# Bacillus Cereus: A Review

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## History

*Bacillus cereus* was first isolated and described by Frankland and Frankland in 1887 as a common inhabitant of the soil (Hutchinson and Taplin 1978, Kramer and Gilbert 1989).

From the beginning of this century, cases of food poisoning caused by *B. cereus* or *B. cereus*-like organisms have been reported in literature. One of the earliest recorded episodes was described in 1906 by Lubenau; a hospital outbreak involving 300 patients and staff who developed symptoms of acute gastroenteritis shortly after eating dinner. It was shown that the meat balls were highly contaminated with an aerobic spore-forming bacterium, most probably a *B. cereus*. In 1913, Seitz reported the isolation of large numbers of *B. cereus*-like micro-organisms from the faeces of a young man suffering from severe enteritis and diarrhoea. Brekenfeld (1926) implicated aerobic sporogenic bacilli as the cause of two separate outbreaks involving vanilla sauce and a jellied meat dish (Kramer and Gilbert 1989).

Early reports of food poisoning associated with *Bacillus* species were somewhat sketchy; quantitative bacteriology was rarely undertaken either on suspect foods or clinical specimens. Moreover, the disorder in *Bacillus* taxonomy led to imprecise classification of isolates. This contributed to the slow recognition of pathogenicity of *B. cereus*. Hauge succeeded in establishing the organism as a recognized food poisoning organism, following publication of four large outbreaks involving 600 people that occured in Norway in 1947-1949. He found that the vanilla sauce consumed with chocolate pudding as dessert, contained high levels ($10^7$ - $10^9$ per ml) of *B. cereus*; nevertheless it was not much changed in odour, taste or consistency. Spores of *B. cereus* (up to $10^4$ per g) were found in corn starch, one of the constituents of the vanilla sauce powder. In order to prove the toxicity of the *B. cereus* isolated from the outbreak, a vanilla sauce was prepared from a dry sterilized sauce powder and inoculated with about $10^4$ cells per ml. After incubation for 24 h at room temperature, Hauge consumed 200 ml of the product containing approximately $10^9$ *B. cereus* $ml^{-1}$. After 13 h of consumption, the symptoms started with severe abdominal pains, diarrhoea and rectal tenusmus, lasting more or less continuously for 8h (Hauge 1955).

Since 1950, increasing awareness and recognition of *B. cereus*-associated illness has resulted in a substantial increase in the number of reports of this type of food poisoning.

The importance of *B. cereus* in the dairy industry has been recognized since 1938, when the occurrence of bitty cream was first recorded. It was demonstrated that this defect could be attributed to *B. cereus* (Stone and Rowlands 1952). In addition, spore-formers, including *B. cereus*, have been associated with sweet curdling of pasteurized milk stored at refrigeration temperatures by Hammer and Babel (1957) and Jayne-Williams and Franklin (1960) (Overcast and Atmaram 1974).

## The Genus *Bacillus*

The genus *Bacillus* is the largest of the *Bacillaceae*, encompassing more than 60 species (Priest 1993). Members of the genus *Bacillus* are aerobic or facultatively anaerobic, gram-positive spore-forming rods (Claus and Berkeley, 1986). However, a great genetic diversity exists in this genus. That is why a subdivision of the genus into six smaller genera, based on studies of phenotypic similarities and range of genome base composition (GC content), is suggested (Priest 1981, Priest 1993).

The *B. cereus* group comprises the closely related species *B. cereus*, *B. anthracis*, *B. mycoides* and *B. thuringiensis*. All four species belong to the larger *B. subtilis* group (Priest 1993). *B. anthracis* has been recognized as non-haemolytic, non-motile, penicillin- and gamma phage-sensitive, encapsulated bacilli causing anthrax in man and animals (Claus and Berkeley 1986). *B. mycoides* can be differentiated from *B. cereus* by its lack of motility and rhizoid colonies. *B. thuringiensis* can be distinguished from *B. cereus* by its ability to produce a crystal protein toxin, toxic to various insects, inside the cell during sporulation (Priest 1993).

In spite of extensive similarities (Priest 1981, Ash *et al.* 1991), the four species are still considered to be distinct species (Claus and Berkeley 1986, Priest 1993, Nakamura and Jackson 1995).

## Characteristics of *B. cereus*

Microscopically, cells of *B. cereus* are seen as large rods that are motile by means of peritrichous flagella. There is a wide strain variation in properties of *B. cereus*. Germination of endospores in laboratory media is observed in a range of -1 to 59 °C, with an optimum at 30 °C (Goepfert *et al.* 1972).

Growth of vegetative cells occurs within the temperature range of 10-50 °C, with an optimum between 28 and 35 °C (Johnson 1984). However, psychrotrophic variants of *B. cereus*, capable of growth at temperatures as low as 5 °C have been identified (Christiansson *et al.* 1989, Väisänen *et al.* 1990). Growth is possible under aerobic or facultative anaerobic conditions (Claus and Berkeley 1986). *B. cereus* can multiply in a pH range from 4.9 to 9.3 (Goepfert *et al.* 1972, Johnson 1984). The minimum water activity for growth is 0.91-0.95 (Raevuori and Genigeorgis 1975, Bryan *et al.* 1981, Johnson 1984). The organism is able to metabolize glucose, fructose and trehalose but no pentoses nor many of the sugar alcohols. Certain strains utilize sucrose, salicin, maltose, mannose, glycerol, m-inositol and lactose. Most actively hydrolyze starch, casein and gelatin (Goepfert *et al.* 1972, Kramer and Gilbert 1989).

Factors that have been shown to have an inhibitory effect on growth of *B. cereus* include high levels of spoilage micro-organisms, nisin, sorbic acid and potassium sorbate. Effective antibiotics

against *B. cereus* are aureomycin, dihydrostreptomycin, terramycin, bacitracin, oxytetracycline, chloramphenicol and gentamycin (Johnson 1984).

The heat resistance of *B. cereus* spores is a factor of primary concern to the food and pharmaceutical industries. Vegetative cells are not very heat-resistant and are destroyed at time-temperature combinations which inactivate non spore-forming micro-organisms. Endospores of *B. cereus* show considerable variation in thermal resistance. D-values determined in skim-milk at 90 °C were 4.4 - 6.6 min (Shehata and Collins 1972). $D_{100}$ values of 2.7 to 3.1 min and 5 min have been reported for spores in skim-milk (Mikolajcik 1970) and in low-acid foods (pH > 4.5) (Ingram 1969), respectively. Using aqueous spore suspensions or phosphate-buffered suspensions z-values ranging from 6.1 to 9.2 °C were obtained (Kramer and Gilbert 1989). It was demonstrated that lipid material had a protective effect on the thermal resistance; in milk the $D_{95}$ is 0.5 - 3.5 min (Shehata and Collins 1972, Bergère and Cerf 1993). In products containing high levels of fat, such as cream, the D-value is much higher (Meer *et al.* 1991, Champagne *et al.* 1994). The occurrence of very heat resistant *B. cereus* spores (in underprocessed canned soups) that had $D_{95}$ values of more than 250 min has also been reported (Kramer and Gilbert 1989).

### Isolation of *B. cereus*

Procedures for the isolation, detection and enumeration of *B. cereus* almost all involve direct agar plating techniques (Van Netten and Kramer 1992). To obtain an indication of the proportion of bacteria present as spores in a sample, is to conduct a viable count of a sample in which the vegetative cells are killed by heat or some other treatment (e.g. air drying, solvent treatment, selective germination followed by pasteurization for specific types of endospore-formers). The most common procedure for *B. cereus* spores is the application of a heating step of 10-15 min at 80 °C. By this heat treatment, vegetative cells will be killed, but it will not harm most endospores (Priest and Grigorova 1990).

In the standard procedures for microbiological examinations the maximum time allowed between preparation of the primary dilution and mixing of dilutions and media is 45 min (e.g. Anonymous 1996a). Strikingly, it was shown that considerable germination (up to 40 %) occurs within 30 min at room temperature in diluents prescribed in the standard procedures resulting in underestimation of the numbers of endospore-forming bacteria present as spores. Therefore, the time between preparation of the primary dilution and the heat-activation step should be limited (< 10 min) and the temperature during the analysis should be as low as possible (e.g. by keeping dilutions in melting ice). In order to prevent underestimation of the numbers of endospore-forming bacteria present as spores (Te Giffel *et al.*, 1995).

Blood agar has been used for isolation of *B. cereus* from foodborne illness outbreaks, but a selective and differential medium is required for detection of low levels of the organism in the presence of other bacteria. Various plating media have been developed based on characteristic features of the organism such as hemolysin production, lecithinase activity, fermentation properties or morphological features. Polymyxin B sulfate is normally incorporated as selective agent to inhibit competitive micro-organisms (Donovan 1958, Mossel *et al.* 1967, Kim and Goepfert 1971a, Holbrook and Anderson 1980). The Mannitol Egg Yolk Polymyxin B sulfate agar (MEYP) is widely used in Europe and the United States to isolate *B. cereus* from food products. MEYP is based on lecithin hydrolysis and mannitol assimilation for differentiation of *B. cereus* from most other commonly occurring *Bacillus* species. Polymyxin B sulfate is added as selective agent to inhibit gram-negative bacteria (Mossel *et al.* 1967).

### Identification of *B. cereus*

Almost all procedures for the isolation, detection and enumeration of *B. cereus* involve direct agar plating techniques on non-selective media such as blood agar or selective media of which Mannitol Egg Yolk Polymyxin B sulfate (MEYP) agar is most commonly used (Mossel *et al.* 1967). For the confirmation of presumptive colonies and differentiation between *B. cereus* and closely related species biochemical tests are employed. According to the ISO Standard Procedure presumptive *B. cereus* colonies should be confirmed by glucose fermentation, reduction of nitrate to nitrite and Voges Proskauer reaction, i.e. production of acetylmethylcarbinol (acetoin) (Anonymous 1992). However, results of sequencing part of the 16S rRNA of '*B. cereus*' isolates indicated that these biochemical tests do not always identify a species correctly. Several isolates, involved in food poisoning, were classified, using sequencing, as *B. thuringiensis* instead of *B. cereus*. These two species are very closely related and can only be differentiated by the production of toxin crystals (Bourque *et al.* 1993). However, this character is plasmid encoded and transmissible to *B. cereus* by conjugation. Moreover, it has been shown recently that strains of *B. thuringiensis* produced a *B. cereus*-diarrhoeal-type of enterotoxin and outbreaks of *B. cereus* foodborne illness from ingestion of vegetables have been reported (Damgaard 1995, Damgaard *et al.* 1996). As *B. thuringiensis* is the most frequently used microbial insecticide worldwide, these results emphasize the need to re-evaluate the taxonomy of these organisms and to develop accurate identification methods. Therefore, a specific and sensitive method was developed, using DNA probes based on the variable regions of the 16S rRNA, to discriminate between *B. cereus* and *B. thuringiensis* (Te Giffel *et al.*, 1997).

### Typing of *Bacillus* species

To differentiate *Bacillus* strains several methods have been applied including biotyping, serological methods, phage typing, gas-liquid chromatography of whole-cell fatty acids, Pyrolysis Gas-liquid Chromatography (Py-GC) or Pyrolysis Mass Spectrometry (Py-MS), plasmid profiling, and Polymerase Chain Reaction (PCR) based methods.

### Biotyping

There has been considerable interest concerning biochemical differentiation between strains. For that purpose, the API system (a standardized, miniaturized version of conventional biochemical tests) has been used in several studies (Logan and Berkeley 1981, Logan and Berkeley 1984). It was found possible to discriminate between *B. cereus* strains causing the diarrhoeal and emetic types of food poisoning on the basis of failure to ferment salicin, dextrin, starch and glycogen (Logan and Berkeley 1981, Shinagawa 1993). In contrast, some workers have found no consistent differences between diarrhoeal and emetic strains (Major *et al.* 1979).

### Serotyping

Serotyping can be based on flagellar, vegetative cell somatic or spore antigens. The most successful method for serotyping of *B. cereus* is based on the flagellar (H) antigens. A typing scheme was developed in the UK which employs more than 20 agglutinating sera (Kramer *et al.* 1982). The scheme was proven of value in the epidemiological investigation of food poisoning (Gilbert and Parry 1977, Kramer *et al.* 1982, DeBuono *et al.* 1988) and in the investigation into the source of a *B. cereus* meningitis (Barrie *et al.* 1992). However, the antisera are not commercially available. Moreover, as many as 54 to 61 % of isolates from food sources may be untypable (Gilbert and Parry 1977, Gilbert and Kramer 1986).

### Phage typing

Phage typing is based on the sensitivity of strains to bacteriophages. Bacteriophage typing has not been extensively used for *Bacillus* species. Where use of bacteriophages has been made, identification tends to be based upon combinations of bacteriophage sensitivities with other types of data, rather than on sensitivity patterns alone. Phage typing has good discrimination, but leaves many strains untypable. Strains of the *B. cereus* group originating from dairy products could be differentiated using phage typing. However, *B. cereus*, *B. mycoides* and *B. thuringiensis* cross-reacted with their respective phages. Even though phage typing could not be used for accurate species identification, it was useful to cluster isolates of the *B. cereus* group, permitting to track routes of contamination (Väisänen *et al.* 1991). The use of phage typing of isolates associated with outbreaks of food poisoning has also been reported (Ahmed *et al.* 1995, Jackson *et al.* 1995)

### Gas chromatographic analysis of cellular fatty acids

This method is based on the extraction of fatty acids (by saponification and methylation) followed by chemical analysis by gas chromatography. It was shown that this was a reproducible method and that it was possible to discriminate between species of the heterogeneous genus *Bacillus*. The technique was, however, not able to distinguish between closely related species, e.g. *B. cereus*, *B. mycoides* and *B. thuringiensis* (Väisänen *et al.* 1991, Wauthoz *et al.* 1995).

### Pyrolysis gas-liquid chromatography (Py-GC) or pyrolysis mass spectrometry (Py-MS)

In these techniques, pyrolysis (the thermal degradation of matter in an inert atmosphere to produce a series of volatile low molecular weight substances characteristic of the original matter) is combined with analytical techniques of gas-liquid chromatography or mass spectrometry. Although both techniques have been used for the identification of *Bacillus* species, there are problems concerning reproducibility and standardization of the technique (Berkeley *et al.* 1984). Moreover, the critical levels at which isolates are distinguished are not known (Pitt 1992). The application of Py-MS to the investigation of outbreaks of food poisoning or non-gastrointestinal infection associated with *Bacillus* species showed that all epidemiologically related isolates were clustered correctly, and that all the single, epidemiologically unrelated isolates were distinguished (Sisson *et al.* 1992)

### Plasmid profiling

Plasmid profiling is based on the extraction of extrachromosomal DNA, which is a variable part of the genome of a bacterium. The sizes and number of plasmids that will be carried by the strains will vary depending on a range of influencing environmental factors. These strain to strain variations can be used as a way of separating the micro-organisms into different types.

Plasmid profiling has been applied in an investigation of an outbreak among patients and staff in a nursing home (DeBuono *et al.* 1988), to differentiate between *B. cereus* strains in an egg pasteurization plant (Ellison *et al.* 1989) and in an epidemiological investigation of *B. cereus* emetic-syndrome food poisoning (Nishikawa *et al.* 1996). The results show that plasmid profiling is a convenient method to differentiate between *B. cereus* strains. Of the strains isolated from the environment 92 % contained plasmid DNA (Ellison *et al.* 1989). The possibility of plasmid loss or gain by a strain is a legitimate concern, but unless there are some strong selective pressures acting, bacterial plasmid DNA is generally stably maintained within the cell by specific mechanisms.

### Polymerase Chain Reaction (PCR) Methods

PCR-based methods rely on the heterogeneity present in the chromosome of a bacterial species due to such factors as small deletions, base changes, rearrangements and randomly distributed insertion sequences.

PCR/RAPD

The randomly amplified polymorphic DNA (RAPD) technique provides a new means for characterization of bacteria. Primers are chosen arbitrarily and the PCR amplifies sets of short sequences, which can be visualised by agarose gel electrophoresis to give multiple banding patterns. The more closely related the genomic DNA of two strains, the more similar the banding patterns. RAPD typing has been used for epidemiological subtyping of *B. licheniformis* (Stephan *et al.* 1994) and *B. thuringiensis* (Brousseau *et al.* 1993). Recently, successful application of the method for the differentiation of *B. cereus* strains in milk processing lines (Nilsson and Christiansson 1996) and baby food (Stephan 1996) has been described. The RAPD-PCR technique provides good discrimination, especially if multiple primers are used (Williams *et al.* 1990).

A major advantage of the technique is its speed. In addition, the reaction is so sensitive that with many species there is no necessity to extract the DNA and cells can be used directly from a plate. However, there are still questions about the reproducibility of the technique (Dodd 1994).

### Ribotyping

Ribotyping, in which a Southern blot of restriction-enzyme-digested chromosomal DNA is hybridized to a probe based on the prokaryotic 16S rRNA gene has provided a useful approach to bacterial typing of *B. thuringiensis* and related bacteria at the species and subspecies level (Priest *et al.* 1994).

This approach offers an advantage over the direct visualization of restriction fragments because the number of bands and, hence the complexity of the fingerprint pattern are reduced and easier to resolve. The length of the procedure makes this method slower than plasmid profiling and the PCR/RAPD methods described above. However, recently, a commercial instrument, the Qualicon L.L.C RiboPrinter™ has become available. This allows automated, simple and convenient typing of bacteria (Banks 1996).

## Ecology of *B. cereus*

The ability to sporulate coupled with adaptation of many species to environmental extremes has enabled endospore-formers to become amongst the most widespread of all bacteria (Claus and Berkeley 1986, Priest and Grigorova 1990, Priest 1993).

*Bacillus* species are ubiquitous and can be isolated from a wide variety of environments, including soil, sediments, dust, natural waters and many food types as listed in Table 1, notably milk and dairy products, cereals, dried foods, spices, meat products and vegetables (Goepfert *et al.* 1972, Johnson 1984, Kramer and Gilbert 1989, Priest 1993). Direct contamination via soil is important for some food products e.g rice. *Bacillus* species are reported to account for about 90 % of the microflora of soil in rice paddies (Asanuma *et al.* 1979). From its natural habitats the organism is easily spread to foods.

**Table 1.** Incidence of *Bacillus cereus* in raw and processed foods

| Food product | % *B. cereus* positive | Range (cfu $g^{-1}$ or $ml^{-1}$) | Reference |
|---|---|---|---|
| **Rice and rice products** | | | |
| Raw rice | 40-100 | $10^2$ - $10^3$ | 1,8,13 |
| Boiled rice | 10-93 | $10^1$ - $10^7$ | 1,8,13 |
| Fried rice | 12-86 | $10^1$ - $10^5$ | 1,8,13 |
| Rice dishes | 3-40 | $10^1$ - $10^5$ | 1,8,10,13 |
| **Milk and dairy products** | | | |
| Raw milk | 7-35 | $10^1$ - $10^2$ | 2,6,7 |
| Pasteurized milk | 2-35 | $10^1$ - $10^3$ | 2,8,9,10,11 |
| Sterilized milk | 40 | nd | 1 |
| UHT milk | 48 | nd | 1 |
| Milk powder | 15-75 | $10^1$ - $10^3$ | 3,4,7,8,11,12 |
| Cream | 5-11 | $10^1$ - $10^5$ | 8,10 |
| Cheese | 2-40 | $10^1$ - $10^3$ | 2,10,12 |
| Ice cream | 40-52 | $10^1$ - $10^4$ | 2,8,11,12 |
| **Dried products** | | | |
| Herbs, spices and seasonings | 10-75 | $10^1$ - $10^6$ | 3,5,8,10 |
| Pulses and cereals | 56 | $10^2$ - $10^5$ | 1 |
| Raw barley | 62-100 | $10^2$ - $10^4$ | 1,8 |
| Wheat flour | 17-90 | nd | 1,8,9 |
| Dried potatoes | 40 | $10^2$ - $10^4$ | 1 |
| Soup | 4-50 | $10^2$ - $10^4$ | 8,9 |
| **Meat and meat products** | | | |
| Raw meat | 2-34 | $10^1$ - $10^3$ | 1 |
| Cooked meat | 22 | $10^1$ - $10^3$ | 1 |
| Sausage | 4-12 | $10^1$ - $10^3$ | 1 |
| **Miscellaneous** | | | |
| Vegetables | 0-100 | $10^1$ - $10^4$ | 1,10,13 |
| Pastries | 6-41 | $10^1$ - $10^4$ | 1 |
| Fish products | 4-9 | $10^1$ - $10^4$ | 1,10 |

**References:**

1 Kramer and Gilbert 1989, 2 Ahmed *et al.* 1983, 3 Kim and Goepfert 1971b, 4 Walthew and Lück 1978, 5 Powers *et al.* 1976, 6 Shehata and Collins 1971, 7 Shinagawa 1993, 8 Johnson 1984, 9 Mosso *et al.* 1989, 10 Van Netten *et al.* 1990, 11 Wong *et al.* 1988, 12 Rangasamy *et al.* 1993, 13 Harmon and Kautter 1991

Spores can survive during food processing and after germination and outgrowth cause spoilage and even outbreaks of foodborne illness (Johnson 1984). The ingestion of *B. cereus* may be regarded as an inevitable consequence of the widespread contamination of our food supply with this organism. In healthy individuals, spores surviving passage through the upper alimentary tract eventually become part of the transitory intestinal flora. The prevalence of *B. cereus* in 14-15 % of faecal specimens from healthy individuals has been reported. Excretion of *B. cereus* probably reflects the dietary intake of the organism, human carriage is not considered to be of any significance in food poisoning (Johnson 1984, Kramer and Gilbert 1989).

## Foodborne Illness due to *B. Cereus*

The first description of the diarrhoeal syndrome was provided by Hauge (1955). He investigated four outbreaks in Norway caused by vanilla sauce, as described before. Since then, there have been several outbreaks in which various products were implicated. *B. cereus* is now well-established as a significant cause of foodborne illness in humans, accounting for 1-23 % of reported outbreaks of known bacterial cause (Hutchinson and Taplin 1978, Kramer and Gilbert 1989, Drobniewski 1993). The WHO Surveillance Program for Control of Foodborne Infections and Intoxications in Europe combined with data on surveillance of foodborne diseases showed that an average of less than 1 to 10 % of reported cases with known aetiology were caused by *B. cereus* (WHO 1992, Todd 1996). In the Netherlands 19 % of the outbreaks was attributed to *B. cereus* in the period 1983-1990 (Notermans and Van de Giessen 1993). Other *Bacillus* species, including *B. licheniformis*, *B. pumilus* and *B. subtilis* have also been associated with incidents of foodborne illness (Kramer and Gilbert 1989, Meer *et al.* 1991).

There are two types of *B. cereus* food poisoning. The characteristics of the two types of food poisoning are listed in (Table 2.)

The first type, having symptoms which are similar to those of *Staphylococcus aureus* food intoxication, is caused by enterotoxin(s), produced during vegetative growth of the organism in the small intestine. The enterotoxin(s) can probably also be preformed in foods, but then the number of cells is at least two orders of magnitude higher than that necessary for food poisoning (Christiansson 1993, Granum 1994). The total infective doses seems to vary between about $10^5$ and $10^{11}$ viable cells or spores, partly due to the large differences in the toxin production by different strains (Hauge 1955, Kramer and Gilbert 1989, Granum 1994). The second type, resembling *Clostridium perfringens* food poisoning, is caused by an emetic toxin, preformed in the food.

**Table 2.** Characteristics of the two types of disease caused by *Bacillus cereus*

| | Diarrhoeal syndrome | Emetic syndrome |
|---|---|---|
| Infective dose | $10^5$-$10^7$ (total) | $10^5$-$10^8$ (cells $g^{-1}$) |
| Toxin produced | small intestine | preformed in food |
| Type of toxin | protein | cyclic peptide |
| Incubation period | 8-16 h | 0.5-5 h |
| Duration of illness | 12-24 h | 6-24 h |
| Symptoms | abdominal pain, watery diarrhoea, occasionally nausea | nausea, vomiting and malaise, (diarrhoea) |
| Principal food vehicles | meat products, soups, vegetables, puddings, sauces, milk (products) | rice(fried and cooked), pasta, pastry and noodles |

(Kramer and Gilbert 1989)

The diarrhoeal type of food poisoning is associated with a wide range of foods including cooked meat dishes, soups, vegetable puddings, milk based products and sauces (Johnson 1984, Kramer and Gilbert 1989, Meer *et al.* 1991). There is still confusion regarding how many different enterotoxins are produced by *B. cereus*. At least two have been characterized, so far (Beecher *et al.* 1995, Agata *et al.* 1995). One is a three component enterotoxin with haemolytic and necrotic activities (Beecher and Wong 1994, Beecher *et al.* 1995), while the other is a single component enterotoxin (Agata *et al.* 1995). Recently, it has been shown that there is at least one enterotoxin complex in addition to the haemolysin BL toxin and enterotoxin T (Granum *et al.* 1996).

There are two commercially available kits for the detection of enterotoxin: a Reversed Passive Latex Agglutination kit (RPLA) and an Enzyme-Linked ImmunoSorbent Assay (ELISA). The problem is that the antibodies used in both kits are not specific for the enterotoxin and detect two different non-toxic proteins (Beecher and Wong 1994).

The emetic toxin, cereulide, consists of a ring structure (dodecapsipeptide) of three repeats of four amino- and/or oxy-acids; $[D\text{-}O\text{-}Leu\text{-}D\text{-}Ala\text{-}L\text{-}O\text{-}Val\text{-}L\text{-}Val]_3$ (Agata *et al.* 1994). The biosynthetic pathway and mechanism of action of the emetic toxin still have to be elucidated. Approximately 95 % of all emetic syndrome episodes have been associated with the consumption of rice. It appears that the two syndromes cannot be related to specific serovars, although most strains (63.5 %) involved in emetic syndrome outbreaks belong to serotype H.1 (Kramer and Gilbert 1989). Strains causing the emetic syndrome have been found to be unable to hydrolyze starch or ferment salicin. However, some studies have observed no differences between diarrhoeal and emetic strains (Johnson 1984).

In addition, *B. cereus* produces several haemolysins and phospholipases. The in vivo role of these enzymes is not entirely clear, but they could be significant virulence determinants in infections (Johnson 1984, Drobniewski 1993).

### *Bacillus cereus* in the dairy environment

*B. cereus* can be introduced into milk from a variety of sources during production, handling and processing. To determine the major contamination sources, the incidence of *B. cereus* on farms, in two dairy processing plants and in pasteurized milk in household refrigerators was investigated. On farms, the major contamination sources were soil and faeces. In winter, when the cows are housed, used bedding probably also participates in this contamination route. The udder will be contaminated, resulting in the presence of the organism in raw milk. *B. cereus* could be detected in 35 % of the raw milk samples analyzed. During processing of raw milk to pasteurized milk or milk powder an increase in the percentage of positive samples was observed, suggesting that *B. cereus* can also be introduced via sources other than raw milk, e.g. equipment. *B. cereus* spores can remain in a heat exchanger, adhere to the surface, germinate and grow out. If these spores detach, the equipment can be a continuous source of contamination. The contamination levels were generally low; the organism could only be isolated after pre-incubation for 6 h at 30 °C. *B. cereus* could be isolated from 40 % of the pasteurized milk samples obtained from household refrigerators without pre-incubation. In 77 % of the positive samples levels of less than 5 per ml were detected. However, in 5 % of the samples numbers were higher than 5000 $ml^{-1}$.

Biochemical characterization, by carbohydrate utilization patterns (API 50 CHB; BioMérieux), and molecular typing, by PCR/RAPD fingerprinting and plasmid profiling of strains, demonstrated that selection of strains occurs in the milk production chain and during storage. This is illustrated by the high percentage of strains present in pasteurized milk in household refrigerators able to utilize lactose as carbon source (20 %) and able to grow at low temperatures (53 %). It was also observed that some *B. cereus* types could be isolated from raw milk, at the various stages of processing and in the end products, indicating that raw milk is an important source of contamination. Other types could only be detected after the pasteurization step in the process supporting the assumption that there are additional sources of contamination during processing.

It is unlikely that the presence of *B. cereus* in the milk production and processing environment can be prevented. The initial level should be low and this can be influenced by cleaning and disinfection. The assessment of sanitizer efficacy tested in the laboratory with non-adherent bacteria can lead to false assumptions as to the true effectiveness under in-use conditions. The results of surface tests reflected these conditions more closely than the suspension tests. It is recommended to include this type of test to evaluate sporicidal efficacy of cleaning and disinfection agents.

Despite a world-wide occurrence of *B. cereus* in milk and milk products relatively few reports on food poisoning caused by these products have been reported. The emetic toxin production by dairy isolates is probably not very significant as very few strains unable to hydrolyze starch or to ferment salicin, properties which have been reported to be related to production of this type of toxin, were found. The diarrhoeal type of food poisoning is caused by ingestion of cells. Thus, strains able to grow at both low temperatures, in the product, and high temperatures, in the human intestine, are of importance. Most *B. cereus* strains isolated on farms and at the two

processing plants, that were able to multiply at 7 °C, grew only slowly at 37 °C, suggesting that these strains may not be very enterotoxigenic. Of the isolates originating from pasteurized milk stored in household refrigerators 53 % was able to grow at 7 °C, 40 % of these strains could also grow at 37 °C. In addition, 27 % of the *B. cereus* obtained from households produced haemolysin BL, possibly a virulence factor. The ability of dairy strains to produce enterotoxin was confirmed by immunoblotting, using antiserum against the purified enterotoxin, in-vivo cell assays on Vero-cells and by PCR, using a set of primers for the B component of the haemolysin BL. *B. cereus* strains able to utilize lactose as carbon source may have a growth advantage in milk, moreover they showed higher enterotoxigenic activity compared with strains unable to ferment this substrate. This may be of significance in highly flavoured dairy products in which spoilage and/or presence of enterotoxin may be masked. However, the role of the carbohydrate metabolism in enterotoxin production remains to be further elucidated. If milk is mixed as an ingredient in other food products and stored incorrectly foodborne infections or intoxications may also occur.

If stored under proper conditions, maximum storage temperature 7 °C, and consumed within the expiry date, the levels of *B. cereus* in pasteurized milk will, in general, not exceed $10^5$ ml$^{-1}$ and cause no problems for healthy adults. Factors that may contribute to the low rate of *B. cereus* infection and intoxication are. visible spoilage occurs before numbers are sufficiently high to cause problems, duration of disease symptoms, unsuitable conditions for growth and toxin production in milk (Te Giffel 1997).

## References


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