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Research Note

Cold Temperature Growth of Clinical and Food Isolates of *Bacillus cereus*‡

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ABSTRACT

A collection of 27 *Bacillus cereus* food and clinical isolates were screened for the ability to grow at cold temperatures. Growth was examined using fluid or solid nutrient media or milk incubated at 10, 7, or 5°C. Fourteen isolates were capable of visible colony formation on brain heart infusion (BHI) agar by day 7 at 10°C; two isolates formed visible colonies by day 10 at 7°C on Trypticase soy agar. Nineteen of the isolates could grow in BHI at 7°C if previously adapted to 7°C over a five-week period. Both food and clinical isolates demonstrated a cold adaptation response. This should be considered when modeling *B. cereus* growth in foods or in assessing shelf life or safety relative to *B. cereus*.

Key words: *Bacillus cereus*, cold temperature growth, adaptation, psychrotroph

Bacillus cereus is recognized as a psychrotrophic organism which may be involved in either an emetic or diarrheal foodborne illness *(10)*. However, not all foodborne isolates are psychrotrophic, and those that are may be affected by prior growth history such that their ability to grow at cold temperature may not be manifested merely by brief incubation at a low temperature. Johnson et al. *(6)* detected growth of some *B. cereus* strains between 15 and 50°C in rice or Trypticase soy broth. The inoculum for these studies was a freshly heat-shocked (70°C, 15 min) spore suspension. A number of researchers have isolated psychrotrophic *B. cereus* from raw or pasteurized milk or milk products where growth was detected at temperatures as low as 8°C *(1)*, 6°C *(4, 5, 12)*, 7°C *(3, 7, 9)*, 5°C *(11)*, 4°C *(13)*, or 1°C *(2)* (although the observation of growth at 1°C was for one strain only and growth at 4°C was more common). Psychrotrophic *B. cereus* strains also have been isolated from other foods or implicated in foodborne illness associated with refrigerated foods *(8, 13)*. Psychrotrophic strains of *B. cereus* may produce enterotoxin *(3, 4, 13)*, and toxin production may occur at refrigeration temperatures *(1, 5)* especially if the growth medium is aerated *(1)*. Griffiths *(5)* and van Netten et al. *(13)* suggested and a recent report by Dufrenne et al. *(3)* proved that the ability of *Bacillus cereus* to demonstrate growth at low temperatures is dictated by prior temperature handling history. Dufrenne et al. *(3)* documented that the lag phase for growth in brain heart infusion broth or skim milk at 7°C was affected by prior growth of cells at 7, 30, or 37°C; the lag phase increased as prior growth temperature increased. Generation times were not significantly affected by prior growth temperature. As part of a larger study on cold stress response, 27 food and clinical isolates of *B. cereus* were screened for their ability to grow at psychrotrophic temperatures on solid and liquid media. The screening included cells held at cold temperature prior to evaluation and those tested after incubation at 30°C.

MATERIALS AND METHODS

B. cereus *strains and inocula preparation*

The identity, source, and origin of the 27 *B. cereus* isolates are presented in Table 1. All cultures were maintained as frozen (−85°C) stocks, which were used to inoculate 5 ml portions of broth media (brain heart infusion [BHI] broth or tryptic soy broth [TSB], as appropriate; Difco Laboratories, Detroit, MI). Inocula were incubated overnight at 30°C prior to use except where noted.

Growth at 10°C on BHI agar

BHI agar (BHIA) plates were tempered to 10°C prior to inoculation. Single loopfuls of the *B. cereus* inocula were streaked for isolation onto separate plates. Plates were incubated at 10°C up to 7 days and observed for growth.

Growth at 10°C in BHI

Seventy-five-milliliter volumes of BHI were inoculated at 1% from the overnight culture inocula, giving initial cell concentrations of 10^5 to 10^6 CFU/ml. Flasks were incubated at 10°C with shaking at 100 rpm. The inoculated medium reached 10°C within

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TABLE 1. *Source, origin, and cold temperature growth characteristics of* Bacillus cereus *strains*

Strain	Source	Origin and information	BHIA, 10°C, 7 days[a]	BHI, 10°C, 9 days[b]	TSA, 7°C, 10 days[a]	BHI, 5°C, 30 days[a]	Skim milk, 5°C, 45 days[a]	Cold-adapted cultures[b]
T	Laboratory culture collection	Laboratory reference strain	+/s	+	−	ND[c]	ND	+
404	F. Busta, Univ. of Minnesota	Unspecified; FDA	+	+	+	−	−	+
M145	F. Busta	Unspecified; FDA	+	+	+	−	−	−
B5ac	G. Clyde, Westreco, Inc.	Food poisoning; D. A. A. Mossel, Louvain, Belgium	−	ND	−	ND	ND	−
B6ac	G. Clyde	Food poisoning; D. A. A. Mossel	−	ND	−	ND	ND	+
B7ac	G. Clyde	Food poisoning; D. A. A. Mossel	+	+	−	−	−	−
B9ac	G. Clyde	Food poisoning; D. A. A. Mossel	−	ND	−	ND	ND	+
5056	G. Clyde	Food poisoning; D. A. A. Mossel	+/s	ND	−	ND	ND	+
F38	G. Clyde	Macaroni and cheese; C. V. Hall Public Health Labs, Seattle, WA	+/s	+	−	ND	ND	+
F39	G. Clyde	Gravy; C. V. Hall Public Health Labs, Seattle, WA	−	ND	−	ND	ND	+
F42	G. Clyde	Gravy; C. V. Hall Public Health Labs, Seattle, WA	−	ND	−	ND	ND	+
F47	G. Clyde	Gravy; C. V. Hall Public Health Labs, Seattle, WA	−	ND	−	ND	ND	+
F77	G. Clyde	Gravy; C. V. Hall Public Health Labs, Seattle, WA	+/s	ND	−	ND	ND	+
Watertown-1	G. Clyde	Chicken salad; Wisconsin State Hygiene Lab	−	ND	−	ND	ND	−
Waukesha 30020 #1	G. Clyde	Hot dogs; Wisconsin State Hygiene Lab	+	+	−	−	−	+
Rhinelander 19668 #1	G. Clyde	Mashed potatoes; Wisconsin State Hygiene Lab	+	+	−	−	−	−
Sheboygan 6102A #1	G. Clyde	Mashed potatoes; Wisconsin State Hygiene Lab	+/s	−	−	ND	ND	+
Milwaukee 1852 #M3	G. Clyde	Gravy; Wisconsin State Hygiene Lab	+/s	+	−	ND	ND	+
F717B/76	G. Clyde	Pasteurized cream; R. J. Gilbert, Central Public Health Laboratory, England	+	+	−	ND	ND	+
F3463/76	G. Clyde	Chicken; R. J. Gilbert	−	−	−	ND	ND	−
F4491/75	G. Clyde	Chinese meal remains; R. J. Gilbert	−	−	−	ND	ND	+
F4548/75	G. Clyde	Chicken in batter; R. J. Gilbert	+/s	−	−	ND	ND	+
F5814/72	G. Clyde	Corned beef; R. J. Gilbert	−	−	−	ND	ND	+
F4810/72	G. Clyde	Emetic; R. J. Gilbert	−	−	−	ND	ND	+
SLH Isolate 1	G. Clyde	Prime rib; Wisconsin State Hygiene Lab	−	−	−	ND	ND	+
KG-7	G. Clyde	Milk isolate	−	−	−	ND	ND	−
MFSE	B. Sheldon, NCSU	Pasteurized egg isolate	+	+	−	ND	ND	−
Summary: number of strains demonstrating growth/number of strains evaluated			14/27	10/18	2/27	0/5	0/5	19/27

[a] +, growth; −, no growth; +/s, slow growth manifested as small colonies.
[b] +, population increased by >1 log cycle; −, population increased by ≤0.5 log cycle or decreased.
[c] ND = not done.

20 min. At 3-day intervals, 1-ml volumes of the experimental cultures were serially diluted in 0.1% peptone water and enumerated by the spread plate procedure on BHIA after 24 h at 30°C.

Growth at 7°C on TSA

TSA plates were tempered to 7°C prior to inoculation by streaking as described above for BHIA. Plates were incubated at 7°C for 10 days and observed for colony formation.

Growth at 5°C in BHI and skim milk

Ten-milliliter volumes of sterile, room temperature skim milk and BHI were inoculated at 1% from the overnight *B. cereus* cultures. The experimental BHI and skim milk cultures were incubated at 5°C (the inoculated medium reached 5°C within 20 min) for 30 and 45 days, respectively, and sampled at ca. 10-day intervals. The 1-ml samples were serially diluted and enumerated as described above.

Growth after adaptation at 7°C

Cultures were propagated from the frozen stocks by overnight growth in BHI for 24 hr at 30°C. Five milliliters of each culture was transferred to 5 ml of fresh BHI which had been tempered to 7°C. These cultures were incubated at 7°C for 1 week. Each culture was transferred weekly as detailed on four additional occasions so that cultures were held and propagated a total of 5 weeks at 7°C. After 5 weeks, 5 ml of each of the BHI cultures was transferred to 45 ml BHI (tempered to 7°C). These cultures were swirled to mix, incubated at 7°C, and sampled immediately and at approximate 3-day intervals for up to 15 days to determine populations by duplicate colony counts. Colony counts were determined by spread plating on TSA with incubation 24 hr at 30°C. Cultures were considered to grow at 7°C under these conditions if populations increased by at least 1 log cycle. Growth after adaptation was assessed using two or three replicate trials with each strain.

RESULTS AND DISCUSSION

The results of the various *B. cereus* cold temperature growth studies are included in Table 1. The initial screening of the 27 isolates at 10°C on BHIA identified 14 *B. cereus* strains that were capable of forming visible colonies in 7 days. Ten of 18 isolates examined exhibited detectable growth in BHI incubated aerobically at 10°C for 9 days. Two isolates demonstrated growth at 10°C on BHIA in 7 days but not on BHI in 9 days. This may reflect differences between the liquid and solid medium relative to oxygen concentration, nutrient availability, diffusion of cellular metabolites, and by-product concentration, as well as other factors. Among the 27 strains screened, growth at 7°C on TSA was detected only for *B. cereus* 404 and M145. Compared to studies using BHI and BHIA at 10°C, the relative inability to grow on TSA at 7°C may have reflected nutrient content of the medium or the reduced temperature. BHI and BHIA are highly nutritious and contain infusions from calf brain and beef heart, proteose peptone, dextrose, and sodium chloride. In our laboratory TSA is routinely used with success to culture thermally injured or healthy cells of *B. cereus* yet lacks sugar and its amino acids are from pancreatic digest of casein and papaic digest of soybean meal. The medium also contains sodium chloride. Growth at 5°C in either BHI or skim milk was not observed for any of the five isolates evaluated, three of which could grow at 10°C on BHIA or BHI and two of which could grow at 7°C on TSA. Nineteen of the cold-adapted cultures demonstrated growth at 7°C in BHI, which is in general agreement with the report by Dufrenne et al. *(3)*, who demonstrated that low-temperature growth of *B. cereus* is influenced by prior adaptation at low temperature. Both food and clinical isolates demonstrated an adaptation response. These data have implications relative to modeling growth of *B. cereus* and for evaluation of the safety and/or shelf life of food products. For example, inoculated pack challenge studies conducted with *B. cereus* should consider the likely temperature history of the natural inoculum and recognize that the results are likely to differ according to whether the inoculum has been adapted at low temperature or not prior to the challenge test. Likewise, useful application of models of *B. cereus* growth in foods must consider temperature history of the cultures used to construct the model.

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