggs, centrifuged to remove shell fragments, and stored frozen) and equipment associated with melange production frequently were contaminated with *B. cereus*. Pasteurized liquid whole eggs as well as bakery products containing pasteurized egg products also were positive for the organism. *B. cereus* was present in the environment of an egg pasteurization facility as well as in raw and pasteurized egg samples obtained from that facility (45). Both Wood and Waites (44) and Ellison et al. (45) reported decreases in *B. cereus* isolated after pasteurization, suggesting a predominance of vegetative cells rather than spores in the product. Ellison et al. also found *B. cereus* in a number of retail products containing eggs; these included frozen omelettes, quiches, flans, and egg custards (45). There is a brief report concerning a *B. cereus* outbreak that occurred in the United Kingdom in which 13 out of 40 people had abdominal pain and diarrhea after eating soft fried egg sandwiches that had been kept warm in insulated boxes for several hours after preparation (46).

It would appear that it is impossible to obtain raw products that are free from *B. cereus* spores. Thus, raw products must be handled with care during storage (for nondry raw foods) and during subsequent formulation into edible foods. The high incidence of *B. cereus* in cooked foods suggests that the foods were stored improperly after preparation.

IV. GROWTH AND SURVIVAL

Considering the incidence of *B. cereus* in various uncooked and cooked foods, it is not surprising that the organism will grow if foods are subjected to improper storage temperature or other types of temperature abuse. If growth does occur, there will be a possibility of toxin production with an ensuing food poisoning incident or outbreak.

The growth of *B. cereus* was studied in pasteurized milk, fruit-flavored reconstituted milk, and fermented milk at 30°C (47). An inoculum of 10⁴ CFU/ml vegetative cells or spores added to pasteurized milk (pH about 6.8) reached a maximum population of 10⁸ CFU/ml in 12 h, but growth was slower in fruit-flavored milk (pH about 4.7), in which *B. cereus* only reached a population of 10⁶–10⁷ CFU/ml in 24 h. There was no germination of spores within 7 days in fermented milk (pH about 3.7) and vegetative cells died in 40 min when added to fermented milk. CHO cells treated with supernatants of each milk type after centrifugation were observed for either cell lysis (enterotoxin) or cytotoxicity (cytotoxin causing cell elongation) after 1-h or 2-h incubations. Toxins produced during *B. cereus* growth at 30°C in pasteurized milk either lysed or caused a cytotoxic reaction in CHO cells. Addition of phosphate buffer to pasteurized milk slightly reduced this effect. Growth of *B. cereus* in apple-flavored reconstituted milk did not result in production of toxic activity. When added to milk simultaneously, *B. cereus* grew at the same rate as lactic acid bacteria up to 12 h, at which time a cytotoxic reaction of CHO cells was noted (pH of the milk was 5.7–5.9 and at maximum counts of 10⁸ CFU/ml, *B. cereus* started to decline); after 72 h (pH of the milk was 4.2–4.3) viable *B. cereus* cells no longer were detected, but a cytotoxic effect still was observed in CHO cells. Samples of each milk type without *B. cereus* were negative in the assays, and acidity in the fermented milk did not affect the CHO cells (47).

When *B. cereus* was inoculated into nonfat milk medium simultaneously with *Streptococcus lactis* (presently referred to as *Lactococcus lactis*), *S. thermophilus*, *Lactobacillus acidophilus*, or *L. bulgaricus*, growth of the lactic acid bacteria was not affected (48). *B. cereus* grew from an initial 10⁴ CFU/ml to 10⁸ CFU/ml in 8–12 h and then declined rapidly to less than 10¹ CFU/ml by 20 h. If *B. cereus* was added to milks that had been fermented by the *Lactobacillus* cultures for 24 h, growth increased by 2 log values

followed by decline to less than 10^1 CFU/ml in 16–20 h; milks fermented by streptococci did not permit growth of *B. cereus* and the numbers of the pathogen decreased to less than 10^1 CFU/ml within 16–20 h. Wong and Chen found that addition of 10^4 CFU/ml *B. cereus* to milks fermented 72 h (streptococci or lactobacilli fermentation) did not lead to growth of the pathogen and *B. cereus* decreased to less than 10^1 CFU/ml over 30–40 min (48).

A higher percentage of *B. cereus* isolates from milk and cream produced cytotoxins against HeLa (human cervix epitheloid carcinoma), Vero, or MRC-5 (human embryonic lung) cells when grown at 30°C for 24 h in milk compared with brain heart infusion (BHI) (49). In the absence of aeration (i.e., shaking), *B. cereus* did not produce cytotoxic activity in milk at 8°C or 15°C. Some strains were acclimated to grow at 8°C; when the relationship between cytotoxicity and growth of these isolates was compared, most became cytotoxic at approximately 10^8 CFU/ml. Dairy isolates that produced cytotoxicity in milk at 8°C under aerated conditions also gave positive VPR with rabbits, which is indicative of *B. cereus* diarrheal toxin. While *B. cereus* commonly is found in milk and other dairy products and milk is a suitable substrate for toxin production even at low temperatures under aerated conditions, the incidence of *B. cereus* food poisoning in milk is low, probably due to insufficient aeration during normal storage (49).

B. cereus spores in rice pudding were not destroyed when inoculated at levels of 10^3 – 10^6 CFU/g or ml into either rice or milk prior to boiling (greater than 30 min at 98°C). When the rice pudding was stored at 4°C or 7°C for 8 days, there was no growth of *B. cereus*, but, depending on the level of inoculation, the population of *B. cereus* reached toxic levels when rice pudding was stored at 22°C for 24–96 h (50).

In vacuum-packaged bologna, *B. cereus* growth does not appear to be inhibited by the growth of the normal saprophytic flora at 12°C storage (51). Co-inoculation of a mixture of lactobacilli (isolated from vacuum-packaged meats) and *B. cereus* resulted in marked reduction of the growth of the pathogen at 12°C; at Day 0, the *B. cereus* count was 10^2 CFU/g, while at 28 days the count was 10^7 CFU/g in the absence of lactobacilli (pH 6.2) and 10^4 CFU/g in the presence of lactobacilli (pH 5.4). The levels of lactobacilli were 10^3 initially and reached 10^9 after 28 days (51).

Finnish liver sausage containing salt (1.7%) and nitrite (120 parts per million [ppm]) was inoculated with approximately 10^3 *B. cereus* spores/g and cooked in water at 80°C for 40 min, followed by cooling to 4.5°C within 75 min. When sausages were stored at 4°C or 8°C, there was no growth of *B. cereus* (52). At 15°C, the sausages reached a *B. cereus* count of 10^6 by 9 days and sausages containing salt, nitrite, and sodium erythorbate (0.5%) reached 10^6 in 7 days; however, when the sausages contained salt, nitrite, erythorbate, citric acid (0.05%), and glucono-delta-lactone (0.15%),

there was no growth of *B. cereus* at 14 days when incubated at 15°C. The authors stated that, at 22°C, the formulation containing glucono-delta-lactone significantly delayed the growth of *B. cereus*. The sausages with glucono-delta-lactone had slightly lower pH values (5.7–5.8) than those lacking glucono-delta-lactone (5.9–6.1), which may have accounted for the delay of *B. cereus* growth.

Several species of *Bacillus* are capable of rapid growth in bread, causing “ropiness” of the product. Kaur found that *B. cereus* often was not present in bread ingredients and when the organism was present, the level was less than or equal to 10² CFU/g (53). When *B. cereus* was inoculated into bread dough at levels of 10⁴–10⁵ CFU/g, there was no growth during the 4-h bread rising period at 27.5°C. Loaves of 400 g were baked at 320°F for 25 min, cooled, and then stored in polyethylene bags at 27.5°C. At the beginning of storage, the *B. cereus* count was less than 50 CFU/g; therefore, baking was quite destructive to the organism. At one-day storage there was little growth, but at 2 days the *B. cereus* counts were 10³–10⁴ CFU/g. Similar results were obtained with 800-g loaves. Addition of calcium propionate delayed spore germination. Kaur felt that *B. cereus* food poisoning from contaminated bread was unlikely. Rizk and Ebeid obtained similar results (54): baking of bread killed most of the *B. cereus* that had been inoculated into the dough. At 25°C, there was little or no growth of *B. cereus* until the bread had been stored for 3 days. Samples of uninoculated wheat flours were found to contain *B. cereus* levels of 10 to 100 CFU/g.

B. cereus was able to grow during rice tape fermentation at 30°C even though the pH reached as low as 3.5; however, more than 48 h was necessary to achieve such a low pH (55). Initial *B. cereus* levels of 10⁶ increased to 10⁸ CFU/g within 12 h and maintained that level of cells for at least 72 h when the substrate was white glutinous, white long-grain, or brown long-grain rice. When black glutinous rice was used for the tape fermentation, however, there was an initial increase in the growth of *B. cereus*, followed by a decrease. The seed coat of black glutinous rice contains tannins, procyanidins, and anthocyanins that may be released during fermentation and probably are inhibitory to *B. cereus*. It is important to use rice with low numbers of *B. cereus* when fermented rice foods are to be prepared.

Since soybean tempeh can be contaminated with *B. cereus*, Nout et al. found that acidification of the soybeans with lactic acid to pH less than or equal to 4.4 prevented *B. cereus* growth and tempeh of good quality was produced (56). However, acidification with acetic acid to pH less than or equal to 5.5 inhibited the growth of both *B. cereus* and the fungal starter, *Rhizopus oligosporus*.

Garcia-Arribas and Kramer studied the effect of glucose, starch, and pH on growth and toxin production by four strains of *B. cereus*: one each of emetic, diarrheal, clinical (wound infection), and veterinary (mastitis) isolates (57). In BHI broth supplemented with 1% glucose and incubated at 30°C, all strains showed maximum growth (from an initial 10^5 CFU/ml to 5×10^8 CFU/ml) in 6–8 h with no lag; the number of cells remained constant over a 70-h period. VPR activity was detected within 6 h and reached maximum levels during the stationary stage of growth; by 70 h, there was a slight decline in VPR activity. Hemolysin activity increased rapidly during the exponential growth phase and reached a maximum at 15–32 h, followed by a rapid decrease in activity. When 1% starch was substituted for glucose, the picture was similar to that exhibited by BHI plus glucose, except that the diarrheal and veterinary strains showed a secondary increase in VPR activity. Secondary production of VPR activity in the presence of starch described by Garcia-Arribas and Kramer is interesting and may be of significance in production of toxin by *B. cereus* in starchy (cereal) foods.

Alkaline conditions (BHI adjusted to pH 8.8), led to a 6–8-h lag in growth and in VPR and hemolysin production. The levels reached for VPR activity were similar to those of BHI plus glucose. The production of hemolysin was half that found with BHI plus glucose.

BHI at pH 5.0 was more inhibitory to the growth of the clinical and veterinary strains of *B. cereus* (maximum population was 5×10^7 CFU/ml) than to the food poisoning strains (maximum population was 5×10^8 CFU/ml), but the log growth rate for all strains was similar and there was no lag. The acid conditions did lead to a lag in the production of hemolysin and VPR activity (6–8 h). The emetic strain produced less VPR activity than the other strains when grown under acid conditions. The wide range of pH (5.0 to 8.8) over which toxin can be produced indicates that toxin could be found in a wide variety of foods.

The mean generation time for four strains of *B. cereus* (1 diarrheal, 2 emetic, and 1 food isolate) on cooked minced chicken breast (pH 6.0) was 3.8 h (range 3.2 to 4.1) at 15°C, 1.4 h (range 1.4 to 1.5) at 22°C, and 0.5 h (range 0.4 to 0.5) at 37°C (38). The mean generation time for the strains on cooked minced chicken leg (pH 6.5) was 3.2 h (range 2.6 to 3.7) at 15°C, 1.2 h (range 1.1 to 1.3) at 22°C, and 0.4 h at 37°C. The diarrheal strain did not grow on chicken at 10°C but the emetic strains did grow slightly at that temperature. None of the strains were able to grow in beef heart infusion broth at 10°C. It would appear that, at low temperatures, meat may be a better substrate than laboratory medium for growth of some strains of *B. cereus*.

Low temperature often is used as a means of limiting bacterial growth, but a few studies have shown that *B. cereus* can grow and produce the diarrheal toxin (and probably the emetic toxin) at low temperatures. Bergann studied the growth temperature requirements of 50 strains of *B. cereus* (58). All strains grew at temperatures ranging from 14°C to 40°C. Half of the strains grew at 45°C and three strains grew at 49°C. More than half of the strains grew at 10°C, six strains grew at 8°C, and one strain grew at 6°C.

Van Netten et al. found that *B. cereus* isolates that were involved in two foodborne outbreaks of diarrheal illness (one in the Netherlands involving vegetable pie and one in Spain involving codfish) were able to grow and produce toxin at 4°C–7°C (49). From a third outbreak (vomiting illness) involving pasteurized milk in the Netherlands, strains of *B. cereus* of the emetic type were isolated that were able to grow at 4°C–7°C, but enterotoxin production was not detected using the Oxoid *Bacillus cereus* enterotoxin–reversed passive latex agglutination (BCET–RPLA) kit (Oxoid Ltd., Basingstoke, England) for diarrheal toxin. It is quite probable that the emetic toxin can be produced at low incubation temperatures, although they did not test for it.

These authors examined the *B. cereus* populations in over 1700 food samples (59). Approximately 16% of 600 *B. cereus* isolates were able to grow at 7°C. More than 95% of the strains that grew at 7°C were not able to grow at 43°C. Isolates that were able to grow at 7°C and the number of samples used were spices (1/72), cream pastry (2/45), mousses/pate (4/4), cooked/chilled foods (4/4), and pasteurized milk (84/155). Toxin production in foods over the temperature range of 4°C–17°C was dependent on a_w (water activity) greater than or equal to 0.95 and pH greater than or equal to 5.8. Under these conditions, diarrheal enterotoxin was detected first on Day 24 at 4°C incubation, 11–12 days at 7°C, and on Day 2 at 17°C.

Detectable toxin levels occurred as *B. cereus* reached greater than $10^{6.6}$ CFU/g. Mimicry of temperature-abuse conditions (insertion of one day at 12.5°C or 2 h at 17°C) after two days of proper storage (7°C) resulted in enterotoxin detection 1–2 days earlier than under nonabuse conditions. Psychrotrophic *B. cereus* strains did not grow or produce toxin over a 20-day period when grown in BHI incubated at 7°C with the pH adjusted to less than or equal to 6.0 with lactic, acetic, or sorbic acids. Van Netten et al. suggested that all cooked/chilled foods be acidified to a pH of at least 6.0 or, alternately, be stored at less than 4°C.

Griffiths found that 71 of 83 psychrotrophic *Bacillus* species produced diarrheal toxin (assayed by the Oxoid kit) at 25°C when grown in BHI (60). *B. cereus* and closely related *Bacillus* species were the dominant enter-

otoxin-producing strains. A strain of *B. mycoides* and three strains of *B. cereus* produced diarrheal toxin when grown in 10% reconstituted skim-milk powder at temperatures ranging from 6°C to 21°C. Griffiths indicated that milk produced under hygienic conditions and kept properly refrigerated probably would not lead to food poisoning by psychrotrophic *B. cereus* since the milk would be consumed before the organisms grew to sufficient numbers.

Psychrotrophic *Bacillus* species were isolated from raw and pasteurized milk samples and approximately 37% were identified as *B. cereus* (61). When growth studies were performed, none of the *B. cereus* or related strains were able to grow at 2°C, but, at 6°C, the lag times for these isolates ranged from 60 h to 132 h and generation times ranged from 12 h to 23 h.

V. INHIBITION AND INACTIVATION

Methods for inhibition and inactivation include the determination of the *D*-values for *B. cereus* spores heated in custard by Bassen et al.; these values were 3.6 min, 2.8 min, and 2.2 min at 90°C, 95°C, and 100°C, respectively (62). When custard was adjusted to pH 6.2, the $D_{90^{\circ}\text{C}}$ decreased to 3.1 min, the $D_{95^{\circ}\text{C}}$ to 2.5 min, and the $D_{100^{\circ}\text{C}}$ to 1.7; increasing the pH to 7.6 increased the $D_{90^{\circ}\text{C}}$ to 3.7 min and the $D_{95^{\circ}\text{C}}$ to 3.0. Addition of benzoate, butylated hydroxyanisole, or butyrate hydroxytoluene to the custard mix facilitated the destruction of *B. cereus* spores by heat.

Chung and Sun determined the *D*-values of spores of six *B. cereus* strains isolated from uncooked rice and found that the mean $D_{92^{\circ}\text{C}}$ was 22 min (range 16 to 36 min) while the mean $D_{100^{\circ}\text{C}}$ value was 4.9 min (range 4.2 to 6.3 min) when heated in rice broth at pH 7.0 (35). When *B. cereus* spores of 25 strains isolated from various dairy products were heated in 0.025 molar (M) phosphate buffer (pH 7.0), the mean $D_{100^{\circ}\text{C}}$ value was 3.5 min with a range of 2.0–5.4 min (34). Kamat et al. determined the $D_{90^{\circ}\text{C}}$ of six environmental strains of *B. cereus* using phosphate buffer at pH 7.0 and found the mean *D*-value for spores was 11.7 min (range 8.0 to 15.0 min) and the mean *D*-value for vegetative cells was 0.43 min (range 0.33 to 0.5 min) (28). Similar variation was found in earlier studies: Parry and Gilbert showed that the spores of 14 strains of food poisoning isolates of *B. cereus* had $D_{95^{\circ}\text{C}}$ values of 2.5–36.2 min whereas 13 strains from uncooked rice gave values of 1.5–19.7 min (spores were heated in water) (63). The authors suggested that inactivation of spores does not occur during the boiling of rice and, in fact, may select for heat tolerance.

Johnson et al. compared heat resistance to spore germination ability of a number of *B. cereus* strains using sodium phosphate buffer (25 millimolar [mM], pH 7.0) (64). The $D_{85^{\circ}\text{C}}$ for spores of one atoxigenic strain

was 77.1 min, that of three emetic strains was 50.1–106.0 min, and that for three diarrheal strains was 32.1–75.1 min; the mean z -value was 9.2°C (range 6.8°C to 13.9°C). The mean spore $D_{95^\circ\text{C}}$ for all of the strains was 1.2–20.2 min. Germination rates (determined by loss of heat resistance) showed that the diarrheal strains tended to germinate faster and at higher maximum percentages than the emetic and atoxigenic strains. The authors, however, could not correlate slow germination with greater heat resistance (64).

Pendurkar and Kulkarni found that spores of *B. cereus* inoculated into uncooked rice were not inactivated completely during the cooking process (approximately 100°C for 30 min) (65). Viable organisms could not be found in fried rice (180°C–190°C for 5–7 min).

Spite showed that microwaving frozen mashed potatoes or macaroni and cheese dishes containing 10^5 to 10^7 CFU/g *Salmonella cubana*, *Staphylococcus aureus*, *Clostridium perfringens*, or *B. cereus* did not lead to elimination of the organisms from the product (66). Increasing the lipid content of the formulation was protective for *C. perfringens*, but not for the other organisms. Overcooking with resultant lowering of quality did eliminate the nonsporeformers. Therefore, microwave cooking of products from the frozen state according to manufacturers' directions will not eliminate these pathogens, including *B. cereus*.

Methods for inhibition and inactivation have been investigated, including treatment by irradiation, gases, and chemicals. The mean D_{10} of spores from six strains of *B. cereus* subjected to gamma radiation was 11.7 kilogray (kGy) (a range from 8.0 to 15.0), while that of vegetative cells was 0.62 kGy (a range from 0.3 to 0.75) when suspended in phosphate buffer, pH 7.0 (28). The D_{10} is the dose in kGy that gives 90% inactivation. Ozone was sporicidal against the spores of *B. cereus* and the effect was dependent on the relative humidity (RH); the presence of moisture probably increased the penetration of the gas into the spores (67). At 3 milligrams/liter (mg/l) ozone, the D -value was 15 min at 95% RH, 23 min at 90% RH, 42 min at 80% RH, and 123 min at 70% RH. Enfors and Molin found that the relative percent inhibition of growth of *B. cereus* in medium in the presence of 100% CO_2 at 1 atmosphere (atm) (as compared to growth in 100% N_2 at 1 atm) was 86% at 20°C, 61% at 30°C, and 42% at 40°C (68). Solubility of CO_2 increases as temperature decreases, thus, at refrigerated temperatures, growth of *B. cereus* could be inhibited in a food packaged in a carbon dioxide atmosphere. D -values for washed spores from five dairy strains of *B. cereus* diluted into 1% H_2O_2 and immediately subjected to ultraviolet (UV) irradiation at 254 nanometers (nm) at an intensity of 750 joules/second/centimeter² (J/s/cm²) ranged from 3.9 to 11.4 min with a mean of 7.8 min (69). Foegeding et al. found that ClO_2 was sporicidal against

B. cereus spores (70). ClO_2 at 50 mg/l reduced 1 to 6×10^6 CFU/ml *B. cereus* spores greater than or equal to 99.9% in 15–38 min. Varying the pH from 4.5–8.5 had little effect on the activity of ClO_2 . Increasing the ClO_2 level to 80 mg/l led to more rapid inactivation of spores.

When the pH of trypticase soy broth (TSB) was adjusted with 1 normal (N) HCl, the critical pH for growth was 5.0 (48). The critical pH for inhibition of growth of vegetative cells of *B. cereus* was 5.6, 6.0, and 6.1 in the presence of 0.1-M lactate, formate, and acetate, respectively. In nonfat milk medium, *B. cereus* and lactic acid bacteria produced primarily lactate and acetate, with little formate produced. Critical concentrations of the organic acids at pH 6.4 (growth not affected) were 0.15 M for formate and acetate and 0.33 M for lactate. Spore germination was more resistant to the organic acids 0.1 M acetate, lactate, or formate in nutrient broth, which respectively gave 50% inhibition of germination at pH 4.2, 4.3, or 4.4. When two strains of *B. cereus* were incubated at 30°C in nutrient broth adjusted to pH 5, 6, 7, or 8 (presumably by addition of HCl or NaOH), the mean generation time was 125 min at pH 5, 63 min at pH 6, 58 min at pH 7, and 60 min at pH 8 (35).

When *B. cereus* spores were added to milks undergoing fermentation at 30°C (by *L. acidophilus*, *L. bulgaricus*, *S. lactis*, or *S. thermophilus*) at either zero time or after 24 h of fermentation, there was no inhibition of germination (48). If, however, the spores were added to milk that had been fermented for 48 h, the mean percent germination of *B. cereus* spores was approximately 38; if the spores were added after 72 h of fermentation, the percent germination was 25. Wong et al. found that there was no inhibition of *B. cereus* spore germination when incubated at 30°C in pasteurized milk (pH 6.8) for 4 h (47). There was 46% inhibition of germination in fruit-flavored reconstituted milk (pH 4.7) and the percent inhibition in fermented milk (pH 3.7) was 71 at 4-h incubation (30°C). Thus, acid conditions inhibit *B. cereus* spore germination. Commitment to germination in *B. cereus* spores occurred during a 0.5-min exposure to L-cysteine or L-alanine even though first visible germination (measured by refractile decrease) did not occur until 15–20 min after commitment (71). The authors determined that inhibition of germination of *B. cereus* at pH 4.5 was due to the inability of the germinant to bind to the germination trigger site.

In BHI broth containing disodium ethylenediaminetetraacetic acid (EDTA), little or no growth from *B. cereus* spores was observed within 48 h at 35°C when the chelator concentration was 300–1000 ppm (72). Inhibition of growth by EDTA was observed at pH values ranging from 5 to 9, with maximum inhibition at pH 7.0. The growth inhibitory effect was reversed by addition of iron, zinc, or calcium ions, but addition of magnesium ions had no effect. EDTA did not affect spore germination nor the

release of calcium from the spores. These data suggest that EDTA inhibits growth of *B. cereus* by binding an ion essential for outgrowth after germination takes place.

Shelef et al. studied the effect of the herb sage on the growth of *B. cereus* in nutrient broth and various foods (73). *B. cereus* was more sensitive to the inhibitory effects of sage than was *S. aureus* or *S. typhimurium*; spore germination was not inhibited, but subsequent outgrowth was inhibited. Sage was most inhibitory to *B. cereus* growth when added to nutrient broth, but addition to rice, a chicken-noodle dish, or to strained beef diminished its activity toward *B. cereus*. The minimum inhibitory concentrations of sage were 0.1%, 0.4%, and 1.0%, respectively, for growth in nutrient broth, rice, and the chicken-noodle dish. Sage at 2.5% concentration had only slight inhibition against *B. cereus* when mixed with strained beef.

Sooltan et al. observed an inhibitory effect on *B. cereus* growth when inoculated simultaneously with competing organisms isolated from a breaded chicken product. After inoculation of approximately 30-CFU/ml *B. cereus* with equal numbers of competitors, recovery of *B. cereus* ranged from 0-9 CFU/ml, whereas without competitors counts ranged from 0-59 CFU/ml, depending on the isolation medium used.

In a review on water activity (a_w), Troller discussed the more dated literature on the effect of various solutes on spore germination and growth of *B. cereus* (74). Spore germination is less sensitive to reduced a_w than is outgrowth or growth of vegetative cells. Peters et al. studied the effects of NaCl, pH, and temperature combinations on the growth of *B. cereus* using two-dimensional gradient plates (75). At 0.5% NaCl, growth occurred over the range of 14°C to 41°C with a pH range of 4.7 to 6.8. Increasing the NaCl level to 3% restricted the growth to temperatures ranging from 15°C to 40°C and to pH values ranging from 4.9 to 6.8. At 5% NaCl, growth was reduced and took place at 21°C-39°C and within the pH limits of 5.5-6.8; there was no growth at 7% NaCl. Bergann studied the effect of NaCl levels on growth of 50 strains of *B. cereus* within 6 days at 30°C (58). All 50 strains grew at 5% NaCl, but growth of all was inhibited at 10%; 45 strains grew at 6% NaCl, 35 grew at 7%, 10 grew at 8%, 8 grew at 9%, and 1 strain was able to grow in the presence of 9.5% NaCl.

Nitrite acted as a membrane-directed sulfhydryl agent against *B. cereus*; nitrite inhibition reversed when the exposure to nitrite was removed (76). The active agent appeared to be a protonated form of nitrite, but it was uncertain just where nitrite acted in the bacterial membrane. The authors showed that the nitrite concentrations that modified membrane sulfhydryl activity were correlated with those that had bacteriostatic effects.

Treatment of *B. cereus* spores with nicotinamide (0.5 mg/ml) produced spores with reduced heat stability (77). Nicotinamide-treated spores

germinated poorly and lost their ability to germinate upon storage for two months at 4°C. Exogenous addition of such reversal agents as dipicolinic acid (also produced by the cells) or L-kynurenine to nicotinamide-treated spores restored heat resistance; however, addition of L-tryptophan had no effect. Kalita and Singh suggested that addition of nicotinamide to foods could prove useful as an anti-*B. cereus* agent.

Although effective against *C. perfringens* and *C. botulinum*, the addition of 0 to 3000 µg/ml sodium hypophosphite to TSB had little inhibitory effect against *B. cereus* at pH values ranging from 5.5 to 7.0 or at salt levels ranging from 1% to 3% (78).

VI. ISOLATION, IDENTIFICATION, AND CHARACTERIZATION

A. Microbiological Media

In their 1989 review, Kramer and Gilbert discussed the use of various isolation and enumeration media for *B. cereus* occurring in foods and clinical specimens (17). Selective media for *B. cereus* exploit the organism's ability or inability to carry out certain activities.

Hemolysin activity can be detected on agars containing erythrocytes of horse, rabbit, sheep, or human origin. Hydrolysis of lecithin by the organism is determined by incorporating egg yolk into the agar. The inability of the organism to utilize mannitol is determined easily by the incorporation of mannitol and an indicator dye into the agar. Polymyxin normally is added to improve selectivity since most gram-negative organisms are inhibited by the antibiotic.

For the enumeration of *B. cereus* in mung bean and wheat sprouts, Harmon et al. compared the ability of three selective agars: mannitol-egg yolk-polymyxin (MYP), polymyxin-pyruvate-egg yolk-mannitol-bromothymol blue (PEMBA), and trypticase soy-polymyxin-sheep blood (79). Rates of recovery of *B. cereus* were not significantly different for the three media; however, the authors suggested that *B. cereus* was differentiated more readily from other microorganisms on MYP and fewer confirmatory tests were necessary. Kim and Goepfert formulated an egg yolk-polymyxin medium (KG) that was comparable to MYP in ability to isolate *Bacillus* species (80). KG contains low levels of peptone and no carbohydrate source and thus is advantageous for spore production within 24 h. These conditions enabled differentiation between *Bacillus* species and identification of presumptive *B. cereus*. Peterz et al. compared the behavior of three different media in isolation of *B. cereus* from dry foods or milk products (81). There were no significant differences among MYP, PEMBA, or 5% beef blood agars in terms of efficiency of isolation, but use of the blood agar

medium did require more confirmatory testing. A rapid *Bacillus* identification system is available utilizing the Analytab Products, Inc. (API) system (Analytab Products, Plainview, NY), which can be used to confirm isolates of *B. cereus* from selective agars (82).

B. Serological Typing

Serological typing techniques of *B. cereus* strains involved in food poisoning are discussed in Kramer et al. (83), Kramer and Gilbert (17), and Shinagawa (84). The flagellar (H) antigens have proven effective for distinguishing between strains. Serological typing is important because it can be used to correlate clinical strains found in an outbreak with the strains isolated from suspect foods. In addition, serological typing is an important epidemiological tool for strain identification during *B. cereus* food poisoning episodes and other diseases caused by *B. cereus* infection. For example, serotyping studies of the outbreak at the Rhode Island nursing home (see Example 1 in the section, "Outbreaks") determined that all of the isolates recovered from affected patients were of the same previously undescribed serotype H-26 (20).

Shinagawa presents a useful scheme diagramming the isolation and identification of *B. cereus* (84). Samples should be subjected to direct plating techniques as well as enrichments. Biochemical testing and morphological examination should be performed on presumptive colonies, followed by H-serotyping, biotyping, phage typing, and toxin assays.

C. Toxins and Toxin Assays

The production, assay, and/or pathological effects of the various toxins produced by *B. cereus* have been reviewed by Kramer et al. (83), Gilbert and Kramer (85), Turnbull (86), Kramer and Gilbert (17), Shinagawa (84), and Jackson (87). Toxins produced include diarrheal and emetic enterotoxins, hemolysins, phospholipase C, and lethal toxins (mouse assay). The enterotoxins (both emetic and diarrheal) appear to be unrelated to hemolysins or phospholipase C.

Spira and Goepfert proposed that diarrhea induced by *B. cereus* was due to intoxication, rather than infection, based on ligated rabbit ileal loop assays (88). Fluid accumulation in ileal loops correlated with introduction of either cell-free supernatants, large inoculum levels of vegetative cells (109), or 2- to 3-fold increases of bacterial levels within the loops. Washed cells resuspended in fresh medium did not induce fluid accumulation, indicating that the reaction occurred only in the presence of enterotoxin.

Hostacka et al. compared culture filtrates of *B. cereus* isolated from ice cream, milk products, bakery products, meats, tea, dye, and cola for

toxic activity (89). Results from 89 culture filtrates revealed that none were positive for the suckling mouse assay, 3 were positive with the rabbit ileal loop assay, 71 with VPR, 59 with Vero cell assay, 31 with mouse lethality, and 55 were positive for lecithinase. All were hemolytic on sheep agar and 81 out of the 89 were hemolytic against 0.5% rabbit erythrocytes. Size of the VPR blueing appeared to relate to other toxic properties. VPR categories 4, 5, 6, and 7 correlated with high percentages (69%–100%) of positive reactions with mouse lethality, hemolysis of rabbit erythrocytes, and lecithinase activity. These VPR categories all correlated with monolayer destruction of Vero cells. No relationship was observed between VPR and the rabbit ileal loop assay.

Shinagawa et al. examined culture filtrates of *B. cereus* strains isolated from Japanese food poisoning outbreaks (19). They found that culture supernatant (CS) fluid from most of the diarrheal toxin strains gave strong VPR, led to accumulation of fluid in both rabbit and mouse ligated ileal loops, and killed mice when injected intravenously. Most of the diarrheal strains (7 out of 9) were able to hydrolyze starch. On the other hand, Shinagawa et al. found that 50 out of 50 emetic strains were starch-hydrolysis negative, CS fluids from only 6 of the 50 strains had moderate VPR, and none of 42 strains led to accumulation of fluid in mouse ileal loops. However, culture filtrates from 3 of 21 emetic strains did lead to accumulation of fluid in rabbit ileal loops, suggesting that the rabbit ileal loop test is more sensitive than the mouse ileal loop test. The emetic strains that were positive in the VPR and led to fluid accumulation in rabbit ileal loops may be able to produce both emetic and diarrheal toxins. Most of the emetic strains (34 of 42) produced the mouse lethality factor. Mouse lethality does not appear to be specific to only one toxin type.

Recently, Shinagawa et al. have described an improved technique for the purification of the diarrheal toxin (90). Enterotoxin was isolated from culture filtrates by ammonium sulfate fractionation, followed by dialysis, and then concentration by ultrafiltration. Chromatofocusing followed by reversed passive latex agglutination revealed that the enterotoxin eluted at pI 5.0–5.7. Hemolysin activity of fractions was distinct from enterotoxin with elution at pI 4.1–4.3. They were able to get approximately 2.1 mg of toxin from 6000 ml of culture fluid with almost 1000-fold purification. The purified preparation was homogenous electrophoretically with molecular weight (MW) of 45,000. The material was positive in VPR, mouse lethality, mouse ileal loop, and cytotoxicity (Vero cells) tests; treatment of the protein with antitoxin antibody led to loss of activity in the various tests. Toxin was sensitive to trypsin and pepsin, stable at pH 6 to pH 9 but unstable at pH less than 3 or greater than 11, stable to heating at 45°C for 10 min but not at 50°C for 5 min, and was stable for 60 days at –20°C but not for 30

days at 4°C. Thus, the diarrheal toxin appears to be a single heat-labile protein. Since a good animal model is not available, the authors did not test the protein to determine if it actually caused diarrhea, but the positive ileal loop test indicated that it would do so.

Shinagawa et al. used monoclonal antibodies against purified enterotoxin for separation by immunoaffinity chromatography (91). The authors were able to separate a single protein (45,000 MW) of electrophoretic and antigenic homogeneity. The protein was active in VPR, mouse lethality, and mouse ligated ileal loop assays; there were no lecithinase or hemolytic activities. The authors thus suggested that the isolated protein was associated with the biological activities of *B. cereus* enterotoxin. They caution that confirmation of biological activity and antigenic homogeneity of enterotoxins from a variety of *B. cereus* strains should be performed utilizing their isolation procedure.

Ting and Banwart compared the rat ligated intestinal loop assay with that of the rabbit for the titration of the diarrheal toxin (92). According to their study, the rat test is not as sensitive as the rabbit test and it was necessary to concentrate culture filtrates of *B. cereus* at least 20-fold (dialysis against carbowax) to obtain the sensitivity of unconcentrated culture fluids used in the rabbit ileal test.

Jackson developed a fluorescent immunodot assay using antiserum raised in rabbits against either purified *B. cereus* diarrheal enterotoxin (PE) or culture supernatant (CS). The assay using PE had a sensitivity of 50 ng and was quite specific to *B. cereus* culture filtrates. PE did not react with CS fluids from other Group 1 bacilli (*B. coagulans*, *B. licheniformis*, *B. firmus*, *B. megaterium*, *B. pumilis*, *B. subtilis*) but antibody raised against *B. cereus* CS fluid containing toxin did react with CS fluids from 2 out of 7 *B. coagulans*, 2 out of 2 *B. licheniformis* and 1 out of 6 *B. subtilis* strains. When the CS fluids from 25 *B. cereus* strains isolated from food poisoning outbreaks were tested by the fluorescent immunodot assay, 18 of 25 gave a greater than three-plus to four-plus (strong) reaction, 5 of 25 gave a greater than two-plus to three-plus (intermediate) reaction, and 2 of 25 gave a zero to less than two-plus (negative/weak) reaction. CS fluid from strains isolated during routine surveillance of foods also were tested via immunodot: 11 of 25 gave a strong reaction, 9 of 25 were intermediate reactors, and 5 of 25 gave a negative/weak reaction. The fluorescent immunodot diarrheal toxin assay as described by Jackson appears to be a simple and rapid assay that can be used for routine determination of the diarrheal toxin produced by *B. cereus*.

Griffiths (61) and van Netten et al. (59) used the Oxoid BCET-RPLA kit to measure the diarrheal toxin. The BCET-RPLA kit is supposed to detect the presence of *B. cereus* enterotoxin (diarrheal) by the use of re-

versed passive latex agglutination. Polystyrene latex particles are sensitized with purified rabbit antitoxin. Samples of food or CS fluids containing the diarrheal toxin will agglutinate the sensitized latex particles, forming a lattice structure. However, van Netten et al stated that the specificity of the Oxoid test for the diarrheal toxin has not been substantiated (59). Using the BCET-RPLA kit, Griffiths found that 33 of 38 strains of *B. cereus* were positive (produced greater than or equal to 2 ng/ml diarrheic toxin) (61); 17 of 17 strains of *B. mycoides*, 6 of 9 strains of *B. circulans*, 4 of 4 strains of *B. cereus* var. *mycoides*, 3 of 3 strains of *B. thuringiensis*, 3 of 4 strains of *B. lentus*, 1 of 2 strains of *B. pumilis*, 1 of 2 strains of *B. polymyxa*, and 2 of 2 strains of *B. carotarum* also were positive in the BCET-RPLA test. In comparison, Jackson, using a fluorescent immunodot assay dependent on purified diarrheal toxin, got no cross-reaction with 7 strains of *B. coagulans*, 1 of *B. firmus*, 2 of *B. licheniformis*, 2 of *B. megaterium*, 3 of *B. pumilis*, or 6 strains of *B. subtilis* (93). Conversely, when Jackson used the immunodot assay developed with antibody raised against CS fluid from a diarrheal toxin-producing *B. cereus* strain, some strains of *B. coagulans*, *B. licheniformis*, and *B. subtilis* gave positive results. Buchanan and Schultz found that the Oxoid BCET-RPLA kit gave positive results for heat-denatured culture filtrates, which were negative for cytotoxicity in tissue culture assays (94). Jackson's results with antisera from purified toxin (93) and positive results obtained with heat-denatured samples (94) suggest that the antigen used in BCET-RPLA test of Oxoid lacks the necessary purity and/or specificity.

The mechanism by which the diarrheal toxin produces diarrhea, that is, leads to fluid accumulation in ligated ileal loops, is unknown. Turnbull reviewed studies on the role of cyclic AMP (adenosine monophosphate) (c-AMP) in the mode of action of the diarrheal toxin (86). It is not possible to determine the role played by c-AMP from the studies described. With the advent of better purification and assay methods for the diarrheal toxin, studies on the mode of action of the toxin should be more feasible and provide needed answers.

The emetic toxin generally is assayed by feeding test materials to Rhesus monkeys. The assay has limited usefulness since very few laboratories have primate facilities and the assay does not lend itself to the routine analysis necessary during toxin isolation and purification studies (83). Hughes et al. tested culture filtrates of *B. cereus* strains associated with emetic-type outbreaks in HEp-2 (larynx carcinoma) tissue cultures. They found that 13 of 15 strains produced vacuoles in the tissue culture cells. Culture filtrates heated at 100°C for 10 min produced vacuolation (the emetic toxin is reportedly heat stable and this heat treatment eliminated cytotoxic or cytotoxic effects); the activity was stable to heating at 126°C

for 70 min. Membrane filtration studies indicated that the toxin had a MW of less than 15,000. The results obtained by Hughes et al. indicated that the vacuolation activity of the culture filtrates may be specific to emetic types of *B. cereus*. All of the emetic-syndrome serotype H-1 isolates tested produced vacuolation, whereas serotype H-1 strains from diarrheal and other sources did not (except one diarrheal isolate from cooked pork). Interestingly, they demonstrated that two enterotoxigenic strains of *S. aureus* did not induce vacuoles in HEp-2 cells even though the enterotoxins of *S. aureus* have emetic properties.

Szabo et al. used Int 407 (embryonic intestine), CHO, HEp-2, Vero, HeLa, Y-1 adrenal, and MA-104 (rhesus embryonic monkey kidney) cell lines to assay emetic toxin from *B. cereus* (96). Vacuolation was apparent in both Int 407 and HEp-2 cells at a titer of 512. Vero, HeLa, and MA-104 were vacuolated to lesser degrees with titers of 64, 256, and 32, respectively. No vacuolation was evident in the CHO or Y-1 cells, but there was a rounding and granulation effect at titers of 512 and 32, respectively. The highest titers of vacuolated Int 407 and HEp-2 cells were obtained when the strains were cultured in milk rather than when cultured in rice and BHI. Partial purification of the toxin indicated that the molecular weight was approximately 14,000 and had a pI of 5.9. Szabo et al. indicated that the toxin they had obtained may be actually an aggregate rather than a single molecule. Jackson used McCoy cell monolayers and found that culture filtrates from *B. cereus* emetic strains progressively destroyed the cell monolayer (87). Jackson analyzed the vomitus and stool specimens from an individual apparently suffering from *B. cereus* emetic food poisoning and found that extracts from vomitus and stools effectively destroyed all the cells in the monolayers. The monolayer destruction activity of the extracts was resistant to autoclaving and to trypsin treatment. The mechanism by which the emetic toxin induces vomiting is unknown.

D. Hemolysins

There are two hemolysins produced by *B. cereus*: a thermolabile, thiol-activated cytolysin called *cereolysin*, and a thermostable secondary hemolysin (86). Gilmore et al. have suggested that the secondary hemolysin is a two-component system consisting of a phospholipase C and a sphingomyelinase that they call *cereolysin AB* (97). They suggested that the heat-labile hemolysin be named *cereolysin O*.

Hemolysin BL from *B. cereus* has been characterized by Beecher and Macmillan (98,99). The hemolysin can be separated into a binding component (B) and lytic components (L₁ and L₂). All three proteins are necessary for hemolysis. The multicomponent system produced edema and blueing in

the VPR test. Since VPR is known to correlate with the activity of the diarrheal toxin, the authors feel that hemolysin BL is identical to the enterotoxin. Unfortunately, Beecher and Macmillan did not have enough material to do an ileal-loop assay, which is indicative of diarrhea (99). Hemolysin BL appears to be distinct from cereolysin O and cereolysin AB. Turnbull has indicated that the diarrheal toxin is not hemolytic (86) and Shinagawa et al. demonstrated that their purified diarrheal toxin lacked hemolytic activity (90,91). Whether or not the diarrheal toxin possesses hemolytic activity obviously needs more study.

Honda et al. have shown that both *B. thuringiensis* and *B. cereus* produce biologically, immunologically, and physicochemically identical thiol-activated hemolysins (cereolysin), which suggests that the two organisms may be related closely (100). Since *B. thuringiensis* is used as a biological agricultural insecticide and produces a hemolysin identical to that of *B. cereus*, which is a known pathogen and cause of food poisoning, the use of the *B. thuringiensis* as a living insecticide should be examined more closely for safety.

E. Differentiation of *B. cereus* from Other *Bacillus* Species

Similarities between species of *Bacillus* exist as exemplified by the identical hemolysins of *B. cereus* and *B. thuringiensis* (100). In the past, species differentiation has been accomplished through morphological examination and biochemical properties. Gordon et al. updated the classification scheme delineated by Smith et al. (102) with examination of properties of most of the known strains of *Bacillus* (101). New methods are available for examination of relationships between *Bacillus* species based on molecular differences.

By comparative analysis of small-subunit ribosomal RNA sequences, Ash et al. were able to deduce the phylogenetic structure of the genus *Bacillus* (103). Five very heterogeneous groups were mapped based on homology of sequences and evolutionary distance values; these groups may be considered distinct enough to be separate genera. *B. anthracis*, *B. cereus*, *B. medusa*, *B. mycoides*, and *B. thuringiensis* formed a distinct clade (a cluster of closely related species) when the technique was used.

Turnbull et al. examined avirulent strains of *B. anthracis* using DNA probes, polymerase chain reaction (PCR), and anthrax toxin detection techniques. *B. anthracis* strains that have lost the ability to form either toxin or capsules are indistinguishable phenotypically from *B. cereus*. Good correlation was found between the presence of virulence factors and DNA hybridization and PCR, however, difficulty in identification arose when both virulence factors were lost (104). Research should be done to develop markers for identification of avirulent *B. anthracis* strains in order to differentiate them from *B. cereus*. Lawrence et al. were able to differentiate *B.*

anthracis from *B. cereus* by using gas chromatographic whole-cell fatty acid analysis (105).

Zahner et al. made a comparative study of enzyme variation in *B. cereus* and *B. thuringiensis* (106). They concluded that *B. cereus* and *B. thuringiensis* should be considered as one species with certain strains able to produce insecticidal parasporal bodies. There are 23 types of flagellar antigens in *B. cereus*; Mikami et al. found a monoclonal flagellar antibody that reacted with all 23 types, thereby finding a common flagellar antigen for *B. cereus* strains (107). The monoclonal antibody for the common flagella antigen should prove useful in separating the organism from closely related species of *Bacillus*. Since Ash et al. have shown the close relationship among *B. anthracis*, *B. cereus*, *B. medusa*, *B. mycoides*, and *B. thuringiensis* strains (103), it appears that more study is needed in defining and differentiating these species.

F. Genetic Studies

Battisti et al. developed a mating system that promoted plasmid transfer among strains of *B. anthracis*, *B. cereus*, and *B. thuringiensis* (108). They were able to transfer the ability to produce parasporal crystals from *B. thuringiensis* to *B. cereus* and *B. anthracis* by plasmid transfer. Cell-cell contact was required for a period of 2–4 h during exponential growth for effective plasmid transfer. A low frequency of chromosomal gene transfer from *B. thuringiensis* to *B. cereus* during cell-mating experiments was demonstrated by Aronson and Beckman (109). DNA isolated from plasmids transformed *B. cereus* vegetative cells by the use of either electroporation (110) or cellophane membranes (111). It would be interesting to determine if the genes for the diarrheal and/or emetic toxins can be transferred from *B. cereus* to other *Bacillus* species.

A physical map of the *B. cereus* chromosome has been described by Kolsto et al. (112). The map was constructed by aligning DNA fragments produced by the use of the restriction enzyme, NotI. The chromosome of *B. cereus* consisted of 11 NotI fragments ranging in size from 200 to 1300 kilobases (kb) to give a genome of 5.7 megabases (Mb). Thus, having a physical map of the *B. cereus* chromosome should facilitate the construction of a genetic map.

VII. OTHER BACILLUS SPECIES INVOLVED IN FOOD POISONING

Bacillus species other than *B. cereus* have been isolated from a variety of foods. Of Nigerian spices, 60% were found to contain *B. subtilis* among other species of *Bacillus* (31). Skim-milk powder contained *B. subtilis*, *B.*

polymyxa, and *B. coagulans* (33); psychrotrophic *B. circulans*, *B. mycoides*, and other *Bacillus* species were isolated from raw and pasteurized milk samples (61). Bacilli other than *B. cereus* occasionally are isolated from food samples suspected to be involved in outbreaks of food poisoning but it is not always easy to determine if they were the responsible agent. The finding of *Bacillus* species in foods may not necessarily mean that they can cause illness, but there appear to be documented outbreaks of food poisoning due to *B. licheniformis*, *B. subtilis*, *B. pumilus*, and *B. brevis* (17,83,84,113). Both diarrhea and vomiting were present in these outbreaks but the nature of the toxins was not determined.

VIII. NONGASTROINTESTINAL INFECTIONS CAUSED BY *BACILLUS CEREUS*

It has been recognized that *B. cereus* can be involved in clinical diseases other than those affecting the gastrointestinal tract. This area has been reviewed by Turnbull et al. (114), Siegman-Igra et al. (115), Logan (116), and Kramer and Gilbert (17). While there is no attempt to be inclusive, a number of recent clinical reports discussing the nongastrointestinal diseases induced by *B. cereus* are presented below. *B. cereus* may prove to be the major organism responsible for disease symptoms in both humans and animals and the finding of the organism on culture plates should not be dismissed idly as an unimportant contaminant.

Posttraumatic endophthalmitis (eye infections) induced by *B. cereus* infection following penetrating trauma to the eye is marked by rapid eye involvement and destructiveness of eye tissue with loss of sight or loss of the eye. Schemmer and Driebe (117), Davey and Tauber (118), and Beer et al. (119) reported cases of *B. cereus* infection following penetration of the eye and the surgical and antibiotic treatments used to save the vision of the patients. In addition, Davey and Tauber presented a review of posttraumatic endophthalmitis and the role of *B. cereus* infection (118). In individuals wearing soft contact lenses, keratitis has been associated with *B. cereus* or *B. subtilis* contamination of contact lens cleansing fluids (120).

Primary cutaneous *B. cereus* infections have been demonstrated in neutropenic patients (121,122). The patients developed vesicular lesions on the extremities and cellulitis was present. *B. cereus* appeared to be a common organism in wound infections of normal people working under tropical conditions (123). Guiot et al. present an interesting and ultimately fatal case of a granulocytopenic patient who fell and suffered a minor wound on his arm that became infected with *B. cereus* (124). *B. cereus* has been isolated from postoperative and posttraumatic orthopedic wounds (125) and the organism was implicated in a case of gangrene (126).

Necrotizing pneumonia and empyema can result from pleuropulmonary infections by *B. cereus* that may end in the death of the patient (127, 128). Colpin et al. described a case of *B. cereus* meningitis in a patient under gnotobiotic (partial sterilization of the gut with antibiotics) care. *B. cereus* colonized the throat of the patient and, despite antibiotic treatment, the infection spread to the meninges, resulting in the patient's death.

Weber et al. have concluded that the incidence of *B. cereus* bacteremia is increasing since there appears to be an increase in the number of isolations of the organism from blood cultures (130). Risk factors identified for *Bacillus* bacteremia include drug addiction, hemodialysis, and cancer (131). *B. cereus* bacteremia was present in cancer patients who had a Hickman catheter in place (132); in such patients, antibiotic therapy was insufficient and it was necessary to remove the catheter. Electron microscopy revealed biofilms of *Bacillus* encasing the internal luminal surface of the catheters and the bacteria in the biofilms are probably less susceptible to antibiotic treatment.

Endocarditis involving a surgically implanted porcine aortic prosthetic valve was caused by *B. cereus* infection (133). Steen and coworkers reported a case of *B. cereus* endocarditis involving six intravenous drug users, and one patient each with a pacemaker, a mechanical heart valve, a porcine aortic valve, and rheumatic heart disease. The intravenous drug users responded to antibiotic treatment; however, two of the patients with valvular heart disease died and the others required valve replacements.

B. cereus mastitis has been demonstrated in both cattle and goats (135) and has caused abortion in cattle (136). It is probable that *B. cereus* can cause such other types of infection in animals as respiratory tract, central nervous system, and wound infections.

IX. DIAGNOSIS AND TREATMENT OF BACILLUS CEREUS FOOD POISONING

Guerrant and Bobak have reviewed infectious gastroenteritis as related to epidemiological settings, diagnosis, clinical identification, and treatments (137). Symptoms of *B. cereus* food poisonings as described above generally resolve within 24–48 h and the illness even may go unreported. Therapy for symptoms and oral rehydration using electrolytes and glucose should be sufficient for otherwise healthy individuals. Diagnosis and treatment of immunocompromised patients could be more difficult due to weakened conditions and predisposition to infection. Vomitus and stool specimens should be submitted routinely for clinical analysis in which protocols for isolation and identification should be followed (17,83,84,137). Guerrant and Bobak emphasize the need to prevent enteric infections, especially in

such institutional settings as hospitals, extended-care facilities, and day-care facilities.

X. CONTROL

The ubiquitousness and environmental stability of *B. cereus* spores and its easy isolation from raw agricultural materials and the food-processing environment demands that the food processor assume that raw materials and processing equipment contain the organism; the food processor also must assume that it will be difficult to manufacture a food product free from *B. cereus*. Similarly, food transportation and distribution organizations, food-service organizations, and the home food preparer must assume that processed foods and the environment contain *B. cereus* spores. They must take steps to control the organisms by preventing their growth through elimination of temperature abuse and preventing contamination or recontamination of prepared foods.

The logical approach to *B. cereus* control in foods is through the use of the hazard analysis critical control point (HACCP) system by all who are involved in the manufacture, distribution, and serving of food. The food processor must consider each step involved in the manufacture of a food product. Critical elements in HACCP evaluation would involve knowledge of the producer of raw materials as well as the microbiological quality of those raw materials. Plant design and layout, equipment design, process control, employee practices, cleansing and sanitizing procedures, and verification of *B. cereus* control must be included in the HACCP evaluation. Examination may indicate that the food-processing plant and the equipment used for food processing may have to be redesigned to ensure that safe products are being manufactured. Sanitation procedures and the types of sanitizer used may have to be changed also. Organizations that distribute foods must ensure integrity of food products under their control. Products must be handled in such a way that the recommended storage temperatures are maintained and that contamination and recontamination are prevented by maintenance of package integrity. Food-service establishments also should have HACCP procedures in place. Beard even has suggested an HACCP approach that consumers should use to assure the safety of their prepared foods (138).

An integral part of preventing *B. cereus* food poisoning is education. Employees in food-processing, food-distribution, and food-service organizations should be exposed to continuing training explaining the dangers associated with temperature abuse of foods containing *B. cereus*. The consumers, too, must be made aware of the potential hazards involved in handling foods and they must receive the knowledge necessary to protect

them from experiencing the unpleasantness of a *B. cereus*-induced food poisoning attack.

REFERENCES

1. Norris, J. R., Berkeley, R.C.W., Logan, N. A., and O'Donnell, A. G. The genera *Bacillus* and *Sporolactobacillus*. In *The prokaryotes*, Vol. 2, edited by M. P. Starro, H. Stolp, H. G. Truper, A. Balows, and H. G. Schlegel, Springer-Verlag, New York, 1981, pp. 1711-1742.
2. Bean, N. H., and Griffin, P. M., *J. Food Prot.*, 53:804-817 (1990).
3. Todd, E.C.D., *J. Food Prot.*, 46:650-657 (1983).
4. Todd, E.C.D., *J. Food Prot.*, 48:990-996 (1985).
5. Todd, E.C.D., *J. Food Prot.*, 48:1071-1078 (1985).
6. Todd, E.C.D., *J. Food Prot.*, 50:420-428 (1987).
7. Todd, E.C.D., *J. Food Prot.*, 50:982-991 (1987).
8. Todd, E.C.D., *J. Food Prot.*, 51:56-65 (1988).
9. Todd, E.C.D., *J. Food Prot.*, 52:436-442 (1989).
10. Todd, E.C.D., *J. Food Prot.*, 52:503-511 (1989).
11. Todd, E.C.D., *J. Food Prot.*, 52:595-601 (1989).
12. Todd, E.C.D., *J. Food Prot.*, 52:586-594 (1989).
13. Turnbull, P.C.B., and Kramer, J. M. *Bacillus*. In *Manual of clinical microbiology*, 5th ed., edited by A. Balows, American Society for Microbiology, Washington, DC, 1991, pp. 296-303.
14. Goepfert, J. M., Spira, W. M., and Kim, H. U., *J. Milk Food Technol.*, 35:213-227 (1972).
15. Gilbert, R. J., *Bacillus cereus* gastroenteritis. In *Food-borne infections and intoxications*, 2d ed., edited by H. Rieman and F. L. Bryan, Academic Press, New York, 1979, pp. 495-518.
16. Johnson, K. M., *J. Food Prot.*, 47:145-153 (1984).
17. Kramer, J. M., and Gilbert, R. J. *Bacillus cereus* and other *Bacillus* species. In *Foodborne bacterial pathogens*, edited by M. P. Doyle, Marcel Dekker, New York, 1989, pp. 22-70.
18. Davies, F. L., and Wilkinson, G. *Bacillus cereus* in milk and dairy products. In *The microbiological safety of food*, edited by B. C. Hobbs and J.H.B. Christina, Academic Press, New York, 1973, pp. 57-67.
19. Shinagawa, K., Matsusaka, N., Konuma, H., and Kurata, H., *Jpn. J. Vet. Sci.*, 47:557-565 (1985).
20. DeBuono, B. A., Brondum, J., Kramer, J. M., Gilbert, R. J., and Opal, S. M., *J. Clin. Microbiol.*, 26:1571-1574 (1988).
21. Auld, M., and Russell, F. E., *Commun. Dis. Scotland Weekly Rept.*, 22(24):5 (1988).
22. Centers for Disease Control, *Morbidity and Mortality Weekly Rept.*, 35(25):408-410 (1986).
23. Moreno, A. J., Orr, C. D., Morales, E., and Weiman, I. M., *Ann. Internal Med.*, 102:868-869 (1985).
24. Public Health Laboratory Service, Communicable Disease Report, No. 84/

21. In *1984 Annual Report of Food Research Institute*, University of Wisconsin, Madison, 1984, p. 236.
25. Singh, R. S., Batish, V. K., Parkash, O., and Ranganathan, B., *J. Dairy Sci.*, 67:513-517 (1984).
26. Holmes, J. R., Plunkett, T., Pate, P., Roper, W. L., and Alexander, W. J., *Arch. Internal Med.*, 141:766-767 (1981).
27. Giannella, R. A., and Brasile, L., *J. Infect. Dis.*, 139:366-370 (1979).
28. Kamat, A. S., Nerkar, D. P., and Nair, P. M., *J. Food Safety*, 10:31-41 (1989).
29. Bhat, R., Geeta, H., and Kulkarni, P. R., *J. Food Prot.*, 50:418-419, 433 (1987).
30. Bachhil, V. N., and Jaiswal, T. N., *J. Food Sci. Technol.*, 25:371-372 (1988).
31. Antai, S. P., *Internat. J. Food Microbiol.*, 6:259-261 (1988).
32. Pafumi, J., *J. Food Prot.*, 49:958-963 (1986).
33. Suzuki, A., Kawanishi, T., Takayama, S., Haruta, M., Shimizu, Y., Ogiwara, H., and Jinbo, K., *J. Food Hygienic Soc. Japan*, 25:106-111 (1984).
34. Wong, H.-C., Chang, M.-H., and Fan, J.-Y., *Appl. Environ. Microbiol.*, 54:699-702 (1988).
35. Chung, K.-T., and Sun, H.-L., *J. Food Sci.*, 51:1208-1212 (1986).
36. Arispe, I., and Westhoff, D., *J. Food Prot.*, 47:27-35 (1984).
37. Mosso, A., Garcia-Arribas, L., Cuenca, J. A., and de la Rosa, C., *J. Food Prot.*, 52:184-188 (1989).
38. Sooltan, J.R.A., Mead, G. C., and Norris, A. P., *J. Food Microbiol.*, 4: 347-351 (1987).
39. Ternstrom, A., and Molin, G., *J. Food Prot.*, 50:141-146 (1987).
40. Konuma, H., Shinagawa, K., Tokumaru, M., Onoue, Y., Konno, S., Fujino, N., Shigehisa, T., Kurata, H., Kuwabara, Y., and Lopes, C.A.M., *J. Food Prot.*, 51:324-326 (1988).
41. Samson, R. A., van Kooij, J. A., and de Boer, E., *J. Food Prot.*, 50:92-94 (1987).
42. Harmon, S. M., Kautter, D. A., and Solomon, H. M., *J. Food Prot.*, 50: 62-65 (1987).
43. Harmon, S. M., and Kautter, D. A., *J. Food Prot.*, 54:372-374 (1991).
44. Wood, S. L., and Waites, W. M., *Food Microbiol.*, 5:103-107 (1988).
45. Ellison, A., Dodd, C.E.R., and Waites, W. M., *Food Microbiol.*, 6:93-98 (1989).
46. Public Health Laboratory Service, *Communicable Disease Report*, No. 87/ 15, Public Health Laboratory Service, London, 1987, p. 5.
47. Wong, H.-C., Chen, Y.-L., and Chen, C.L.F., *J. Food Prot.*, 51:707-710 (1988).
48. Wong, H.-C., and Chen, Y.-L., *Appl. Environ. Microbiol.*, 54:2179-2184 (1988).
49. Christiansson, A., Naidu, A. S., Nilsson, I., Wadstrom, T., and Pettersson, H.-E., *Appl. Environ. Microbiol.*, 55:2595-2600 (1989).
50. Sahsamanoglou, M., Karaioannoglou, P., Papageorgiou, D., and Koidis, P., *Milchwissenschaft*, 43:640-642 (1988).

51. Nielsen, H.-J. S., and Zeuthen, P., *J. Food Prot.*, 48:28-34 (1985).
52. Asplund, K., Nurmi, E., Hill, P., and Hirn, J., *Internat. J. Food Microbiol.*, 7:349-352 (1988).
53. Kaur, P., *J. Appl. Bacteriol.*, 60:513-516 (1986).
54. Rizk, I.R.S., and Ebeid, H. M., *Die Nahrung*, 33(9):839-844 (1989).
55. Cook, P. P., Themba, M. M.-L., and Campbell-Platt, G., *Lett. Appl. Microbiol.*, 13:78-81 (1991).
56. Nout, M.J.R., Beernink, R. G., and Bonants-van Laarhoven, R.M.G., *Internat. J. Food Microbiol.*, 4:293-301 (1987).
57. Garcia-Arribas, M., and Kramer, J. M., *Internat. J. Food Microbiol.*, 11: 21-34 (1990).
58. Bergann, T., *Mh. Vet.-Med.*, 44:23-25 (1989).
59. van Netten, P., van de Moosdijk, A., van Hoensel, P., Mossel, D.A.A., and Perales, I., *J. Appl. Bacteriol.*, 69:73-79 (1990).
60. Griffiths, M. W., *J. Food Prot.*, 53:790-792 (1990).
61. Griffiths, M. W., and Phillips, J. D., *J. Soc. Dairy Technol.*, 43:62-66 (1990).
62. Bassen, M. K., Gupta, L. K., Jolly, L., and Tewari, R. P., *MIRCEN J.*, 5: 511-516 (1989).
63. Parry, J. M., and Gilbert, R. J., *J. Hyg. (Camb.)*, 84:77-82 (1980).
64. Johnson, K. M., Nelson, C. L., and Busta, F. F., *J. Food Sci.*, 47:1268-1271 (1982).
65. Pendurkar, S. H., and Kulkarni, P. R., *Die Nahrung*, 34(2):177-180 (1989).
66. Spite, G. T., *J. Food Prot.*, 47:458-462 (1984).
67. Ishizaki, K., Shinriki, N., and Matsuyama, H., *J. Appl. Bacteriol.*, 60:67-72 (1986).
68. Enfors, S.-O., and Molin, G., *Can. J. Microbiol.*, 27:15-19 (1981).
69. Valsanen, O. M., Mwaisumo, N. J., and Salkinoja-Salonen, M. S., *J. Appl. Bacteriol.*, 70:315-324 (1991).
70. Foegeding, P. M., Hemstapat, V., and Giesbrecht, F. G., *J. Food Sci.*, 51: 197-201 (1986).
71. Blocher, J. C., and Busta, F. F., *Appl. Environ. Microbiol.*, 50:274-279 (1985).
72. Bulgarelli, M. A., and Shelef, L. A., *J. Food Sci.*, 50:661-664 (1985).
73. Shelef, L. A., Jyothi, E. K., and Bulgarelli, M. A., *J. Food Sci.*, 49:737-740, 809 (1984).
74. Troller, J. A., *J. Food Prot.*, 49:656-670 (1986).
75. Peters, A. C., Thomas, L., and Wimpenny, J. W. T., *FEMS Microbiol. Lett.*, 77:309-314 (1991).
76. Buchman, G. W., and Hansen, J. N., *Appl. Environ. Microbiol.*, 53:79-82 (1987).
77. Kalita, D. K., and Singh, R. P., *J. Basic Microbiol.*, 7:355-359 (1987).
78. Rhodehamel, E. J., and Pierson, M. D., *Internat. J. Food Microbiol.*, 11: 167-178 (1990).
79. Harmon, S. M., Kautter, D. A., and McClure, F. D., *J. Food Prot.*, 47:65-67 (1984).

80. Kim, H. U., and Goepfert, J. M., *Appl. Microbiol.*, 22:581-587 (1971).
81. Peterz, M., Wiberg, C., and Norberg, P., *J. Food Prot.*, 48:969-970 (1985).
82. Logan, N. A., and Berkeley, R.C.W., *J. Gen. Microbiol.*, 130:1871-1882 (1984).
83. Kramer, J. M., Turnbull, P.C.B., Munshi, G., and Gilbert, R. J. Identification and characterization of *Bacillus cereus* and other *Bacillus* species associated with foods and food poisoning. In *Isolation and identification methods for food poisoning organisms*, edited by J.E.L. Corry, D. Roberts, and F. A. Skinner, Society of Applied Bacteriology, Technical Series 17, Academic Press, London, 1982, pp. 261-286.
84. Shinagawa, K., *Internat. J. Food Microbiol.*, 10:125-142 (1990).
85. Gilbert, R. J., and Kramer, J. M., *Biochem. Soc. Transactions*, 12:198-200 (1984).
86. Turnbull, P.C.B. *Bacillus cereus* toxins. In *Pharmacology of bacterial toxins*, edited by F. Dorner and J. Drews, Pergamon Press, New York, 1986, pp. 397-448.
87. Jackson, S. G., *J. Assoc. Off. Anal. Chem.*, 74:704-706 (1991).
88. Spira, W. M., and Goepfert, J. M., *Appl. Microbiol.*, 24:341-348 (1972).
89. Hostacka, A., Kosiarova, A., Majtan, V., and Kohutova, S., *Zbl. Bakt.*, 276:303-312 (1992).
90. Shinagawa, K., Sugiyama, J., Terada, T., Matsusaka, N., and Sugii, S., *FEMS Microbiol. Lett.*, 80:1-6 (1991).
91. Shinagawa, K., Takechi, T., Matsusaka, N., and Sugii, S., *Can. J. Microbiol.*, 38:153-156 (1992).
92. Ting, W.-T., and Banwart, G. J., *J. Food Safety*, 7:57-63 (1985).
93. Jackson, S. G., *J. Immunol. Methods*, 120:215-220 (1989).
94. Buchanan, R. L., and Schultz, F. J., *J. Food Prot.*, 55:440-443 (1992).
95. Hughes, S., Bartholomew, B., Hardy, J. C., and Kramer, J. M., *FEMS Microbiol. Lett.*, 52:7-12 (1988).
96. Szabo, R. A., Speirs, J. I., and Akhtar, M., *J. Food Prot.*, 54:272-276 (1991).
97. Gilmore, M. S., Cruz-Rodz, A. L., Leimeister-Wachter, M., Kreft, J., and Goebel, W., *J. Bacteriol.*, 171:744-753 (1989).
98. Beecher, D. J., and Macmillan, J. D., *Infect. Immun.*, 58:2220-2227 (1990).
99. Beecher, D. J., and Macmillan, J. D., *Infect. Immun.*, 59:1778-1784 (1991).
100. Honda, T., Shiba, A., Seo, S., Yamamoto, J., Matsuyama, J., and Miwatani, T., *FEMS Microbiol. Lett.*, 79:205-210 (1991).
101. Gordon, R. E., Haynes, W. C., and Hor-Nay Pang, C., *The genus Bacillus*, Agriculture Handbook No. 427, Agricultural Research Service, USDA, Washington, DC, 1973.
102. Smith, N. R., Gordon, R. E., and Clark, F. E., *Aerobic sporeforming bacteria*, U.S. Department of Agriculture Monograph 16, USDA, Washington, DC, 1952.
103. Ash, C., Farrow, J.A.E., Wallbank, S., and Collins, M. D., *Lett. Appl. Microbiol.*, 13:202-206 1991.
104. Turnbull, P.C.B., Hutson, R. A., Ward, M. J., Jones, M. N., Quinn, C. P., Finnie, N. J., Duggleby, C. J., Kramer, J. M., and Melling, J., *J. Appl. Bacteriol.*, 72:21-28 (1992).

105. Lawrence, D., Heitefuss, S., and Seifert, H.S.H., *J. Clin. Microbiol.*, 29: 1508-1512 (1991).
106. Zahner, V., Momen, H., Salles, C. A., and Rabinovitch, R., *J. Appl. Bacteriol.*, 67:275-282 (1989).
107. Mikami, T., Hiraoka, K., Murakami, T., Boon-Long, J., Matsumoto, T., and Suzuki, M., *Microbiol. Immunol.*, 34:709-714 (1990).
108. Battisti, L., Green, B. D., and Thorne, C. B., *J. Bacteriol.*, 162:543-550 (1985).
109. Aronson, A. I., and Beckman, W., *Appl. Environ. Microbiol.*, 53:1525-1530 (1987).
110. Belliveau, B. H., and Trevors, J. T., *Appl. Environ. Microbiol.*, 55:1649-1652 (1989).
111. Sabelnikov, A. G., and Ulyashova, L. V., *FEMS Microbiol. Lett.*, 72:123-126 (1990).
112. Kolsto, A.-B., Gronstad, A., and Oppegaard, H., *J. Bacteriol.*, 172:3821-3825 (1990).
113. Lund, B. M., *Lancet*, 336:982-986 (1990).
114. Turnbull, P.C.B., Jorgensen, K., Kramer, J. M., Gilbert, R. J., and Parry, J. M., *J. Clin. Pathol.*, 32:289-293 (1979).
115. Siegman-Igra, Y., Lavochkin, J., Schwartz, D., and Konforti, N., *Israel J. Med. Sci.*, 19:546-561 (1983).
116. Logan, N. A., *J. Med. Microbiol.*, 25:157-165 (1988).
117. Schemmer, G. B., and Driebe, W. T., *Arch. Ophthalmol.*, 105:342-344 (1987).
118. Davey, R. T., Jr., and Tauber, W. B., *Rev. Infect. Dis.*, 9:110-123 (1987).
119. Beer, P. M., Lugwig, I. H., and Packer, A. J., *Am. J. Ophthalmol.*, 110: 212-213 (1990).
120. Donzis, P. B., Mondino, B. J., and Weissman, B. A., *Am. J. Ophthalmol.*, 105:195-197 (1988).
121. Henrickson, K. J., Shenep, J. L., Flynn, P. M., and Pui, C.-H., *Lancet*, 18: 601-603 (1989).
122. Henrickson, K. J., *Internat. J. Dermatol.*, 29:19-20 (1990).
123. Dryden, M. S., *J. Roy. Soc. Med.*, 80:4809-481 (1987).
124. Guiot, H.F.L., de Planque, M. M., Richel, D. J., and van't Wout, J. W., *J. Infect. Dis.*, 153:1186 (1986).
125. Akesson, A., Hedstrom, S. A., and Ripa, T., *Scand. J. Infect. Dis.*, 23:71-77 (1991).
126. Johnson, D. A., Aulicino, P. L., and Newby, J. G., *J. Trauma*, 24:267-270 (1984).
127. Bekemeyer, W. B., and Zimmerman, G. A., *Am. Rev. Respir. Dis.*, 131: 466-469 (1985).
128. Carbone, J. E., and Stauffer, J. L., *West. J. Med.*, 143:676-677 (1985).
129. Colpin, G.G.D., Guiot, H.F.L., Simonis, R.F.A., and Zwaan, F. E., *Lancet*, ii:694-695 (1981).
130. Weber, D. J., Saviteer, S. M., Rutala, W. A., and Thomann, C. A., *South. Med. J.*, 82:705-709 (1989).

131. Cotton, D. J., Gill, V. J., Marshall, D. J., Gress, J., Thaler, M., and Pisso, M., *J. Clin. Microbiol.*, 25:672-674 (1987).
132. Banerjee, C., Bustamante, C. I., Wharton, R., Talley, E., and Wade, J. C., *Arch. Intern. Med.*, 148:1769-1774 (1988).
133. Oster, H. A., and Kong, T. Q., *South. Med. J.*, 75:508-509 (1982).
134. Steen, M. K., Bruno-Murtha, L. A., Chaux, G., Lazar, H., Bernard, S., and Sulis, C., *Clin. Inf. Dis.*, 14:945-946 (1992).
135. Jones, T. O., and Turnbull, P.C.B., *Vet. Record*, 108:271-274 (1981).
136. Schuh, J., and Weinstock, J., *J. Am. Vet. Med. Assoc.*, 187:1047-1048 (1985).
137. Guerrant, R. L., and Bobak, D. A., *New England J. Med.*, 325:327-340 (1991).
138. Beard, T. D., III, *Food Technol.*, 45(6):123-124 (1991).

5981

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