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Discrimination of Psychrotrophic and Mesophilic Strains of the *Bacillus cereus* Group by PCR Targeting of Major Cold Shock Protein Genes

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Detection of psychrotrophic strains (those able to grow at or below 7°C) of the *Bacillus cereus* group (*Bacillus cereus*, *Bacillus thuringiensis*, and *Bacillus mycoides*) in food products is at present extremely slow with conventional microbiology. This is due to an inability to discriminate these cold-adapted strains from their mesophilic counterparts (those able to grow only above 7°C) by means other than growth at low temperature, which takes 5 to 10 days for detection. Here we report the development of a single PCR assay that, using major cold shock protein-specific primers and appropriate annealing temperatures, is capable of both rapidly identifying bacteria of the *B. cereus* group and discriminating between psychrotrophic and mesophilic strains. It is intended that this development help to more accurately predict the shelf life of refrigerated pasteurized food and dairy products and to reduce the incidence of food poisoning by psychrotrophic strains of the *B. cereus* group.

Psychrotrophic (psychrotolerant) bacteria have been recognized as a recurring problem in the refrigerated storage and distribution of perishable food products, which is particularly pertinent to the dairy industry (13, 15). Research into the bacterial spoilage of dairy products has focused predominantly on postpasteurization contaminants, such as *Pseudomonas*. However, improved processing conditions for milk and other dairy products have decreased interest in these non-heat-resistant contaminants, with more attention being directed towards psychrotrophic sporeformers such as *Bacillus* (2, 8, 13, 17). It has been estimated that 25% of all shelf life problems associated with conventionally pasteurized milk and cream products in the United States may be linked to this class of thermoduric bacteria, with a large number of the contaminants being psychrotrophic *Bacillus cereus* and *Bacillus mycoides* (13). Moreover, in many European countries, where the average storage temperature of milk products is several degrees higher than that in the United States, psychrotrophic *Bacillus* species, especially *B. cereus*, have proven to be an even greater problem to the dairy industry and consumers (1, 8, 16).

B. cereus, *Bacillus thuringiensis*, and *B. mycoides* are very closely related bacteria that, in the case of the former two species at least, are known to cause food poisoning through the production of enterotoxins (3, 7, 14). Differentiation of this bacterial group, which also includes *Bacillus anthracis*, the causative agent of anthrax in humans and animals, has been achieved by using a number of biochemical and molecular techniques (9, 18). However, since all psychrotrophic strains of the *B. cereus* group can proliferate at refrigeration temperatures (i.e., at or below 7°C), there is also a need to distinguish these cold-adapted strains from their mesophilic counterparts. Early detection and quantification of such psychrotrophic bacteria might allow food manufacturers to more accurately predict the shelf life of perishable products and thus reduce the incidence of food poisoning by strains of the *B. cereus* group.

The detection of psychrotrophic strains of the *B. cereus* group in dairy products is at present extremely slow (5 to 10 days), due to the inability to discriminate psychrotrophic and mesophilic strains by means other than growth at low temperature. In order to develop a rapid detection method for such bacteria, at least some molecular aspects of psychrotrophic adaptation need to be known. Studies directed at understanding the molecular mechanisms adopted by *Bacillus* for dealing with growth at low temperature (i.e., cold adaptation) have recently focused on the role played by a family of small polypeptides termed the major cold shock proteins (5, 11). Certain of these proteins, which are widespread in bacteria (4), are significantly induced by a temperature downshift and appear to play a fundamental role in the bacterium's survival at low temperature (6). These proteins were therefore made the focus of our attention, since it was suspected that subtle differences in their regulation and/or structure might contribute to the psychrotrophic growth of strains of the *B. cereus* group. Prior studies of the family of major cold shock proteins found in *B. cereus* have shown CspA to be the homologue most significantly induced by cold shock (11); therefore, initial studies were directed at this particular major cold shock protein.

Psychrotrophic and mesophilic *B. cereus* and *B. mycoides* organisms were isolated from milk samples obtained from three dairies in southern Germany (G1, G2, and G3 [Table 1]). Strains were initially isolated on *B. cereus* selective agar (PEMBA; Oxoid) and tested for the ability to grow at or below 7°C in liquid culture (plate count [PC] medium; Merck). Isolates were then individually characterized according to a range of criteria, including colony morphology, biochemical profiling, and phage typing (12). From these data 28 isolates (9 psychrotrophic *B. cereus* isolates, 16 mesophilic *B. cereus* isolates, and 3 psychrotrophic *B. mycoides* isolates) were characterized as

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TABLE 1. Origins, minimum growth temperatures, and PCR data of *B. cereus* group strains examined in this study[a]

Strain	Strain code	Dairy	Product contaminated	Growth at 7°C	Signature[b]		Product amplification		Deduced phenotype strain
					5′ *cspA*	3′ *cspA*	*cspA*	*cspF*	
B. c	WSBC10201	G1	Milk	+	**ACAGTT**	**T**TAGAA	+	+	Psychrotrophic
B. c	WSBC10202	G2	Milk	+	**ACAGTT**	**T**TAGAA	+	+	Psychrotrophic
B. c	WSBC10204	G1	Milk	+	**ACAGTT**	**T**TAGAA	+	+	Psychrotrophic
B. c	WSBC10205	G1	Milk	+	**ACAGTT**	**T**TAGAA	+	+	Psychrotrophic
B. c	WSBC10206	G1	Milk	+	**ACAGTT**	**T**TAGAA	+	+	Psychrotrophic
B. c	WSBC10208	G1	Milk	+	**ACAGTT**	**T**TAGAA	+	+	Psychrotrophic
B. c	WSBC10209	G1	Milk	+	**ACAGTT**	**T**TAGAA	+	+	Psychrotrophic
B. c	WSBC10210	G2	Milk	+	**ACAGTT**	**T**TAGAA	+	+	Psychrotrophic
B. c	WSBC10211	G1	Milk	+	**ACAGTT**	**T**TAGAA	+	+	Psychrotrophic
B. c	WSBC10027	G1	Milk	−	**GCAGTA**	**C**TAGAA	−	+	Mesophilic
B. c	WSBC10028	G1	Milk	−	**GCAGTA**	**C**TAGAA	−	+	Mesophilic
B. c	WSBC10029	G1	Milk	−	**GCAGTA**	**C**TAGAA	−	+	Mesophilic
B. c	WSBC10030	G1	Milk	−	**GCAGTA**	**C**TAGAA	−	+	Mesophilic
B. c	WSBC10031	G1	Milk	−	**GCAGTA**	**C**TAGAA	−	+	Mesophilic
B. c	WSBC10032	G1	Milk	−	**GCAGTA**	**C**TAGAA	−	+	Mesophilic
B. c	WSBC10033	G1	Milk	−	**GCAGTA**	**C**TAGAA	−	+	Mesophilic
B. c	WSBC10034	G1	Milk	−	**GCAGTA**	**C**TAGAA	−	+	Mesophilic
B. c	WSBC10035	G1	Milk	−	**GCAGTA**	**C**TAGAA	−	+	Mesophilic
B. c	WSBC10036	G1	Milk	−	**GCAGTA**	**C**TAGAA	−	+	Mesophilic
B. c	WSBC10037	G1	Milk	−	**GCAGTA**	**C**TAGAA	−	+	Mesophilic
B. c	WSBC10038	G1	Milk	−	**GCAGTA**	**C**TAGAA	−	+	Mesophilic
B. c	WSBC10039	G1	Milk	−	**GCAGTA**	**C**TAGAA	−	+	Mesophilic
B. c	WSBC10040	G1	Milk	−	**GCAGTA**	**C**TAGAA	−	+	Mesophilic
B. c	WSBC10041	G1	Milk	−	**GCAGTA**	**C**TAGAA	−	+	Mesophilic
B. c	WSBC10042	G1	Milk	−	**GCAGTA**	**C**TAGAA	−	+	Mesophilic
B. c	HER1399	NK	NK	−	**GCAGTA**	**C**TAGAA	−	+	Mesophilic
B. c	HER1414	NK	NK	−	**GCAGTA**	**C**TAGAA	−	+	Mesophilic
B. c	ATCC 7064	NK	NK	−	**GCAGTA**	**C**TAGAA	−	+	Mesophilic
B. c	ATCC 27877	NK	NK	−	**GCAGTA**	**T**TAGAA	−	+	Mesophilic
B. m	WSBC10276	G3	Milk	+	**ACAGTT**	**T**TAGAA	+	+	Psychrotrophic
B. m	WSBC10278	G3	Milk	+	**ACAGTT**	**T**TAGAA	+	+	Psychrotrophic
B. m	WSBC10279	G3	Milk	+	**ACAGTT**	**T**TAGAA	+	+	Psychrotrophic
B. t	HER1357	NK	NK	−	**GCAGTA**	**C**TAGAA	−	+	Mesophilic
B. t	HER1078	NK	NK	−	**GCAGTA**	**C**TAGAA	−	+	Mesophilic
B. t	HER1410	NK	NK	−	**GCAGTA**	**C**TAGAA	−	+	Mesophilic
B. t	HER1418	NK	NK	−	**GCAGTA**	**C**TAGAA	−	+	Mesophilic
B. c	WSBC10289	G3	Milk	+	ND	ND	+	+	Psychrotrophic
B. c	WSBC10286	G4	Cream	−	ND	ND	−	+	Mesophilic
B. c	WSBC10287	G4	Cream	−	ND	ND	−	+	Mesophilic
B. c	WSBC10228	G5	UHT Cream	−	ND	ND	−	+	Mesophilic
B. c	WSBC10227	G6	UHT Milk	−	ND	ND	−	+	Mesophilic
B. c	WSBC10181	G7	Milk	−	ND	ND	−	+	Mesophilic
B. c	WSBC10288	G8	UHT Shake	−	ND	ND	−	+	Mesophilic
B. c	WSBC10045	G9	Milk	+	ND	ND	+	+	Psychrotrophic
B. c	WSBC10088	G9	Milk	−	ND	ND	−	+	Mesophilic
B. c	WSBC10089	G9	Milk	+	ND	ND	+	+	Psychrotrophic
B. c	WSBC10290	G10	Milk	−	ND	ND	−	+	Mesophilic
B. c	CW1	G11	Milk Powder	−	ND	ND	−	+	Mesophilic
B. c	CW2	G11	Milk Powder	−	ND	ND	−	+	Mesophilic
B. c	CW3	G11	Milk Powder	−	ND	ND	−	+	Mesophilic
B. c	CW4	G11	Milk Powder	−	ND	ND	−	+	Mesophilic
B. c	CW5	G11	Milk Powder	−	ND	ND	−	+	Mesophilic
B. c	CW6	G11	Milk Powder	−	ND	ND	−	+	Mesophilic
B. c	CW7	G11	Milk Powder	−	ND	ND	−	+	Mesophilic
B. c	CW8	G11	Milk Powder	−	ND	ND	−	+	Mesophilic
B. c	CW9	G11	Milk Powder	+	ND	ND	+	+	Psychrotrophic
B. c	WSBC10244	Den	Milk	−	ND	ND	−	+	Mesophilic
B. c	PG1	Nor	Cream	+	ND	ND	+	+	Psychrotrophic
B. c	PG2	Nor	Cream	−	ND	ND	−	+	Mesophilic
B. c	PG3	Nor	Butter	−	ND	ND	−	+	Mesophilic
B. c	PG4	Nor	Cream	−	ND	ND	−	+	Mesophilic
B. c	ATCC 14579^T	NK	NK	−	ND	ND	−	+	Mesophilic
B. m	WSBC10291	Tha	Soil	−	ND	ND	−	+	Mesophilic
B. m	WSBC10292	Tha	Soil	−	ND	ND	−	+	Mesophilic
B. m	WSBC10293	Tha	Soil	−	ND	ND	−	+	Mesophilic
B. m	ATCC 6462^T	NK	NK	+	ND	ND	+	−	Psychrotrophic
B. t	HER1211	NK	NK	−	ND	ND	−	+	Mesophilic
B. t	HER1230	NK	NK	−	ND	ND	−	+	Mesophilic
B. t	HER1231	NK	NK	−	ND	ND	−	+	Mesophilic
B. t	HER1232	NK	NK	−	ND	ND	−	+	Mesophilic
B. t	HER1233	NK	NK	−	ND	ND	−	+	Mesophilic
B. t	HER1236	NK	NK	−	ND	ND	−	+	Mesophilic

Continued on following page

TABLE 1—*Continued*

Strain	Strain code	Dairy	Product contaminated	Growth at 7°C	Signature[b]		Product amplification		Deduced phenotype strain
					5′ *cspA*	3′ *cspA*	*cspA*	*cspF*	
B. t	HER1380	NK	NK	−	ND	ND	−	+	Mesophilic
B. t	HER1387	NK	NK	−	ND	ND	−	+	Mesophilic
B. t	HER1404	NK	NK	−	ND	ND	−	+	Mesophilic
B. t	PO1	Pol	Soil	−	ND	ND	−	+	Mesophilic
B. t	PO2	Pol	Soil	−	ND	ND	−	+	Mesophilic
B. t	PO3	Pol	Soil	−	ND	ND	−	+	Mesophilic
B. t	PO4	Pol	Soil	−	ND	ND	−	+	Mesophilic
B. t	PO5	Pol	Soil	−	ND	ND	−	+	Mesophilic
B. t	PO6	Pol	Soil	−	ND	ND	−	+	Mesophilic
B. t	PO7	Pol	Soil	−	ND	ND	−	+	Mesophilic
B. t	PO8	Pol	Soil	−	ND	ND	−	+	Mesophilic
B. t	PO9	Pol	Soil	−	ND	ND	−	+	Mesophilic
B. t	PO10	Pol	Soil	−	ND	ND	−	+	Mesophilic
B. t	PO11	Pol	Soil	−	ND	ND	−	+	Mesophilic
B. t	PO12	Pol	Soil	−	ND	ND	−	+	Mesophilic
B. t	PO13	Pol	Soil	−	ND	ND	−	+	Mesophilic
B. t	PO14	Pol	Soil	−	ND	ND	−	+	Mesophilic
B. t	PO15	Pol	Soil	−	ND	ND	−	+	Mesophilic
B. t	PO16	Pol	Soil	−	ND	ND	−	+	Mesophilic
B. t	PO17	Pol	Soil	−	ND	ND	−	+	Mesophilic
B. t	PO18	Pol	Soil	−	ND	ND	−	+	Mesophilic
B. t	PO19	Pol	Soil	−	ND	ND	−	+	Mesophilic
B. t	PO20	Pol	Soil	−	ND	ND	−	+	Mesophilic
B. t	PO21	Pol	Soil	−	ND	ND	−	+	Mesophilic
B. t	PO22	Pol	Soil	−	ND	ND	−	+	Mesophilic
B. t	PO23	Pol	Soil	−	ND	ND	−	+	Mesophilic
B. t	PO24	Pol	Soil	−	ND	ND	−	+	Mesophilic
B. t	ATCC 10792[T]	NK	NK	−	ND	ND	−	+	Mesophilic

[a] Growth of *B. cereus* (*B. c*), *B. thuringiensis* (*B. t*), and *B. mycoides* (*B. m*) at 7°C (plus, growth; minus, no growth) was determined by inoculating a 5-ml volume of PC medium (Oxoid) with a 10 μl volume of overnight culture and then monitoring it for turbidity after 5 to 10 days of incubation with agitation at this temperature. Strains prefixed by WSBC were isolated and characterized from dairy samples by R. Mayr (12) and form part of the Weihenstephan Bacillus Collection. Strains prefixed by HER, CW, PG, and PO are from H. Ackermann (Reference Center for Bacterial Viruses, Quebec, Canada), C. Wiebe (Bundesanstalt für Milchforschung, Kiel, Germany), P. Granum (Norwegian College of Veterinary Medicine, Oslo, Norway), and E. Lonc (Wroclaw University, Wroclaw, Poland [10]), respectively. G1 to G11 denote dairies situated throughout Germany, Den is a dairy in Denmark, and Nor represents dairy isolates from Norway. Pol and Tha denote soil samples from Poland and Thailand, respectively. Strains where the source is not known (NK) are marked accordingly. The 5′ and 3′ *cspA* signatures correspond to the nucleotide positions 4 to 9 and 124 to 129 of the *B. cereus cspA* coding sequence (11), respectively. Strains in which these signatures were not determined (ND) are marked accordingly. Amplification of PCR products corresponding to *cspA* (160 bp) and *cspF* (284 bp), examples of which can be seen in Fig. 1, are indicated for each strain by a plus if a product was seen and a minus in the absence of a product (see the text for PCR conditions). UHT, ultrahigh temperature-treated product.

[b] Boldface represents signature bases allowing PCR discrimination.

different strains. *cspA* was PCR amplified from each of these 28 strains and additionally from 4 mesophilic *B. cereus* strains and 4 mesophilic *B. thuringiensis* strains gained from other sources (i.e., those strains with *cspA* signatures shown in Table 1). PCR was performed with a Techne Progene automated thermocycler with 0.2-ml thin-walled PCR tubes (Advanced Biotechnologies). Reactions were carried out in 50-μl volumes containing 5 μl of 10× PCR buffer (supplied with *Taq* DNA polymerase; Eurogentec), 2.0 mM $MgCl_2$, 50 pmol of each oligonucleotide primer (see below), 0.2 mM each deoxynucleotide triphosphate (dATP, dCTP, dGTP, and dTTP; Eurogentec), 1 U of *Taq* DNA polymerase (Eurogentec), and a pinhead-sized aliquot of bacteria picked from a PEMBA plate. Bacterial cells were lysed by heating the mixture at 95°C for 5 min. Amplification of *cspA* was then attempted, with 30 cycles at 95°C for 15 s, 50°C for 30 s, and 72°C for 30 s, followed by a final extension step at 72°C for 2 min. The oligonucleotide primers used to amplify *cspA* from the above strains were BcAF2, CGA ATT TGA TAA TGT GTG GAT TC, an oligonucleotide that is specific to a 5′ noncoding region of *cspA* from the psychrotrophic *B. cereus* strain WSBC10201 (11), and CSPU3, CCC GGA TCC GGT TAC GTT A(G/C)C (A/T)GC T(G/T)(G/C) (A/T/C)GG (G/A/T)CC (degeneracies shown in parentheses), a universal major cold shock protein oligonucleotide that hybridizes at the 3′ end of many bacterial *cspA* homologues, including *B. cereus* (4).

Alignment of *cspA* gene sequences obtained from each of the above-mentioned psychrotrophic and mesophilic strains (28 sequences in all) showed that, although the sequences are extremely homologous (alignment of complete sequences not shown), there are three distinct nucleotide positions within the coding region of this gene at which the two thermal groups of bacteria vary consistently (positions 4, 9, and 124 of the *cspA* coding sequence [11]). These nucleotide differences allowed us to design the discriminatory primers BcAPF1 (GAG GAA ATA ATT ATG **A**CA GT**T**) and BcAPR1 [CTT (C/T)TT GGC CTT CTT CTA **A**] (discriminatory signature bases are shown in boldface, with a single degeneracy shown in parentheses), which favor the amplification of *cspA* sequences from psychrotrophic strains as opposed to those from mesophilic strains (Table 1). PCR performed with the primers at an annealing temperature of 55°C (with the remainder of the PCR conditions identical to those described previously) allowed a 160-bp DNA fragment of *cspA* to be amplified only from psychrotrophic strains of the *B. cereus* group. Identical results were achieved with an annealing temperature of 52°C, providing a 3°C margin of error (e.g., allowing for temperature variations between PCR blocks). In addition to these psychrotrophic *cspA* primers, a third primer was designed to allow the detection of all strains of the *B. cereus* group (both psychrotrophic and mesophilic strains), providing a positive control to confirm the presence of mesophilic strains that were not am-

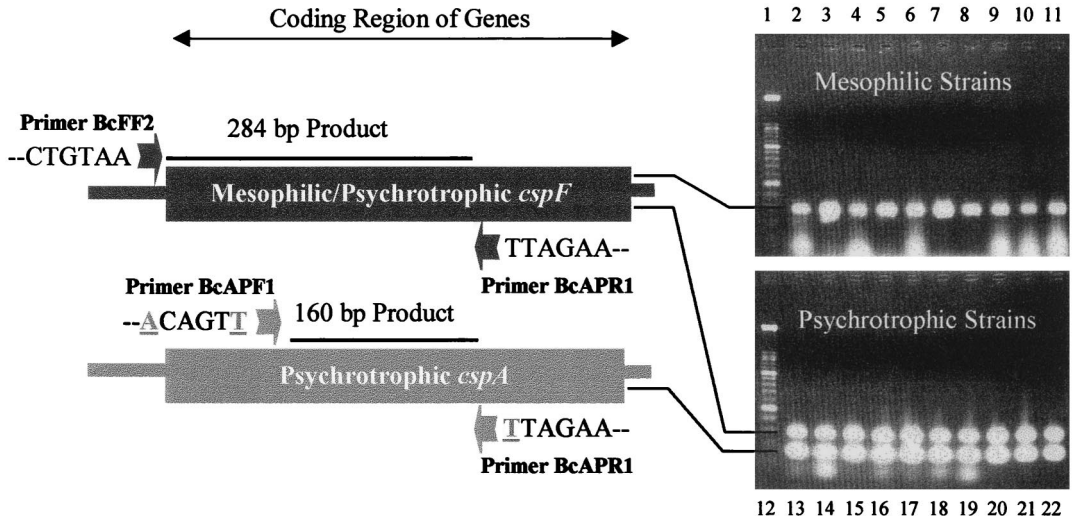

FIG. 1. Schematic diagram of *cspA* and *cspF*, the two genes used as PCR target sequences for the differentiation of psychrotrophic and mesophilic strains of the *B. cereus* group (left). The approximate position at which each of the three primers hybridizes to each of the genes and the sizes of the resulting PCR products are indicated. Signature bases, allowing PCR amplification of psychrotrophic *cspA* only, are underlined and shown in grey. The right-hand side of the figure shows two pictures of an ethidium bromide-stained agarose gel, with examples of DNA amplification from psychrotrophic and mesophilic strains of the *B. cereus* group obtained with our PCR assay. The top picture shows amplified DNA products from 10 mesophilic strains (lanes: 2, WSBC10027; 3, WSBC10028; 4, HER1399; 5, ATCC 7064; 6, ATCC 27877; 7, HER1357; 8, CW1; 9, ATCC 14579^T; 10, ATCC 6462^T; 11, ATCC 10792^T). The bottom picture shows amplified DNA products from 10 psychrotrophic strains (lanes: 13, WSBC10201; 14, WSBC10202; 15, WSBC10276; 16, WSBC10278; 17, WSBC10289; 18, WSBC10045; 19, WSBC10089; 20, CW9; 21, PG1; 22, ATCC 6462^T). Lanes 1 and 12 were loaded with 100-bp DNA ladders. PCR was performed as described in the text, with an annealing temperature of 55°C.

plified by BcAPF1 and BcAPR1. This third primer, designated BcFF2 (GAG ATT TAA ATG AGC TGT AA), was designed from a 5′ noncoding region found in both psychrotrophic and mesophilic *B. cereus cspF* genes (our unpublished data). Due to the high degree of similarity found between major cold shock protein homologues (11), BcAPR1 is able to hybridize not only to psychrotrophic *cspA* sequences but also to *cspF* at a comparable region (the positioning of the primers is shown in Fig. 1). Furthermore, BcAPR1 hybridizes equally to both psychrotrophic and mesophilic *B. cereus cspF* sequences (both have identical complementary sequences). Hence, BcAPR1 in combination with BcFF2 can amplify a 284-bp *cspF* DNA fragment from both psychrotrophic and mesophilic strains of the *B. cereus* group whereas BcAPR1 in combination with BcAPF1 can only amplify a 160-bp *cspA* DNA fragment from psychrotrophic strains of this group (Fig. 1). Again, the PCR conditions used to accomplish the above amplifications were identical to those described previously, with the exception that the annealing temperature was 55°C (52°C worked equally well).

In addition to testing the PCR assay described above with those strains employed in its construction (i.e., the strains with *cspA* signatures shown in Table 1), a large number of additional *B. cereus*, *B. thuringiensis*, and *B. mycoides* strains that were isolated from a wider variety of sources (in this study, unless otherwise stated) were also screened by the same PCR procedure (Table 1). Additional *B. cereus* strains were isolated from dairy products originating from nine other dairies situated throughout Germany (G3 to G11), as well as from dairy products from Denmark and Norway (P. Granum, Norwegian College of Veterinary Medicine, Oslo, Norway). Additional strains of *B. thuringiensis* were obtained from two other sources (E. Lonc, Wroclaw University, Wroclaw, Poland [10], and H. Ackermann, Reference Center for Bacterial Viruses, Quebec, Canada), while other *B. mycoides* strains were isolated from soil from Thailand. Type strains of *B. cereus*, *B. thuringiensis*, and *B. mycoides* were also included. Table 1 shows a summary of the data gained from these different strains, demonstrating in every case a correlation between the ability to grow at 7°C and the occurrence of a second 160-bp DNA fragment amplified by the above PCR assay.

To ensure that this PCR assay was specific for the detection of bacteria of the *B. cereus* group (i.e., *B. cereus*, *B. thuringiensis*, and *B. mycoides*) at an annealing temperature of 55°C (or 52°C), a large number of dairy-associated bacteria (both *Bacillus* and non-*Bacillus* strains) were also screened by the above-mentioned PCR procedure. The *Bacillus* species tested were *B. brevis* (ATCC 8246^T), *B. circulans* (ATCC 4513^T), *B. coagulans* (ATCC 7050^T), *B. firmus* (ATCC 14575^T), *B. laterosporus* (ATCC 64^T), *B. lentus* (ATCC 10840^T), *B. licheniformis* (ATCC 14580^T), *B. macerans* (ATCC 8244^T), *B. megaterium* (ATCC 14581^T), *B. polymyxa* (ATCC 842^T), *B. pumilis* (ATCC 7061^T), *B. sphaericus* (ATCC 14577^T), and *B. subtilis* (ATCC 6051^T). The non-*Bacillus* species tested were *Alcaligenes faecalis* (ATCC 8750^T), *Arthrobacter nicotianae* (ATCC 15236^T), *Brevibacterium linens* (WS 1978), *Clostridium perfringens* (ATCC 13124^T), *Corynebacterium glutamicum* (ATCC 14020), *Enterobacter cloacae* (ATCC 13047^T), *Enterococcus faecalis* (ATCC 19433^T), *Escherichia coli* (ATCC 11775^T), *Flavobacterium flavescens* (ATCC 8315), *Klebsiella pneumoniae* (ATCC 9997), *Lactobacillus plantarum* (ATCC 14917^T), *Lactococcus lactis* subsp. *lactis* (WS1042), *Listeria monocytogenes* (WSLC1364), *Microbacterium lacticum* (WS2024), *Micrococcus luteus* (WS1513), *Proteus vulgaris* (ATCC 13315^T), *Pseudomonas fluorescens* (DSM6147), *Salmonella amersfoort* (WS2686), and *Staphylococcus aureus* (ATCC 6538). No strains other than *B. cereus*, *B. thuringiensis*, and *B. mycoides* gave any amplification of PCR products. Moreover, 16S ribosomal DNA control PCRs, conducted in parallel, confirmed that PCR was capable of occurring efficiently for each bacterium (data not shown).

We believe that development of the above-mentioned PCR assay will significantly reduce the time necessary for screening pasteurized food and dairy products for contamination by psychrotrophic strains of the *B. cereus* group. It is also possible that employment of this assay during food processing will allow

the number of such contaminating bacteria to be more accurately estimated, reducing the incidence of food poisoning by these potential pathogens.

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