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A Research Note

Incidence and Growth Potential of *Bacillus cereus* in Ready-to-Serve Foods

STANLEY M. HARMON and DONALD A. KAUTTER*

Division of Microbiology, Food and Drug Administration, Washington, DC 20204

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ABSTRACT

To simulate temperature abuse, 106 test portions of ready-to-serve moist foods, 12 test portions of rehydrated powdered infant formula, and 18 test portions of nonfat dry milk were incubated for 20 and 24 h at 26°C, and then examined for *Bacillus cereus*. Of the ready-to-serve moist foods, 88 of 106 were positive for *B. cereus* at levels ranging from 0.25 to 8.5 x 10^6/g after 20 h of incubation and from 0.1 to 58 x 10^6/g after 24 h. All of the powdered milk and 12 of the 15 units of infant formula, representing five brands, were positive, with counts ranging from 0.15 to 5.0 x 10^6/g in 20 h and 5.0 to 49 x 10^6 after 24 h. *B. cereus* counts in the powdered products were low, ranging from 0.09/g for one of two soy-based products to an average of 0.29/g for milk-based products. However, these levels were sufficient to initiate growth of *B. cereus* in almost every 2-oz serving. Similar results were obtained for rehydrated nonfat milk, with initial *B. cereus* counts ranging from 0.29 to 1.5/g; at 26°C the counts averaged 3.3 x 10^7 after 20 h and 5.5 x 10^7 after 24 h. Counts ranged from 2.0 x 10^4 to 1.1 x 10^5 after 9 h in milk and were in excess of 10^6/g after 10.5 h.

Although *Bacillus cereus* had been recognized as a cause of food poisoning for more than 20 years (*6,7,15,18*), little information is available about its occurrence and growth potential in many ready-to-serve moist foods. Most work on this topic has focused on the contamination of raw or unprocessed foods (*1-4,10,12,13,16,20*); however, *B. cereus* frequently contaminates dairy products (*1,5,17,20*) and a few cooked foods (e.g., rice) which often serve as vehicles for food poisoning (*6,15,16*). We recently demonstrated *B. cereus* contamination in several common ready-to-serve moist foods simply by incubating the foods for 20 to 24 h at 26°C to simulate temperature abuse and examining them by standard culturing techniques (*14*).

A total of 108 samples of selected ready-to-serve moist foods were collected and examined. Because this sporeforming aerobic bacterium is often implicated as the cause of diarrheal illness in infants (*8*), five brands of reconstituted infant formula and three brands of nonfat dry milk were also examined. The level of *B. cereus* contamination in these products was determined before and after deliberate temperature abuse.

*Author to whom requests for reprints should be addressed.

MATERIALS AND METHODS

Purchase and incubation of foods

Ready-to-serve moist foods were purchased at serving lines in four different cafeterias in Washington, DC. In most instances, single servings were purchased. The ready-to-serve foods included noodles, mashed potatoes, rice, succotash, lima beans, cauliflower, skim milk, egg and chicken salads, and beef and turkey gravies. The foods were dispensed in styrofoam or plastic carry-out containers, covered with food wrap, and immediately transported to the laboratory for incubation at 26°C. The pasteurized skim milk consisted of one-half pint cartons packed by three different dairies in the vicinity; they were warmed quickly to 26°C and incubated in the original carton in the same manner as the other foods. The nonfat dry milk and powdered infant formulas consisted of popular packaged brands purchased at grocery stores in the DC area. Nonfat dry milk was rehydrated with sterile distilled water (or 0.1% peptone to determine initial counts). Infant formula was reconstituted in boiled and cooled tap water according to the manufacturer's instructions. When thoroughly dissolved, it was incubated and examined in the same manner as the other foods.

Effects of temperature abuse

Each food was held at 26°C for 20 h and checked for obvious signs of spoilage, such as a change in appearance or odor. Plate counts of *B. cereus* were then made on a suitable amount of each product. The remaining portion was reincubated for an additional 4 h and then retested.

Enumeration and confirmation

B. cereus was found and enumerated in the temperature-abused foods by plating on mannitol-egg yolk-polymyxin (MYP) agar as specified in the method of the Association of Official Analytical Chemists (AOAC) (*14*). Five isolates from each sample were confirmed as members of the *B. cereus* group by lipid globule staining and spore determination as described by Holbook and Anderson (*11*) and, when necessary, by biochemical testing as specified in the AOAC method (*14*). Isolates were identified as *B. cereus* by the differential tests recommended by Harmon (*9*). Initial populations of *B. cereus* in nonfat milk and infant formula were determined by a modification of the AOAC most probable number method (*14*). Portions of each product, weighing 10, 1, and 0.1 g, were cultured in triplicate in 0.1% peptone or in trypticase-soy-polymyxin broth as specified in the AOAC method. A 25-g portion of each solid food was weighed aseptically and then homogenized with 225 ml of Butterfield's phosphate buffer in a Model 400 Colworth Stomacher (Dynatech Laboratories, Alexandria, VA).

The homogenized material was diluted and tested as specified by AOAC (*14*).

RESULTS AND DISCUSSION

Incidence and population levels of *B. cereus* in the various foods after incubation for 20 and 24 h at 26°C are shown in Table 1. Of the 106 ready-to-serve moist food test portions examined, 88 were positive for *B. cereus* (Table 1). Typically, the *B. cereus* count exceeded 10^5/g after 20 h of incubation at 26°C and usually increased to several million/g within 24 h. Except in milk and infant formulas, the proliferation of *B. cereus* to levels associated with food poisoning was not accompanied by changes in the appearance or organoleptic quality of the food. This was true especially for starchy foods such as mashed potatoes, noodles, and rice unless these items had been combined with other food components such as meat or tomatoes. This observation is consistent with the experience of food poisoning victims who obviously did not find foods contaminated with large populations of *B. cereus* unpalatable.

Clotting was the most common indication of spoilage in pasteurized fluid milk, reconstituted nonfat dry milk, and milk-based infant formula. Soy-based infant formula was less likely to exhibit visual signs of spoilage than the milk-based product. Gas bubbles were often noted in milk and milk-based infant formulas as an early indication of spoilage; however, clotting of infant formula and nonfat milk usually did not occur for a considerable period of time (usually 2 or 3 h) after the appearance of gas bubbles. The *B. cereus* count had reached several million/ml by the time clotting occurred.

The results obtained in this study are in general agreement with those of previous studies concerning the widespread *B. cereus* contamination of foods such as rice and milk (*1,4-6,12,16,17,19*). The higher incidence of *B. cereus* that we found in powdered infant formula was due, no doubt, to the substantially larger amount of sample that we tested. Assuming that an individual serving of food was the minimum amount that might be subject to risk, the single serving portion was examined in all experiments, including those with milk and infant formulas.

Initial levels of contamination found in three brands of nonfat dry milk and five brands of powdered infant formula are shown in Table 2. The growth response of *B. cereus* at 26°C in the nonfat dry milk and infant formulas is shown in Fig. 1. The growth response values for *B. cereus* (Fig. 1) are similar to those of Becker et al. (*2*) and those we found with inoculated products (unpublished data). Generation times in the different products varied from 43 to 58 min at 26°C.

As shown in this study, an incubation period of 12 h or longer must occur before the *B. cereus* population exceeds 10^4/g in any of the milk or infant formulas tested. Because these foods are generally perceived as being perishable, ordinary temperature abuse would probably not equal that used in our experiments and may explain why so few instances of foodborne *B. cereus* illness in this country have been associated with these foods, despite widespread low level contamination (*1,2,5,19,20*).

TABLE 1. B. cereus *in ready-to-serve moist foods before and after simulated temperature abuse at 26°C.*

Food	Examined	No. positive (%)	*B. cereus* counts/g (x10^6) after incubation 20 h Range	20 h Mean[a]	24 h Range	24 h Mean
Noodles	8	7 (88)	6.1-8.5	6.7	19-50	32
Mashed potatoes	10	10 (100)	0.25-3.1	1.4	5.3-60	24.8
Rice	12	11 (92)	0.35-8.4	1.9	9-26	15.5
Succotash	10	7 (70)	0.5-7.2	2.4	16-30	20.7
Lima beans	6	5 (83)	0.25-3.7	1.6	12-28	19.1
Skim milk	24	24 (100)	0.8-4.8	3.2	11-27	16.7
Infant formula[b] (powdered)	12	9 (75)	0.15-5.0	2.9	5.0-49	6.9
Nonfat milk[c]	18	18 (100)	22-51	33	43-69	55
Chicken salad	4	0 (0)	<0.001	<0.001	<0.001	<0.001
Egg salad	4	1 (25)	--	0.5	--	--
Turkey gravy	4	3 (75)	0.3-7.0	3.4	3-38	16.6
Beef gravy	4	1 (25)	--	--	--	--
Cauliflower	2	1 (50)	--	--	--	--

[a]Arithmetic mean.
[b]Reconstituted with sterile water.
[c]Reconstituted with boiled water.

TABLE 2. B. cereus *contamination of nonfat dry milk and infant formula.*

Product	Brand	*B. cereus* count/g of dry product[a] Range	Average[b]
Nonfat dry milk	A	0.23-1.5	0.72
	B	0.49-2.1	1.09
	C	0.23-0.93	0.53
Infant formula	A	0.23-0.43	0.30
	B	0.09-0.23	0.16
	C	0.43-0.93	0.60
	D[c]	--	<0.03[d]
	E[c]	0.04-0.15	0.09

[a]Most probable number with 10-g test sample. Three units of each product were examined.
[b]Three units of each brand were examined.
[c]Soy-based product. Brands A and B and C were milk bases.
[d]*B. cereus* was not found in any of the 9 g portions examined.

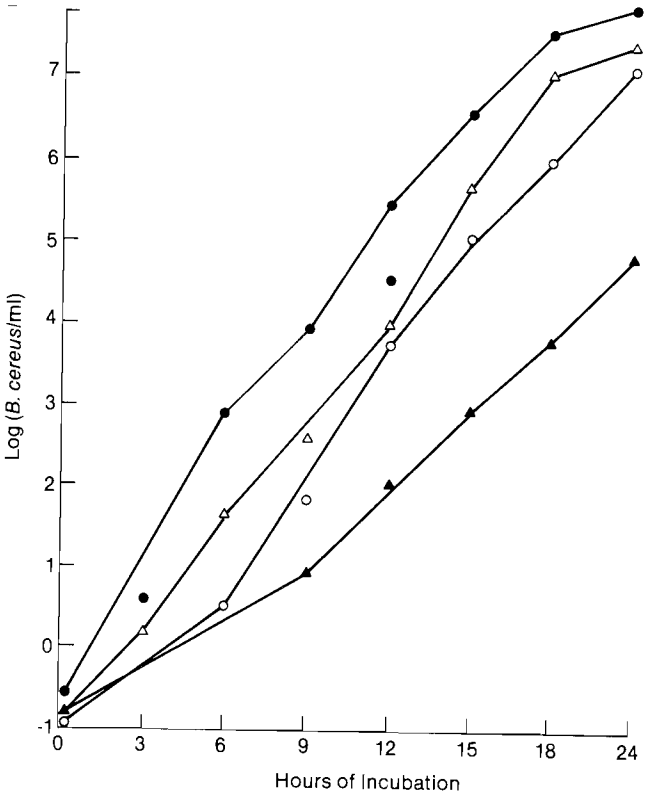

Figure 1. *Growth of naturally occurring* B. cereus *in nonfat dry milk and three brands of infant formula:* ●, *nonfat dry milk (brand B); infant formulas:* Δ, *brand A;* ○, *brand B:* ▲, *brand C.*

Rice has long been recognized as a vehicle of *B. cereus* food poisoning (*7,15,16,18*). The results presented in Table 1 confirm the well-known hazards associated with rice and show that other common ready-to-serve starchy foods such as mashed potatoes and noodles also have the potential to serve as food poisoning vehicles if temperature-abused. Pulses such as lima beans are an additional risk either when served alone or in combination with corn. Although these foods have seldom been incriminated, Blakey and Priest (*3*) have demonstrated their potential as vehicles of *B. cereus* food poisoning.

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