The two forms of illness are principally caused by two significantly different moieties: the diarrhoeagenic toxin and the emetic toxin. The diarrhoeagenic factor (Thompson *et al.*, 1984; Kramer and Gilbert, 1989) is a protein with a molecular weight of about 38,000–46,000 and a pI of 5.1–5.6. The toxin is produced by actively growing cells and is inactivated by trypsin and pronase, as well as by exposure to 56°C for 30 min. The toxin is antigenic and can cause vascular permeability alterations in rabbit skin and dermonecrotic lesions in guinea pigs (Glatz *et al.*, 1974). The vascular permeability (VP) test correlates well with fluid-inducing activity in ligated ileal loops and is substantially easier to perform than the test for fluid-inducing activity in screening isolates for diarrhoeagenic activity. Interpretation of the zones of bluing and necrosis is the major problem with the VP assay (Thompson *et al.*, 1984). The toxin is also toxic to Vero cells and a high correlation has been observed between its toxic effects on Vero cells and a positive ligated ileal-loop assay (Thompson *et al.*, 1984). The toxin has been shown to have activity in the adenyl cyclase-cyclic AMP system.

The mechanism of pathogenicity of the *B. cereus* diarrhoeagenic form of illness has not been clearly elucidated. Studies by Spira and Goepfert (1972) revealed that *B. cereus* cultures introduced directly into ilea did not elicit diarrhoea, and that fluid accumulation was associated with an increase in bacterial numbers within ileal loops, whereas cell numbers decreased for strains that were negative in this model. However, ileal loop-positive strains grown in broth before introduction into ileal loops failed to elicit a response following growth *in vivo*. It was concluded that the increase in cell numbers was a consequence rather than a cause of fluid accumulation; that intraluminal multiplication was not involved in fluid accumulation; and that *B. cereus*-induced diarrhoea was probably the result of an intoxication rather than infection.

Conversely, Thompson *et al.* (1984) determined through monkey feeding studies that diarrhoeal toxin is apparently degraded in the gastrointestinal tract. They observed that buffering systems (bicarbonate buffer followed by BHI) used in previous monkey feeding studies (Goepfert, 1974) caused diarrhoea in the absence of bacterial metabolism. The authors were not able to elicit diarrhoea using large amounts of crude or partially purified material in the absence of neutralizing buffer. They suggested that humans develop diarrhoea caused by *B. cereus* from toxin produced in the intestine after the ingestion of large numbers of bacterial cells. The relatively long incubation period (8–16 h) observed before the onset of diarrhoea substantiates this.

In contrast, the emetic toxin is a small peptide (mol. wt. < 5000) that is not antigenic, but is extraordinarily resistant to heat (126°C for 90 min), extremes in pH (2–11) and enzymatic digestion, i.e. refractory to both trypsin and pepsin (Melling and Capel, 1978). This toxin is produced well into the stationary phase of growth, and its association with sporulation is uncertain at this time. More rapid progress towards understanding the biology and pharmacology of the toxin is hampered by the present lack of a convenient assay method other than monkey feeding.

Detection and enumeration

B. cereus food poisoning can presently be identified only by the isolation and enumeration of *B. cereus*, preferably in both the implicated food and the faeces or vomitus of the victims. Toxin analysis procedures are not yet widely available. Almost all procedures for the isolation and enumeration of *B. cereus* involve direct agar plating techniques. Many different plating media have been developed, based on the principle of providing conditions permitting the organism to exhibit haemolysis production, lecithinase activity, fermentation properties or morphological features characteristic of the species. Selective agents are usually incorporated to inhibit competitive microorganisms and facilitate enumeration.

Primary selective plating media generally use polymyxin B as the selective agent and rely on the expression of lecithinase on the egg yolk incorporated into the agar. The media most commonly used are mannitol-egg yolk-polymyxin agar (MYP) (Mossel *et al.*, 1967) or polymyxin-pyruvate-egg yolk mannitol-bromothymol blue agar (PEMBA) (Holbrook and Anderson, 1980). Dilutions of samples plated on MYP are incubated for 20–24 h at 30–32°C, and colonies typical of *B. cereus*, which have a dry, flat, 'ground-glass' appearance that may be translucent to creamy-white with a violet-red background surrounded by a readily visible zone of egg yolk precipitate, are counted. PEMBA exploits the mannitol-negative, lecithin-hydrolysing nature of *B. cereus*. After 18 h growth on PEMBA at 37°C, *B. cereus* forms flat, crenate to slightly rhizoid colonies that are turquoise to peacock-blue and have a 'ground-glass' surface appearance. Most strains have egg yolk precipitation reactions that form a zone up to 5 mm wide around each colony. Three or more presumptive colonies of *B. cereus* are transferred to nutrient agar and confirmed by

morphological and biochemical tests. Plating on blood agar is used primarily in the examination of faecal samples; on this medium characteristic bacillus-type colonies are surrounded by a zone of complete haemolysis.

Identification

Very few organisms produce similar reactions on egg yolk - polymyxin-containing media to those elicited by *B. cereus*, hence differentiation from non-*Bacillus* species is rarely required. Specific identification of *B. cereus* requires only differentiation from *B. mycoides* (on the basis of colonial morphology), *B. thuringiensis* (*B. cereus* lacks a parasporal insect-toxic crystal), and *B. anthracis* (*B. cereus* is motile). Various biochemical tests can be used, but these are not as rapid or effective as the colonial and microscopic examinations already mentioned. Biochemical tests used for confirmation include the anaerobic production of acid from glucose, nitrate to nitrite reduction, acetylmethyl carbinol production, L-tyrosine decomposition, haemolysis, growth in the presence of 0.001% lysozyme, rhizoid growth, motility, susceptibility to gamma-phage (*B. cereus* negative, *B. anthracis* positive) and lack of fermentation of mannitol, arabinose or xylose after 5 days' incubation at 36°C (Lancette and Harmon, 1980; Kramer and Gilbert, 1989).

Serotyping of *B. cereus* strains from food-related outbreaks is important to achieving an understanding of the epidemiological aspects of outbreaks. A serotyping scheme for differentiating *B. cereus* strains on the basis of their flagellar antigens has been developed (Taylor and Gilbert, 1975; Gilbert and Parry, 1977; Kramer *et al.*, 1982). Twenty-three serotypes have been identified, but some are more frequently involved in outbreaks than others (Shinagawa, 1990). Although some strains remain untypable using existing antisera, about 70% of the strains that have been associated with emetic-type outbreaks are of serotype 1. In contrast, there is no consistent serotype pattern among the diarrhoeagenic types (Gilbert and Parry, 1977).

Biotyping using a combination of biochemical properties, including Voges-Proskauer reaction, nitrate reduction, citrate utilization, urea decomposition, starch hydrolysis and fermentation of sucrose and salicin, has also been a useful epidemiological tool (Kozasa *et al.*, 1977). Biotyping has not been standardized for routine use, hence its application is dependent on previously established individual criteria.

Alternative methods

A *Bacillus* identification kit is available from Analytab Products, Inc. (API) (Plainview, New York, USA) that can rapidly confirm isolates of *B. cereus* from selective agars. Oxoid (Unipath Ltd, Basingstoke, Hampshire, UK) developed the BCET-RPLA kit that was designed to detect the presence of *B. cereus* diarrhoeal toxin by reverse passive latex agglutination. This kit was subsequently determined to lack specificity for *B. cereus* diarrhoeal toxin and yield false-positive results.

Distribution in nature: importance in foods

B. cereus is widely distributed in nature. It is readily isolated from soil, dust, cereal crops, vegetation, animal hair, fresh water and sediments (Kramer and Gilbert, 1989). Consequently, it is not surprising to find the organism in or on virtually every raw agricultural commodity, although its presence and incidence in/on fish is not well established.

A survey by Nygren (1962) of the incidence of *B. cereus* in food materials revealed that 52% of 1546 food ingredients, 44% of 1911 cream and dessert dishes and 52% of 431 meat and vegetable products were contaminated, illustrating its widespread distribution. The organism is also a frequent contaminant of milk and dairy products, between 9 and 48% of such products, including UHT-treated (48%) milk, being contaminated with *B. cereus*.

The ability to form spores ensures survival through all stages of food processing short of retorting and the organism is present in most raw materials used in food manufacture. Under normal circumstances, *B. cereus* is found in food at concentrations $<10^3$/g and mostly $<10^2$/g. In such numbers, the organisms may be considered innocuous since the minimum level required to cause illness has been estimated to be $>10^5$/g (Hobbs and Gilbert, 1974).

Every well-documented report of *B. cereus* intoxication has described time/temperature abuse that has enabled relatively low (innocuous) levels of *B. cereus* in foods greatly to increase. In most incidents studied, the food vehicle has been a cereal or has contained cereal or spice ingredients.

Surveys have revealed 46–100% raw rice and 10–53% spices to be contaminated with *B. cereus* (Kramer and Gilbert, 1989). Cereal products frequently undergo processing, which greatly reduces the vegetative cell flora. They are also cooked before serving, which leaves a residual flora of spores. In the absence of competitive microorganisms, *B. cereus* is able to proliferate readily if the cooked product is held within the growth range of the organism. Therefore, primary control of this type of intoxication consists in prevention of time and temperature abuse, particularly in cooked products.

Any microorganism present in food and capable of causing illness must *a priori* be considered important. The number of incidents of *B. cereus* poisoning is increasing if considered globally. However, it must be remembered that it is only in the past 10–15 years that sufficient attention has been paid to this organism when investigating outbreaks of foodborne illness. Given the relative paucity of information available compared with that for more established foodborne illnesses, such as botulism, salmonellosis and staphylococcal intoxication, caution in attributing illness to *B. cereus* on finding elevated numbers of the organism in foods is advised. More knowledge is required about the growth of *B. cereus* with toxin production in foods and specific methods for detecting the toxin in food must be developed before true estimates of this foodborne illness can be made. Furthermore, the mild nature and short duration of the symptoms that characterize both forms of intoxication from *B. cereus* compared with the severity of other foodborne infections and intoxications relegate this organism to a status of lesser importance.

Growth and survival characteristics

Strains of *B. cereus* vary widely in their growth and survival characteristics (*Table B*). Some strains are psychro-trophic, being able to grow at 4–5°C but not at 30–35°C, whereas other strains are mesophilic and can grow between 15°C and 50 or 55°C. The optimal temperature for growth ranges from 30 to 40°C (*Table 1a*). Similarly, the minimal pH for growth varies among strains and also depends on the acidulant; in general, growth does not occur at pH 4.8 in media acidulated with HCl, or at pH 5.6 in media acidulated with lactic acid (*Table 3a*).

The effect of water activity on the growth of food poisoning strains is not well documented. Present data indicate that *B. cereus* will not grow at a_w 0.92–0.93 with NaCl as the humectant; however, the organism can grow at a_w 0.93 but not at a_w 0.92 in media containing glycerol as the humectant (*Table 2*). Although research is generally lacking on the effect of preservatives on *B. cereus*, 0.26% sorbic acid at pH 5.5 and 0.39% potassium sorbate at pH 6.6 are inhibitory of growth (*Table 5b*).

Destruction of the spores of *B. cereus* has been of concern to the food industry, and has received considerable attention. In general, the heat resistance of *B. cereus* spores is similar to that of most mesophilic spore-forming bacteria (*Table 1c*). However, there is considerable strain variability, the spores of a few strains having D-values at an equivalent temperature 15 to 20 times greater than spores of the most heat-sensitive strains. In addition, spores of *B. cereus* have no unusual resistance to irradiation (*Table 4*), or disinfectants compared with other spore-forming bacteria (*Table 7*).

The interactive effect of pH and water activity (NaCl concentrations) on the death of *B. cereus* has been studied by Raevuori and Genigeorgis (1975). Death is greatly influenced by both parameters; however, rates of death are relatively slow in substrates containing NaCl in brine concentrations $\leq 5\%$ and having a pH of 6.1 or 7.5 (*Table 8*).

Data on the effect of many factors, such as pH and water activity, on growth are remarkably scanty. Research is needed in these areas to provide a more complete understanding of factors influencing *B. cereus* control in food environments.

Studies of the fate of *B. cereus* in bread-making have revealed that the addition of 0.2% calcium propionate to bread dough delayed germination of the organism and subsequent growth sufficiently to render the risk of food poisoning due to the presence of *B. cereus* in bread negligible (Kaur, 1985).

Toxin characteristics and production

Methods for detecting *B. cereus* enterotoxins are neither highly specific nor quantitative. Hence, many of the data on these toxins are questionable. It has been reported that diarrhoeal toxin can be produced in ground beef and lasagna within 24 days at 4°C and within 12 days at 7°C; however, these studies will have to be confirmed using improved methods for detection. The optimal