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Maltodextrin Stimulates Growth of *Bacillus cereus* and Synthesis of Diarrheal Enterotoxin in Infant Milk Formulae

N. J. ROWAN* AND J. G. ANDERSON

Department of Bioscience and Biotechnology, University of Strathclyde, Glasgow, Scotland

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One hundred reconstituted milk-based infant formulae (IMF) representative of 10 leading brands available in many European Economic Community countries were examined for *Bacillus cereus* and for the presence of diarrheal enterotoxin. Sixty-three reconstituted IMF supported growth of the organism after 14 h at 25°C, and in 4 of these, which contained maltodextrin, enterotoxin was detected. Reconstituted IMF (and basal synthetic media) supplemented with ≥0.1% maltodextrin supported both growth of *B. cereus* and diarrheal toxin production when incubated for 14 h or more at 25°C.

Reconstituted infant foods are considered to be a food class of high risk due to the susceptibility of infants to enteric bacterial pathogens, their severe response to enterotoxins, and their increased mortality (14). There is, however, no requirement that these foods should be sterile, and many powdered milk products are subjected only to pasteurization prior to spray drying (11). Of particular concern is the occurrence of enterotoxigenic *Bacillus cereus* in these products; Becker et al. (2) reported that 54% of 261 samples of infant food examined (distributed in 17 countries) were contaminated with this organism, reaching levels of 0.3 to 600 cells g^{-1}.

While the infectious dose of *B. cereus* is considered to be in the range of 10^5 to 10^7 cells ml^{-1} (8), outbreaks of food-borne illness associated with infants and other vulnerable groups have been attributed previously to the consumption of foods containing low numbers of this bacterium, in the range of 10^3 to 10^5 cells g^{-1} (6). Fortunately, despite the inherent ability of *B. cereus* spores to survive harsh environmental conditions (12) and the increasing number of food-related illnesses associated with the consumption of either proteinaceous (13) or farinaceous (9) foods containing this organism, the incidence of *B. cereus* food poisoning attributable to milk and milk products remains remarkably low (2). The low incidence of *B. cereus* food intoxication from these products may be due in part to milk being a poor medium for toxin production, as it has a very low free-amino-acid content and lacks glucose (2, 12). Christiansson et al. (4) reported however, that milk inoculated with *B. cereus* strains showed cytotoxicity in culture supernatant after periods of storage at different temperatures.

There is insufficient evidence to establish conclusively whether *B. cereus* diarrheal-type food intoxication results from consumption of preformed toxin (1, 12) or from production of enterotoxin by ingested cells or spores in the ileum (7). However, all of the studies on the stability of the diarrheal enterotoxin that are cited in the literature have been performed in nonfood systems, and recent evidence suggests that this stability may be significantly greater when the toxin is preformed in foods such as milk (1).

Many leading baby food companies now incorporate ingredients of a high calorific value, such as maltodextrin (a derivative of starch hydrolysis), in infant formula products which are intended for premature and weaning babies (1, 14). Here, we present evidence that maltodextrin stimulates growth of *B. cereus* in reconstituted infant milk-based formulae (IMF) (and in basal synthetic media) and synthesis of enterotoxin.

A correlation was made between maltodextrin content (kindly supplied by Cerestar Gruppo Ferruzzi) and synthesis of diarrheal enterotoxin by using three diarrheagenic *B. cereus* II strains, namely, *B. cereus* SU11 and SU58, isolated from analyzed IMF, and *B. cereus* NCTC 11145, obtained from the National Collection of Type Cultures. *Bacillus licheniformis* NCTC 10341 was used as a negative control for enterotoxin production. Cultures were grown at 30°C and maintained on nutrient agar (Oxoid Products); they were subcultured every 2 weeks. Fisher's exact test was used to compare levels of diarrheal enterotoxin (in nanograms per milliliter) produced in test media by *B. cereus*. All significant differences were reported at the 95% level of confidence ($P < 0.05$).

An examination of 100 IMF for *B. cereus* diarrheal enterotoxin. One hundred IMF (i.e., products in which the only protein source was cow's milk), representative of 10 leading brands available in many European Economic Community countries, were analyzed for the presence of *B. cereus* and for diarrheal enterotoxin production. Triplicate 25-g IMF samples were reconstituted in 225 ml of sterile water at 56°C (±0.2°C) by shaking 25 times through an excursion of 30 cm and were then incubated at 25 and 30°C for periods up to and including 24 h. Samples were spread and spiral plated (Spiral plater model B, Spiral Systems Inc.) onto tryptone soy agar (supplemented with 0.6% yeast extract), blood agar (supplemented with 0.7% defibrinated horse blood), nutrient agar (supplemented with 0.5 mg of $MnSO_4H_2O$ $liter^{-1}$ to aid sporulation), and *B. cereus* selective agar (Oxoid Products) and were incubated for up to 72 h at 30°C. *B. cereus* isolates were identified by establishing the following morphological and biochemical properties: positive Gram and catalase reactions; cell width (>1 μm) and length (>3 μm) (Solitaire 512 Image Analyzer; Seescan Plc); motility and lecithovitellin/lecithinase production; β-hemolytic reaction and gross colony morphological appearance (millimeters); an ellipsoidal endospore, centrally or subterminally positioned with nondistention of the sporangium; hydrolysis of starch, casein, and gelatin; and growth in the presence of 7.5% NaCl or 0.001% lysozyme. The identity of each isolate was confirmed by using the API 50 CHB and API 20 E galleries (Biomérieux Ltd.).

Infant foods contaminated with *B. cereus* were assessed for the presence of diarrheal enterotoxin with the *B. cereus* enter-

* Corresponding author. Mailing address: Department of Bioscience and Biotechnology, University of Strathclyde, Royal College Building, 204 George St., Glasgow, Scotland. Phone: 0044 41 552 4400, ext. 2085. Fax: 0044 41 553 1161. E-mail: n.j.rowan@pop-hub.strath.ac.uk.

TABLE 1. Influence of duration of storage at ≥25°C on number of reconstituted IMF contaminated with *B. cereus* strains, with or without diarrheal enterotoxin

Duration of storage (h)	No. of IMF contaminated with:[a]			
	B. cereus I	*B. cereus* II	Diarrheagenic *B. cereus* II	Diarrheagenic *B. cereus* II and diarrheal toxin
0	10	7	6	0
8	30	34	10	0
14	31	37	10	4
24	31	38	10	4

[a] One hundred IMF were examined for *B. cereus* and for the presence of diarrheal enterotoxin.

otoxin-reverse passive latex agglutination assay kit (BCET-RPLA TD950; Oxoid) after a nonturbid, fat-free extract was initially obtained via the Filtron stirred cell ultrafiltration system fitted with an Omega 300-kDa cutoff polyethersulfone membrane (Filtron Technology Corporation). *B. cereus* isolates producing enterotoxin after overnight cultivation in brain heart infusion (BHI) broth supplemented with 0.1% glucose (BHIG) at 30°C were designated as potentially enterotoxigenic.

B. cereus was detected in 17 IMF examined immediately after reconstitution at levels up to 4.8×10^2 CFU g^{-1} (mean, 1.3×10^2 CFU g^{-1}); only 6 (35.3%) of these contained the enterotoxigenic form of this organism (Table 1). Diarrheal enterotoxin, however, was not detected in any dried IMF product. In agreement with other studies (2, 12), these experiments demonstrated that the dried IMF available in many European Economic Community countries is of acceptable bacteriological quality, having *B. cereus* counts lower than the safety limit of 10^3 CFU g^{-1} proposed by the Association of Dietetic Food Industries of the European Community (data not shown) (2). Subsequent reconstitution and storage resulted in 63 infant foods containing this organism, where 6 products contained both *B. cereus* I and II (Table 1). Of the 38 IMF supporting growth of *B. cereus* II (of which 10 isolates were shown to be enterotoxigenic after cultivation in BHIG), diarrheal enterotoxin was detected in only 4 formulations after 14 h at ≥25°C (Table 1). The level of enterotoxin in cultured IMF varied over the range of 16 to ≥124 ng ml^{-1}, where the reported sensitivity of the BCET-RPLA test in detecting this enterotoxin is 2 ng ml^{-1} (3). All four infant formulations supporting enterotoxin production contained maltodextrin (three also had lactose), while the six toxin-free baby foods containing enterotoxigenic *B. cereus* II had lactose as their sole carbohydrate source. These studies showed that improperly stored IMF containing maltodextrin may pose a threat to consumer safety, as they may be contaminated with diarrheagenic *B. cereus* capable of both growth and enterotoxin production in these products.

Effect of maltodextrin on growth of *B. cereus* and synthesis of diarrheal enterotoxin. To confirm the possible link between maltodextrin and stimulation of diarrheal enterotoxin production, additional studies, using three enterotoxigenic *B. cereus* II isolates (i.e., *B. cereus* SU11, SU58, and NCTC 11145) and a nonenterotoxigenic *B. licheniformis*, NCTC 10341, were carried out. After an initial 24-h cultivation period at 125 rpm in BHI broth at 30°C, each test organism was washed three times in phosphate-buffered saline (0.01 M sodium phosphate [pH 7.2]–0.15 M NaCl) and centrifuged at $11,500 \times g$ for 10 min at 4°C (Microcentaur MSE). Decimal serial dilution of the suspended cells was carried out in phosphate-buffered saline, and a 1-ml sample of the 10^{-4} dilution was inoculated into either basal synthetic media or tyndallized IMF test media to give an initial cell count of 10 to 100 cells ml^{-1}.

Inoculated basal synthetic media (pH 7.2) and tyndallized IMF test media, supplemented with 0.01, 0.1, 1.0, or 3.52% filter-sterilized maltodextrin, glucose, or lactose, were incubated for 14 and 24 h at 25°C. Confirmation that each test sugar had passed through the 0.45-μm-pore-size membrane filter (Millipore) was achieved by carrying out separate sugar assays with a 10% stock solution of each sugar before and after filtration. Following cultivation at 25°C, filtrates obtained from the test cultures (via the Filtron stirred cell ultrafiltration system) were examined for the number of cells present (CFU per milliliter) and for the presence of enterotoxin as described earlier. Reconstituted IMF used in these enterotoxin studies were tyndallized to sterility prior to supplementation with the above-mentioned sugars.

Basal synthetic media (Table 2) and reconstituted IMF (Table 3) supplemented with ≥0.1% maltodextrin both supported growth of *B. cereus* SU11, SU58, and NCTC 11145 and stimulated diarrheal enterotoxin production (Tables 2 and 3). Higher levels of enterotoxin (in nanograms per milliliter) were produced in basal synthetic media containing 1% maltodextrin or glucose (Table 2) and in reconstituted IMF containing 1.6% maltodextrin (Table 3) than in media containing either higher or lower concentrations of these sugars. While lactose supported growth of each test culture, no enterotoxin was produced in these cultured media (Tables 2 and 3). IMF used in these toxin studies either contained or were supplemented with infant formula-grade maltodextrin at levels normally found in certain brands of these products (Table 3). It was not unexpected that maltodextrin (a derivative of starch hydrolysis) had the potential to stimulate diarrheal enterotoxin production, as

TABLE 2. Diarrheal enterotoxin production by *B. cereus* strains at 30°C in basal synthetic media containing different carbohydrate sources

Test organism	ng of enterotoxin ml^{-1}[a] (no. of cells[b]) in basal media containing:									
	Maltodextrin				Glucose				Lactose	
	3.52%	1.0%	0.1%	0.01%	3.52%	1.0%	0.1%	0.01%	3.52%	≤1.0%
B. cereus SU11	2 (7.01)	32 (7.23)	—[c] (6.81)	— (6.75)	2 (7.24)	32 (7.08)	— (6.89)	— (6.79)	— (7.06)	— (6.98)
B. cereus SU58	4 (6.91)	32 (7.20)	2 (6.86)	— (6.78)	4 (7.26)	32 (7.29)	2 (6.97)	— (6.87)	— (6.98)	— (6.92)
B. cereus NCTC 11145	4 (7.04)	32 (7.18)	4 (7.01)	— (6.92)	8 (7.36)	32 (7.18)	4 (7.08)	— (6.98)	— (7.01)	— (6.98)
B. licheniformis NCTC 10341	— (6.80)	— (6.78)	— (6.61)	— (6.38)	— (6.85)	— (6.78)	— (6.66)	— (6.49)	— (6.68)	— (6.61)

[a] Detected by the BCET-RPLA system.
[b] Measured as $\log_{10}$ CFU per milliliter.
[c] —, no toxin detected.

TABLE 3. Diarrheal enterotoxin production by *B. cereus* strains in a variety of commercially available IMF containing or supplemented with maltodextrin

IMF (% maltodextrin)	ng of enterotoxin ml^{-1}[a] produced (no. of cells[b]) in test media			
	B. cereus SU11	*B. cereus* SU58	*B. cereus* NCTC 11145	*B. licheniformis* NCTC 10341
A (3.78)	16 (7.37)	32 (7.29)	64 (7.36)	—[c] (6.90)
B (1.6)	32 (7.32)	64 (7.27)	≥128 (7.37)	— (6.90)
C (3.52)	32 (7.39)	64 (7.28)	64 (7.34)	— (6.88)
D (0)	— (7.29)	— (7.27)	— (7.30)	— (6.86)
D+[d] (3.52)	32 (7.30)	64 (7.30)	64 (7.32)	— (6.86)
E (0)	— (7.22)	— (7.25)	— (7.31)	— (6.90)
E+ (1.6)	32 (7.28)	64 (7.26)	≥128 (7.32)	— (6.91)

[a] Detected by BCET-RPLA assay in fat-free IMF extract.
[b] Measured as $\log_{10}$ CFU per milliliter.
[c] —, no toxin detected.
[d] +, composition of original infant formulations altered by the addition of maltodextrin.

starch has been shown previously to be a suitable carbohydrate source for both growth of *B. cereus* and subsequent diarrheal toxin production (5).

Despite worldwide occurrence of diarrheagenic *B. cereus* in milk, there are remarkably few reports of food intoxication in which this organism was implicated as the etiological agent (2, 12). While the mechanism of *B. cereus* diarrheal-type food poisoning remains unclear, one factor contributing to this low incidence is the unsuitability of milk as a medium for enterotoxin production, as it lacks glucose and free amino acids (12). This study has shown that *B. cereus* occasionally contaminated dried IMF at acceptably low levels and should not present a health problem to the consumer population if IMF is reconstituted under hygienic conditions and consumed within 4 h of preparation. However, improperly stored formulations containing maltodextrin may pose a threat to consumer safety, as they may be contaminated with diarrheagenic *B. cereus* capable of both growth and diarrheal toxin production in these products. By supplementing IMF with maltodextrin in order to enhance the nutritive value of this product (2), food companies may have unwittingly produced a suitable environment in which improperly stored infant foods containing diarrheagenic *B. cereus* may produce enterotoxin.

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