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Prevalence, characterization and growth of *Bacillus cereus* in commercial cooked chilled foods containing vegetables

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C. CHOMA, M.H. GUINEBRETIÈRE, F. CARLIN, P. SCHMITT, P. VELGE, P.E. GRANUM AND C. NGUYEN-THE. 2000. In cooked-chilled and pasteurized vegetable products, initial numbers of *Bacillus cereus* were below 10 cfu g^{-1}. Before the appearance of spoilage, numbers reached 6–8 log cfu g^{-1} at 20 °C and 4–6 log cfu g^{-1} at 10 °C. *Bacillus cereus* was not detected in samples stored at 4 °C. Ten percent of strains isolated from the products were able to grow at 5 °C and 63% at 10 °C. *Bacillus cereus* strains unable to degrade starch, a feature linked to the production of emetic toxin, did not grow at 10 °C and had a higher heat resistance at 90 °C. Using immunochemical assays, enterotoxin was detected in the culture supernatant fluid of 97·5% of the strains. All culture supernatant fluids were cytotoxic but important variations in the level of activity were found. Psychrotrophic isolates of *B. cereus* were unable to grow in courgette broth at 7 °C whereas they grew in a rich laboratory medium. At 10 °C, these isolates grew in both media but lag time in courgette broth was 20-fold longer than in the rich laboratory medium.

INTRODUCTION

Cooked chilled foods (also known as REPFEDs, Refrigerated Processed Foods of Extended Durability) are becoming increasingly popular in Europe. They are generally processed with a mild heat treatment and stored at refrigeration temperatures (2–4 °C) for shelf-lives ranging from a few days to three months. Products based on vegetables account for an important part of REPFEDs, either as recipe dishes intended for the retail market or as precooked ingredients intended for caterers or the food industry. The mild heat treatment kills vegetative forms of micro-organisms, but is not severe enough to kill bacterial spores. Among spore-forming bacteria, *Bacillus cereus* has been responsible for food poisoning (Granum 1997) and has been frequently isolated from raw and processed food products such as rice, milk and dairy products, spices, vegetables (Roberts *et al*. 1982), meat products and farinaceous foods (Kramer and Gilbert 1989). Some strains of *B. cereus* are able to grow at 5 or 7 °C (van Netten *et al*. 1990; Dufrenne *et al*. 1994) and could be of concern in refrigerated, pasteurized foods. The aim of this work was to determine the prevalence of *B. cereus* in two different type of REPFEDs based on vegetables, and to characterize the population of *B. cereus* found in the products with respect to food processing and food safety.

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MATERIALS AND METHODS

Media

Ingredients for culture media were purchased from BioMérieux (Marcy-l'Etoile, France) unless specified otherwise. J-agar (JA) and Anaerobic Agar were prepared as described by Claus and Berkeley (1986). J-broth (JB) had the same composition as JA but did not contain agar. The selective media for *Bacillus cereus* were Polymyxin-Eggyolk-Mannitol-Bromothymol Blue-agar (PEMBA) (Oxoid) and Mannitol-Eggyolk-Phenol red-agar (MYP) prepared as described by Lancette and Harmon (1980). Soil extract agar contained JA mixed with the same volume of sterile soil infusion in deionized water and was prepared as described by Gordon *et al*. (1973). To prepare courgette broth (CB), fresh courgettes were washed, steam-cooked,

homogenized with a stomacher (Seward Medical, London, UK) for 1 min and centrifuged at 10 000 *g* for 10 min at room temperature. The supernatant fluid was filtered through cheese cloth, dispensed into 10 ml vials and clamped with a rubber stopper. Vials were heated three times for 1 h in a water-bath at 80 °C at 1-day intervals.

Cooked chilled foods containing vegetables

Two types of cooked chilled foods containing vegetables were analysed: (i) vegetable purées produced for the retail market, heated in their final package at 80 °C for 10–20 min; and (ii) cooked vegetables produced for caterers, cooked at 98 °C for 10–15 min and packaged under high care conditions (Varoquaux and Nguyen-the 1994). Purées (carrot, broccoli, split peas, courgette and potato) were vacuum-packaged in polymeric trays in 400 g units. Cooked vegetables (leek, carrot and courgette) were packaged into sealed polymeric trays in 500 g units. Samples were transported to the laboratory immediately after processing. Products were then either analysed or stored at target temperatures. Spoilage of stored product was detected by swelling of the package and/or off-odours.

Enumeration of micro-organisms from vegetable products

Samples (50 g) of vegetable purées and cooked vegetables were homogenized using a Stomacher in 100 ml sterile distilled water. For unstored products, 100 μl of homogenates were spread onto five replicate plates of JA for mesophilic bacteria, and PEMBA for *B. cereus*, and appropriate decimal dilutions of homogenates of stored products were spread onto the same media with a spiral plater (Interscience, Saint-Nom la Bretèche, France). Media were incubated at 30 °C for 24 h. The numbers of *B. cereus* were determined after enumeration of the colonies having the characteristic appearance of *B. cereus*, i.e., precipitation of hydrolysed lecithin and the failure to utilize mannitol (Lancette and Harmon 1980).

Isolation and confirmation of *B. cereus* isolates

For each type of product and after each time of storage, approximately five colonies with the typical morphology of *B. cereus* were isolated from PEMBA plates. In addition, 10 colonies were sampled at random from JA plates and used to count mesophilic bacteria. The colonies were sub-cultured on MYP plates and those having the characteristic appearance of *B. cereus* were selected. Isolates were then sub-cultured on JA plates to check purity and kept frozen in a 30% (v/v) glycerol solution at −20 °C. Identification of isolates to the *B. cereus* group isolates was confirmed by Gram-staining, catalase reaction, shape and position of spores under phase contrast microscopy (×1000), growth in anaerobic medium (Claus and Berkeley 1986), ISO 7932 procedure (AFNOR 1996a) and NF V 08–058 procedure (AFNOR 1996b). Isolates were further characterized by API 50 CHB and API 20 E strips using APILAB Plus software version 3.2.2. (BioMérieux). To test starch hydrolysis, strains were grown on a starch agar medium (Ombui *et al.* 1997). After 3 and 5 days at 30 °C, hydrolysis was revealed by flooding plates with 95% ethanol according to Claus and Berkeley (1986).

Growth at various temperatures

All isolates were grown at 2, 5, 10, 30, 37 and 42 °C in 20 ml tubes containing 5 ml JB as described by Claus and Berkeley (1986). Cultures were observed for visible growth after 2 and 5 days at temperatures of 37 and 42 °C, and after 3, 7, 14 and 21 days at temperatures of 2–30 °C. Positive cultures were checked for purity by streaking a loop of medium on JA and checking spore production and spore morphology.

Measure of spore heat resistance

Spores produced on soil extract agar incubated at room temperature for 5 days were collected from the agar surface with a sterile cotton swab and suspended in 1·5 ml sterile distilled water. Spore suspensions were immediately placed in ice and kept for less than 1 h before measuring heat resistance. Observations under phase contrast microscopy (×1000) showed that spores did not form aggregates. Heat-resistance was measured at 90 °C in water as performed by Gilbert *et al.* (1974) and Rajkowski and Mikolajcik (1987), using the submerged tube procedure described by Peck *et al.* (1992). Hungate tubes (Flobio, Courbevoie, France) containing 9·9 ml sterile distilled water were submerged in a water-bath, equilibrated at 90 °C, and 100 μl of the spore suspension injected through the septum. At the end of the heating period, tubes were cooled in iced water. The initial spore concentration was determined after heating at 70 °C for 10 min. Surviving spores were enumerated on JA plates with a spiral plater apparatus as described above. The log numbers of surviving spores were plotted against time and D values were calculated from the slope of the linear phase of spore destruction as the time in minutes to reduce spore number by one log cycle (ICMSF 1980). A control tube containing a temperature probe (Thermocouple J connected to an Almemo 2290–8 recorder, AS Technologies, Nîmes, France) was treated in the same way. The time taken to cool hungate tubes from 90 to 30 °C was 60 s, equivalent to 5–6 s at 90 °C assuming a Z value for spores

of 10 °C (ICMSF 1980), and was therefore not taken into account to calculate D_{90} values.

Enterotoxin production and cytotoxic activity

Enterotoxin production was tested in the culture filtrates of isolates grown in BHI (Biokar, Paris, France) using the *B. cereus* Diarrheal Enterotoxin Visual Immuno Assay (TECRA-BDE) (TECRA, Roseville New South Wales, Australia) and the *B. cereus* Enterotoxin reverse Passive Latex Agglutination test from Oxoid (BCET-RPLA) (Unipath, Basingstoke, UK) following the instructions of the manufacturers.

To measure cytotoxic activity, *B. cereus* isolates were grown in BHI at 32 °C for 6 h on a rotary shaker (300 rev min^{-1}). Preliminary experiments had shown levels of cytotoxicity of culture filtrates grown for 6 and 18 h at 32 °C to be similar. CaCo2 cells were cultivated on microplates at 37 °C in 5% CO_2 for 3–4 days in Dulbecco's modified Eagle's medium containing: glucose, 25 mmol l^{-1}; L-glutamin, 4 mmol l^{-1}; fetal calf serum, 10% (v/v); penicillin, 100 IU ml^{-1}; streptomycin, 100 μg ml^{-1}; amphotericin B, 2·5 μg ml^{-1}. Cultures of *B. cereus* were centrifuged and filtrated through 0·2 μm sterile filter units. Filtrates were diluted one, two, four and eightfold in modified Dubelcco's medium, and 50 μl of each dilution were deposited onto CaCo2 cells. Micro-plates were incubated (3 h at 37 °C) and gently shaken for 5 min to remove killed CaCo2 cells. Surviving cells were fixed with paraformaldehyde (2% in PBS, v/v) at 4 °C for 30 min and stained for 20 min at room temperature with 100 μl of a crystal violet solution (violet crystal, 0·13% w/v; ethanol, 5% v/v; paraformaldehyde, 2% v/v in PBS). Cells were rinsed five times with distilled water and the crystal violet was released from the cells by adding 100 μl 50% (v/v) ethanol in water. The amount of dye released was measured at 620 nm and was inversely related to the cytotoxic activity of culture filtrates. Filtrates were considered cytotoxic whenever optical density represented less than 50% of that of cells incubated with BHI alone. Culture filtrates of each of the *B. cereus* isolates were heated at 58 °C for 40 min to inactivate enterotoxins (Granum 1997) and used as a control. Tests were done in duplicate and whenever the two results were different, a third test was performed.

Culture filtrates which were not cytotoxic were concentrated 10 times by ammonium sulphate precipitation and tested on Vero cells by the method described previously (Sandvig and Olsnes 1982; Granum *et al*. 1993).

Growth kinetic of *B. cereus*

Spore suspensions from five strains of *B. cereus* (three strains able to grow at 5 °C and two strains unable to grow at 10 °C) were prepared as described above (heat resistance method). Spores were washed three times in sterile distilled water by centrifugation (2000 *g*) at 10 °C for 5 min, enumerated, and stored at −20 °C in a sterile glycerol solution (30% in distilled water, v/v). Aliquots of the thawed supensions were mixed in order to obtain a mixture containing the same proportion of each strain. A second solution containing a thawed suspension of one of the psychrotrophic strains was also prepared. Both suspensions were diluted in sterile distilled water to a concentration of 10^4 cfu ml^{-1}, and 100 μl of these dilutions were inoculated into 10 ml JB and CB. Inoculated vials were incubated at 19·5 ± 0·5 °C, 14·2 ± 0·8 °C, 9·6 ± 0·4 °C, 6·5 ± 0·4 °C and 4·0 ± 0·0 °C. Temperatures within the incubation cabinets were recorded at each sampling time or at daily intervals to calculate mean temperatures and standard deviations. Experiments with the mixture of strains and with the single strain were conducted simultaneously. For each storage temperature, one vial was sampled at regular intervals to enumerate *B. cereus* on JA plates using the spiral plater apparatus. To calculate generation time and lag time, growth curves were fitted to the Baranyi equation (Baranyi *et al*. 1993).

RESULTS

Enumeration of *B. cereus* in food samples

Bacillus cereus was detected in 20% of unstored vegetable purées pasteurized in their final package (Table 1) at less than 10 cfu g^{-1}. When spoilage was detected in products stored at room temperature (20–25 °C), i.e., between four and 12 days, 70% of samples were found to be positive and contained $10^{6·2}$–$10^{8·5}$ cfu g^{-1}. After 20 days at 10 °C, 50% of samples were positive for *B. cereus*, containing 10^4 to 10^6 cfu g^{-1} and showing no spoilage. *Bacillus cereus* was not detected on PEMBA selective medium from samples stored at 4 °C. However, two strains among colonies sub-cultured from JA plates were identified as *B. cereus* (Table 2). After 20 days at 10 °C, numbers of *B. cereus* on selective media were between two- and 100-fold lower than those of mesophilic bacteria on JA. At room temperature, numbers of *B. cereus* were similar to those of aerobic mesophilic bacteria (data not shown).

Cooked vegetables packaged under high care conditions spoiled rapidly and were stored only at 10 and 4 °C for 2 and 3 weeks, respectively. Numbers of *B. cereus* were always lower than 100 cfu g^{-1} (data not shown).

Identification of isolates

One hundred and sixty-nine colonies with the typical morphology of *B. cereus* on MYP medium were isolated. They

Table 1 Density ($\log_{10}$ cfu g^{-1}) and frequency of *Bacillus cereus* in pasteurized vegetable purées stored at 10 °C and at room temperature

Days at room temperature*	Number of samples		Vegetable purées				
			Broccoli	Carrot	Courgette	Potato	Split peas
0	4	% Positive samples	25	25	25	0	25
		Cell density‡	0·8	0·75	0·8	< 0·8	0·8
5–12†	5	% Positive samples	40	60	20	60	20
		Cell density‡ ± S.D.	7·4 ± 0·5	6·2 ± 0·4	8·5 ± 0·6	6·5 ± 0·1	7·8 ± 0·2
Days at 10 °C							
0	4	% Positive samples	25	25	25	0	25
		Cell density‡	0·8	0·7	0·8	< 0·8	0·8
20	5	% Positive samples	0	60	100	80	20
		Cell density‡ ± S.D.	< 3·8	4·5 ± 0·1	5·0 ± 0·6	5·7 ± 1·4	5·8
32	5	% Positive samples	0	20	0	40	20
		Cell density‡ ± S.D.	< 4·8	5·2	< 5·1	5·9 ± 0·0	6·1

Samples stored at 4 °C were all negative for *B. cereus* over 46 days.
* 20–25 °C.
† Samples were analysed when spoilage was first visible: 4 days for courgette purée, 5 days for broccoli purée and split peas purée, 12 days for carrot purée and potato purée.
‡Mean of the numbers found in positive samples.

were all Gram-positive and catalase-positive, and they produced endospores and grew under anaerobic condition. Spore position was central or subterminal and did not swell the sporangium. Among these isolates, 96 and 98% were confirmed as *B. cereus* by ISO 7932 and NF V 08–058 procedures, respectively; 83 isolates confirmed as *B. cereus* by both procedures were selected to represent each kind of product and storage condition, and were characterized by API-50CHB and API-20E strips (Table 2). Most strains belonged to the API-profile *B. cereus* 1 and to the profile intermediate between *B. cereus* and *B. mycoides*. These latter strains formed a homogenous group which all had similar API-profiles. These two profiles were found among strains isolated from purées stored at both 10 °C and room

Table 2 Number and origin of *Bacillus cereus* isolates from each API-profile

API Profiles	Pasteurized purées*				Cooked chilled vegetables†	Total
	Unstored product	Stored at 4 °C	Stored at 10 °C	Stored at 20–25 °C		
B. cereus 1	1	2	2	6	21	32
B. cereus 2‡	0	0	0	0	8	8
B. cereus 1/*B. mycoides*	1	0	13	22	1	37
B. cereus/*B. thuringiensis*	1	0	0	0	0	1
B. cereus/*B. laterosporus*	0	0	3	0	0	3
B. mycoides	0	0	0	1	0	1
B. cereus/*B. anthracis*§	0	0	0	0	1	1

All strains had been previously confirmed as *Bacillus cereus* by ISO 7932 and NF V 08–058 procedures.
*Numbers of *B.cereus* in the products are presented in Table 1.
†Numbers of *B. cereus* in cooked chilled vegetables were always lower than 100 cfu g^{-1}
‡Isolates did not hydrolyse starch.
§Isolates were haemolytic.

Table 3 Numbers of *Bacillus cereus* strains* which grew at temperatures ranging from 5 to 42 °C

Growth observed at temperatures of (°C)	API profiles							
	B. cereus 1	*B. cereus* 2	*B. cereus* 1/ *B. mycoides*	*B. cereus*/ *B. thuringiensis*	*B. cereus*/ *B. laterosporus*	*B. mycoides*	*B. cereus*/ *B. anthracis*	Total
5, 10, 30, 37	5	0	1	0	0	0	0	6
5, 10, 30, 37, 42	1	0	0	0	0	0	1	2
10, 30, 37	5	0	1	0	2	0	0	8
10, 30, 37, 42	14	0	20	0	1	0	0	36
30, 37	0	0	2	0	0	0	0	2
30, 37, 42	7	8	12	1	0	1	0	29

*Strains isolated from pasteurized purées and cooked chilled vegetables.

temperature (Table 2). Strains with the profile *B. cereus* 2 were unable to use starch and were isolated only from cooked vegetables. Strains with the API profile *B. cereus* 1 were isolated from both vegetable purées and cooked vegetables, whereas strains with a profile intermediate between *B. cereus* and *B. mycoides* were isolated mostly from vegetable purées.

Growth temperature

None of the isolates of *B. cereus* were able to grow at 2 °C; 10% grew at 5 °C and 63% at 10 °C. All the strains with the API profile *B. cereus* 2 were unable to grow at 10 °C or below (Table 3). All strains grew at 37 °C and 81% at 42 °C (Table 3). This latter group was mostly strains unable to grow at 5 °C, or to grow rapidly at 10 °C (i.e., giving a turbid culture within 3 or 7 days).

Heat resistance of *B. cereus* spores

Fifty-two strains from the various API profiles and the two products were analysed for heat resistance. The D_{90} values ranged from 0·7 to 5·9 min. Strains with the profile *B. cereus* 1 were less heat-resistant (D_{90} values from 0·7 to 2·4 min) whereas strains with the profile *B. cereus* 2 were more heat-resistant (D_{90} values from 3·3 to 5·9 min). Strains able to grow at 5 °C were the least heat-resistant, with D_{90} values lower than 1·5 min (Fig. 1). Strains unable to grow at 10 °C or below had D_{90} values ranging from 0·9 to 5·9 min

Enterotoxin production and cytotoxic activity

The 83 strains characterized by API strips were tested for enterotoxin production; 67 (81%) were positive with the BCET-RPLA test (Oxoid) and 72 (87%) were positive with the TECRA-BDE test. Fifty-nine strains (71%) were positive and two strains were negative (2%) with both tests (Table 4).

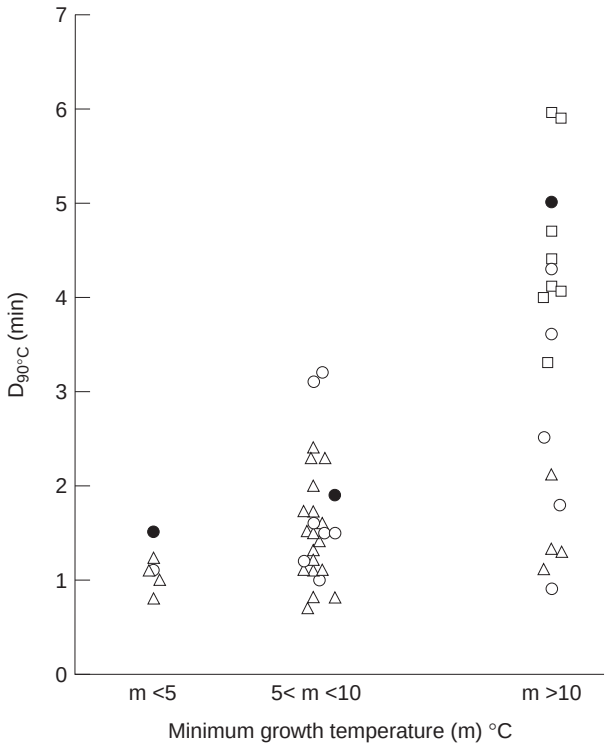

Fig. 1 Heat resistance at 90 °C and minimal growth temperatures of *Bacillus cereus* isolated from pasteurized vegetable purées and cooked chilled vegetables. API profile *B. cereus* 1 (△); API profile *B. cereus* 2 (□); API profile *B. cereus* 1/*B. mycoides* (○); other API profiles (●)

Table 4 Reaction of *Bacillus cereus* strains to the enterotoxin detection tests TECRA-BDE and the RPLA-BCET

		TECRA-BDE test		
	Number of strains	Positive	Negative	Total
RPLA-BCET	Positive	58	9	67
	Negative	14	2	16
Total		72	11	83

For 79% of strains, crude supernatant fluids, not concentrated by ammonium sulphate precipitation, were cytotoxic on Caco cells. Once diluted twofold, 30% of the supernatant fluids were cytotoxic. Supernatant fluids of two strains were still active after an eightfold dilution. Supernatant fluids heated for 40 min at 58 °C lost cytotoxicity. Most strains able to grow at 5 °C and 10 °C within 3 days, and unable to grow at 42 °C, were not cytotoxic to Caco cells (Table 5). Strains isolated from pasteurized purées were more cytotoxic than strains isolated from cooked chilled vegetables; 44% of the strains from purées were cytotoxic even when their supernatant fluid was diluted (10% of the strains from cooked chilled vegetables), and all the strains active with supernatant fluid diluted fourfold and eightfold were from the pasteurized purées stored at room temperature (data not shown). Out of the 15 non-cytotoxic strains, 10 were positive by both TECRA-BDE and BCET-RPLA tests, and three were positive by one test. Therefore, the majority of these negative strains were nevertheless producing enterotoxins. When supernatant fluids were concentrated 10-fold by ammonium sulphate precipitation, all the negative strains became cytotoxic, confirming that enterotoxins were produced, but at a low level.

Growth of *B. cereus* in courgette broth

The strains of *B. cereus* tested did not grow in JB at 4 °C but grew at 6·5 °C with a generation time of 7 h (Table 6). In courgette broth, no growth occurred within 32 days at 4·0 and 6·5 °C. At 9·6 °C, *B. cereus* grew in both media but lag time in courgette broth was 10–15 times longer than in JB. At 19·5 and 14·2 °C, differences between growth parameters in the two media were less pronounced.

DISCUSSION

Bacillus cereus was present in 30% of raw vegetables according to Roberts *et al.* (1982), which approximates to the frequency of contamination among cooked vegetables and vegetable purées reported here. Numbers of *B. cereus* in the unstored products did not exceed 10 cfu g^{-1}, whereas in raw foods of plant origin such as seeds, legumes, cereals and flour, numbers of *B. cereus* reported were usually higher than 100 cfu g^{-1} (Harmon *et al.* 1987; Kramer and Gilbert 1989). Processing steps such as washing, peeling and cooking presumably reduced the number of *B. cereus* in the final product. The bacterium was not detected on selective media in products stored at 4 °C.

Table 5 Cytotoxicity of supernatant fluids of *Bacillus cereus* isolates in relation to their growth temperatures

Growth observed at temperatures of (°C)	Number of strains for which the maximum cytotoxic dilution of the supernatant was:				
	NC*	1	1/2	1/4	1/8
5, 10, 30, 37	6	0	0	0	0
5, 10, 30, 37, 42	0	2	0	0	0
10, 30, 37	6	2	0	0	0
10, 30, 37, 42	4	18	12	0	2
30, 37	0	1	1	0	0
30, 37, 42	1	17	8	3	0
Total	17	40	21	3	2

*Raw, undiluted supernatant fluid was not cytotoxic but was cytotoxic after concentration 10 times by ammonium sulphate precipitation.

Table 6 Growth parameters of a psychrotrophic strain of *Bacillus cereus* (Single strains) and of a mixture of five strains of *Bacillus cereus* (Mixed strains) in J-broth and in Courgette broth inoculated with spores

	Generation time (h)				Lag phase (h)			
	J-broth		Courgette broth		J-broth		Courgette broth	
Temperature (°C)	Mixed strains	Single strain	Mixed strains	Single strain	Mixed strains	Single strain	Mixed strains	Single strain
19·5	1·6	1·5	3·1	2·5	Not detected	Not detected	Not detected	19·4
14·2	2·9	4·1	5·1	4·4	Not detected	5·6	5·4	15·5
9·6	4·0	3·2	8·7*	23·8*	17·2	18·2	333	184
6·5	6·7	7·4	No Growth	No Growth	70·9	56·5	No Growth	No Growth
4·0	No Growth	No Growth	No Growth	No Growth	No Growth	No Growth	No Growth	No Growth

Limit of detection of lag phase was 5 h.
Growth parameters were calculated with the Baranyi model (Baranyi *et al.* 1993) except those marked with * which were calculated on the graph as the maximum slope of the growth curve.

This is consistent with the observation that psychrotrophic strains of *B. cereus* were unable to grow at 7 °C and below in a broth made with vegetable. As 4 °C is the recommended storage temperature for these products in France (Anon 1991), *B. cereus* should not be a hazard whenever refrigeration is properly maintained throughout the shelf-life of the product. At 10 °C, numbers of *B. cereus* found in pasteurized purées after 3 weeks were above 10^4 cfu g^{-1}, the maximum limit recommended in France for some processed vegetables (Jouve 1996). Three weeks was the normal shelf-life of this product, and no spoilage was visible at this stage to warn consumers that the product had been temperature abused and was unfit for consumption. In contrast, cooked vegetables packaged after heating spoiled rapidly, presumably because recontamination after cooking occurred in spite of the high care conditions used for packaging, and shelf-life was too short to allow a significant growth of *B. cereus.*

Numbers of *B. cereus* found in pasteurized purées stored at 10 °C indicate that it grew at a rate of approximately 4–5 log cycles in 3 weeks. In a rich laboratory medium at 10 °C, *B. cereus* grew to the same extent in only 3–4 days. In contrast, the growth observed in courgette broth was consistent with the results found in pasteurized purées. The slower growth of *B. cereus* in vegetable broth at 10 °C and below indicates that vegetables presumably lack some nutrients or factors needed for growth at low temperatures. In other food products, such as pasteurized milk, growth of *B. cereus* is similar to that observed in a rich laboratory medium; the bacterium grew at 10 °C from 0·1 to 10^5 cfu ml^{-1} in 4 days (Notermans *et al.* 1997). However, at 7 °C, Larsen and Jorgensen (1999) reported generation times of *B. cereus* in milk varying from 8 h (a result similar to that reported here in a rich laboratory medium) to over 24 h. Predicting the fate of *B. cereus* in foods from results obtained in rich medium may therefore frequently lead to an important over-estimation of the number of *B. cereus*.

Nearly all colonies isolated from the selective medium for *B. cereus* were confirmed as *B. cereus* by ISO 7932 and NF V 08–058 procedures. The two procedures gave the same results. A large diversity in API profiles was found among the *B. cereus* isolates, with differences between the two products. A large diversity was also observed among growth profiles, heat resistance at 90 °C and the level of cytotoxicity. The range of heat resistance found here was lower than that reported by Gilbert *et al.* (1974), Bradshaw *et al.* (1975), Rajkowski and Mikolajcik (1987), Dufrenne *et al.* (1994) and Faille *et al.* (1997), but similar to that found by Shehata and Collins (1972), Bassen *et al.* (1989) and Arinder *et al.* (1999). The most heat-resistant strains were not psychrotrophic, and the strains growing at 5 °C were the least heat-resistant. However, besides these few strains with extreme profiles, there was no clear relation between heat resistance of spores and temperature of growth, as observed by Dufrenne *et al.* (1994). It is worth noting that the strains from the two API profiles *B. cereus* 1 and *B. cereus* 2 demonstrated distinct heat resistance profiles.

With the exception of two, all strains were positive with at least one of the enterotoxin detection kits, a result similar to that reported by Rusul and Yaacob (1995). The BCET-RPLA test detects one protein of the haemolytic toxic complex and the TECRA-BDE detects one protein of the non-haemolytic toxic complex (Granum and Lund 1997). Most strains were positive with both tests and pre-

sumably had the two toxic complex components. The toxins produced were presumably active because culture supernatant fluids of strains were toxic to Caco and Vero cells (Granum *et al*. 1996). The inactivation of the cytotoxic effect by mild heating supported the assumption that it was due to the heat-labile enterotoxin (Granum 1997). The strains found in the two products were therefore potential food-borne pathogens. However, the intensity of the cytotoxic activity varied among the strains and it is interesting to note that the most psychrotrophic strains demonstrated least cytotoxicity.

Emetic strains of *B. cereus* are usually unable to degrade starch (Kramer and Gilbert 1989; Agata *et al*. 1996). The few strains from cooked vegetables which belonged to the API profile *B. cereus* 2 did not hydrolyse starch and could be emetic strains. These strains were unable to grow at low temperature and were among those demonstrating some heat resistance at 90 °C. Furthermore, these strains were not found in both products, which suggests that *B. cereus* 2 may be less common than other profiles of *B. cereus*.

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