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Production and Characterization of Monoclonal Antibodies against Vegetative Cells of *Bacillus cereus*

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Two monoclonal antibodies (MAbs) against *Bacillus cereus* were produced. The MAbs (8D3 and 9B7) were selected by enzyme-linked immunosorbent assay for their reactivity with *B. cereus* vegetative cells. They reacted with *B. cereus* vegetative cells while failing to recognize *B. cereus* spores. Immunoblotting revealed that MAb 8D3 recognized a 22-kDa antigen, while MAb 9B7 recognized two antigens with molecular masses of approximately 58 and 62 kDa. The use of MAbs 8D3 and 9B7 in combination to develop an immunological method for the detection of *B. cereus* vegetative cells in foods was investigated.

Vegetative cells and spores of *Bacillus cereus* are present in the environment and can frequently be found in many raw, dried, and processed foods (4, 13, 19, 23, 25, 31, 41). This bacterium has been implicated in two different types of food poisoning, namely emetic and diarrheal (26, 28), and is responsible for numerous cases of food spoilage because of the production of lipases and proteases (7, 35). In addition, certain strains of *B. cereus* can grow at temperatures as low as 4 to 6°C (41, 42), and these psychrotrophic *B. cereus* strains are a health risk to the consumer since vegetative cells can produce enterotoxins mainly in the exponential phase (8, 15–17). It is impossible for the food industry to exclude *B. cereus* from their products because, as many studies have shown, *B. cereus* cells can survive heat processing and can grow in foods kept at refrigerated storage conditions. Thus, it is important to develop methods to detect the presence of *B. cereus* in order to eliminate the threat of food poisoning. Several selective plating methods described for detecting *B. cereus* require, with confirmatory testing, up to 4 days to perform (21, 24, 32, 33, 39, 40). Other efforts in *B. cereus* research have focused on detection of the organism by detection of enterotoxin-producing cells (1, 22, 29), and commercial kits designed to detect enterotoxic *B. cereus* via immunoassays have been developed (6, 7, 9). In the food industry, immunoassays are also used to detect spore-forming and non-spore-forming bacteria. Commercial immunoassay-based kits that use either polyclonal antibodies or monoclonal antibodies (MAbs) are available to detect *Salmonella*, *Listeria*, and other organisms. Immunoassays have been developed for the detection of *Bacillus* and *Clostridium* spores by using polyclonal antibodies and MAbs in enzyme-linked immunosorbent assays (ELISAs) (11, 36, 37). Immunoassays have also been developed for the vegetative cells of both sporeformers and nonsporeformers (20). To date, however, there are no commercially available ELISAs for the rapid detection of the vegetative cells of *B. cereus* in food products.

In this paper, we describe the production and characterization of two MAbs against *B. cereus*. These antibodies were selected by ELISA for their reactivity with *B. cereus* vegetative cells. The specificity of the selected antibodies was tested against bacterial cells of a variety of species within and across genera and spores of *B. cereus*. These antibodies, which are specific for vegetative cells, can be used to develop a rapid and sensitive method for the detection of strains of *B. cereus* in foods that potentially cause food poisoning.

Bacterial strains and culture conditions. The bacterial strains used in this study are shown in Table 1. All bacteria were grown at 30°C in Trypticase Soy medium (bioMerieux, Marcy l'Etoile, France). A spore suspension of *B. cereus* cells was prepared from an overnight culture of *B. cereus* vegetative cells that was inoculated on the sporulation medium described by Faille et al. (10). To remove vegetative cell remnants, spores were treated with a solution of thimerosal as described by Norris and Wolf (34). After centrifugation at 10,000 × *g* for 10 min, spores were then incubated in TEL buffer containing 100 mM Tris-HCl (pH 8), 5 mM EDTA, and 0.5% lysozyme at 50,000 U/mg for 1 h at 37°C.

For immunization procedure and immunochemical techniques, all bacterial cells in an exponential growing stage and *B. cereus* spores were harvested by centrifugation at 10,000 × *g* for 10 min at 4°C and washed twice in phosphate-buffered saline (PBS).

Production of MAbs. The MAbs were produced by the procedure described by Galfre and Milstein (12). Vegetative cells of *B. cereus* LMG 6923 (10^8/ml) were injected into BALB/c mice. Hybridomas were screened for antibody production by ELISA with vegetative cells of *B. cereus* LMG 6923 as antigens. Selected hybridomas were cloned at least twice by limiting dilution method. The MAb-secreting clones were propagated as ascitic fluid by the procedure of Harlow and Lane (18). The isotyping of MAbs was performed with a mouse monoclonal isotyping kit according to the manufacturer's instructions. Antibodies were concentrated by ammonium sulfate precipitation of ascites, and immunoglobulin G (IgG) antibody was purified by using a protein A column (18).

Immunochemical techniques. (i) ELISA. The ELISA was done according to the method described by Harlow and Lane (18), with a few modifications. Briefly, microtiter plates (Immulon-1; Dynatech, Chantilly, Va.) were coated overnight at 4°C with 10^8 bacterial cells or *B. cereus* spores per ml. After blocking with PBS containing 3% nonfat dry milk and 0.05% Tween 20, the plates were incubated with the MAbs. Perox-

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TABLE 1. Specificities of MAbs for *B. cereus* vegetative cells, as assessed by ELISA

Species and strain	Source[a]	A_{490} of cell culture[b]	
		MAb 8D3	MAb 9B7
Bacillus cereus			
LMG 6923	LMG	2.106	1.907
DSM31	DSM	2.210	2.060
IB32	LCC	1.570	1.955
IIID49a	LCC	1.458	0.944
ID44	LCC	1.067	0.944
IB16	LCC	1.211	0.983
IVD9b	LCC	1.517	1.955
IVc37	LCC	0.962	0.988
ID12	LCC	0.955	0.925
IIID9	LCC	1.337	1.433
IVC2	LCC	1.422	1.118
ID7	LCC	0.984	0.969
PC42	LCC	1.164	0.933
PC21	LCC	1.127	0.959
PC	LCC	1.414	0.969
LC22	LCC	1.414	0.962
LC21	LCC	1.721	0.985
LC41	LCC	1.176	0.988
RC	LCC	1.255	0.911
RC31	LCC	1.644	1.157
RC42	LCC	0.948	0.964
LMG 6923 spores	LMG	0.080	0.102
Bacillus thuringiensis			
DSM 2046	DSM	0.581	0.168
subsp. *berliner*	LCC	1.097	0.578
subsp. *israelensis*	LCC	0.444	0.174
subsp. *kurstaki*	LCC	1.213	0.549
Bacillus mycoides DSM 2048	DSM	0.532	0.124
Bacillus circulans ATCC 4513	ATCC	0.129	0.155
Bacillus simplex LMG 1160	LMG	0.192	0.155
Bacillus polymyxa DSM 292	DSM	0.133	0.135
Bacillus licheniformis LMG 6933	LMG	0.061	0.130
Bacillus megaterium DSM 32	DSM	0.184	0.201
Bacillus subtilis ATCC 6633	ATCC	0.172	0.122
Bacillus pumilus DSM 27	DSM	0.197	0.101
Salmonella enteritidis	LCC	0.099	0.144
Escherichia coli	LCC	0.164	0.083
Proteus vulgaris	LCC	0.124	0.083
Citrobacter freundii	LCC	0.175	0.192
Pseudomonas stutzeri	LCC	0.103	0.171
Micrococcus luteus	LCC	0.123	0.099
Listeria monocytogenes	LCC	0.165	0.161

[a] Bacterial strains LCC were obtained from our Laboratory Culture Collection. The other bacterial strains were obtained from the Laboratorium voor Microbiologie (LMG, Ghent, Belgium), the German Collection of Microorganisms (DSM, Braunschweig, Germany), or the American Type Culture Collection (ATCC, Manassas, Va.).

[b] A_{490}, absorbance at 490 nm. Values are based on triplicate experiments. A value of >0.2 is considered positive.

idase-conjugated goat anti-mouse IgG+IgM (Jackson Immunoresearch, Immunotech, Marseille, France) and *o*-phenylenediamine (Sigma Chemical Co., St. Quentin Fallavier, France) were used as secondary antibodies and substrate, respectively. Absorbance was read at 490 nm by using a microtiter plate reader (Metertech ε 960 Instrument; BioBlock).

The ELISA procedure was also performed on plates coated with trypsin-protease-treated *B. cereus* vegetative cells to determine MAb specificity for protein antigens: wells at 10^8 cells/ml were treated with 1 mg of trypsin at 37°C for 4 h and were treated again with 100 μg of protease at 37°C overnight.

(ii) SDS-PAGE and immunoblotting. *B. cereus* LMG 6923 (10^8 vegetative cells) was extracted by the method of Matar et al. (30) and was subjected to electrophoresis through sodium dodecyl sulfate (SDS)–12% polyacrylamide gels as described by Laemmli (27).

Proteins separated by SDS-polyacrylamide gel electrophoresis (PAGE) were electroblotted by the method of Harlow and Lane (18). After transfer for 3 h at 1.2 A, the membrane was blocked for 1 h in PBS containing 5% nonfat dry milk and 0.5% Tween 20. MAbs and peroxidase-conjugated goat anti-mouse IgG+IgM (Jackson Immunoresearch) were used as primary and secondary antibodies, respectively. The blots were visualized with chemiluminescence (DuPont Co., Newtown, Conn.).

For glycoprotein determination, proteins separated by SDS-PAGE were exposed to periodic acid-Schiff staining (38). The proteins which stained positive as glycoproteins were determined and compared to the antigens determined by immunoblotting.

Periodate oxidation was used to determine MAb specificity for carbohydrate determinants (2). *B. cereus* cells exposed to various concentrations of sodium metaperiodate (0 to 0.05 M) were subjected to immunoblotting as indicated above.

(iii) ELISA capture system. ELISA capture system was performed with MAb 9B7 used as a specific capture antibody and with biotinylated MAb 8D3 used as a detector antibody (18). Briefly, microtiter plates were coated with 2 μg of MAb 9B7 for 2 h at 37°C. All dilutions were performed in ELISA buffers. Vegetative cell cultures of *B. cereus* were applied to wells for 1 h, and detection of bound antigen was performed by application of biotinylated MAb 8D3. Streptavidin-peroxidase (Sigma S 5512) was applied for 0.5 h. Absorbance was read at 490 nm after addition of the substrate *o*-phenylenediamine (Sigma Chemical Co.).

A total of nine hybridomas were screened by ELISA for their reactivities with *B. cereus* LMG 6923 vegetative cells. Of these, only two hybridomas secreted antibodies reactive with *B. cereus*. These MAbs, designated 8D3 and 9B7, were found to be IgG1 and IgM, with kappa light chains.

The specificity of the MAbs was examined by ELISA with a panel of select bacteria (Table 1). The results showed that the MAbs recognized not only vegetative cells of *B. cereus* LMG 6923, which was used for immunization, but also the vegetative cells of *B. cereus* originating from food or environmental samples. MAb 8D3 reacted strongly with all *B. cereus* strains and

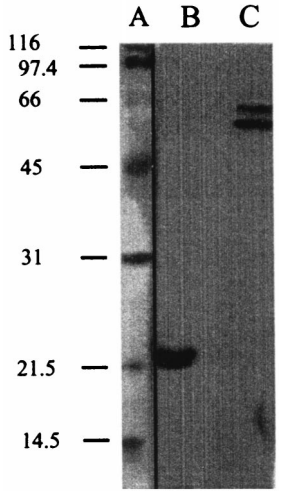

FIG. 1. Immunoblotting with MAbs against *B. cereus* LMG 6923 vegetative cells. Shown are molecular mass standards (in kilodaltons) (A) and *B. cereus* vegetative cells with MAbs 8D3 (B) and 9B7 (C).

TABLE 2. Detection of vegetative cells of *B. cereus* in pure culture by ELISA capture system

Inoculum (cells/ml)	A_{490}[a]
0	0.050
10	0.086
10^2	0.224
10^3	0.310
10^4	0.362
10^5	0.573
10^6	0.754
10^7	1.172
10^8	1.805

[a] A_{490}, absorbance at 490 nm. Values are based on triplicate experiments.

with *Bacillus thuringiensis* subsp. *berliner* and *B. thuringiensis* subsp. *kurstaki*. MAb 8D3 reacted weakly with *B. thuringiensis* DSM 2046, *B. thuringiensis* subsp. *israelensis*, and *Bacillus mycoides*. MAb 9B7 reacted strongly with *B. cereus* species and reacted weakly with *B. thuringiensis* subsp. *berliner* and *B. thuringiensis* subsp. *kurstaki*. However, *B. cereus* is closely related to *B. thuringiensis* and *B. mycoides* (3, 5). These data could explain the cross-reactivity of the MAbs with vegetative cells of *B. thuringiensis* or *B. mycoides*. None of these antibodies reacted with *B. cereus* LMG 6923 spores. In addition, both antibodies showed no reactivity to several members of the family of *Enterobacteriaceae* (*Salmonella enteritidis*, *Escherichia coli*, *Proteus vulgaris*, *Citrobacter freundii*) and other bacteria (*Micrococcus luteus*, *Pseudomonas stutzeri*, *Listeria monocytogenes*).

The antigenic reactivity of the MAbs was destroyed when ELISA plates were coated with trypsin-protease-treated vegetative cells of *B. cereus*. These results show that the antigens recognized by MAbs 8D3 and 9B7 are proteinaceous in nature.

MAbs were analyzed for the antigenic specificity by SDS-PAGE followed by immunoblotting (Fig. 1). *B. cereus* LMG 6923 vegetative cell extracts were used for electrophoretic studies. MAb 8D3 recognized an antigen with a molecular mass of 22 kDa. The antigens which react with MAb 9B7 have molecular masses of approximately 58 and 62 kDa. Periodic acid-Schiff staining indicated that the proteins which react with MAb 9B7 may be glycoproteins. Treatment of *B. cereus* cells with sodium metaperiodate had no effect on the detection of *B. cereus* by MAb 9B7, as assessed by immunoblotting. This indicates that the antigens which react with MAb 9B7 are not carbohydrates.

ELISA experiments indicate that the proteins recognized by MAbs 8D3 and 9B7 are specific to vegetative cells, since the antibodies did not react with spores of *B. cereus*. Furthermore, as shown by SDS-PAGE and immunoblotting analysis, MAbs 8D3 and 9B7 react with different proteins on the vegetative cells. This result indicates that these MAbs are able to capture vegetative cells of *B. cereus* in an immunoassay. Our future research will focus on the use of these antibodies to develop immunoassays which will detect *B. cereus* cells in food products.

We need a rapid and reliable method to detect vegetative cells of *B. cereus*, since checking for this pathogenic organism is critical to ensure food safety. It is impossible for the food industry to completely avoid the presence of *B. cereus* in their products, and the consumption of foods containing 10^5 vegetative cells of *B. cereus* per ml will result in food poisoning. Thus, the detection method should be sensitive enough to be able to detect low numbers of *B. cereus* organisms (14).

We have therefore developed the following method to detect vegetative cells of *B. cereus*, which we tested in pure culture. We used MAbs 8D3 and 9B7 to develop an ELISA capture system. MAb 9B7 was used as a specific capture antibody and MAb 8D3 was used as a detector antibody. The results show that the ELISA capture system can detect and quantify vegetative cells of *B. cereus* (Table 2). We determined that the lower detection limit was on the order of 10^2 cells per ml and the upper detection limit was on the order of 10^8 cells per ml, with absorbance values of 0.224 to 1.805. So, this work appears to demonstrate the feasibility of detecting this pathogenic organism in food products with our ELISA capture system.

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REFERENCES

1. **Andersson, M. A., R. Mikkola, J. Helin, M. C. Andersson, and M. Salkinoja-Salonen.** 1998. A novel sensitive bioassay for detection of *Bacillus cereus* emetic toxin and related depsipeptide ionophores. Appl. Environ. Microbiol. **64:**1338–1343.
2. **Arnold, F., L. Bedouet, P. Batina, G. Robreau, F. Talbot, P. Lecher, and R. Malcoste.** 1998. Biochemical and immunological analyses of the flagellin of *Clostridium tyrobutyricum* ATCC 25755. Microbiol. Immunol. **42:**23–31.
3. **Ash, C., J. A. E. Farrow, M. Dorsch, E. Stackebrandt, and M. D. Collins.** 1991. Comparative analysis of *Bacillus anthracis*, *Bacillus cereus*, and related species on the basis of reverse transcriptase sequencing of 16S rRNA. Int. J. Syst. Bacteriol. **41:**343–346.
4. **Becker, H., G. Schaller, W. von Wiese, and G. Terplan.** 1994. *Bacillus cereus* in infant foods and dried milk products. Int. J. Food Microbiol. **23:**1–15.
5. **Bourque, S. N., J. R. Valero, M. C. Lavoie, and R. C. Levesque.** 1995. Comparative analysis of the 16S to 23S ribosomal intergenic spacer sequences of *Bacillus thuringiensis* strains and subspecies and of closely related species. Appl. Environ. Microbiol. **61:**1623–1626.
6. **Buchanan, R. L., and F. J. Schultz.** 1994. Comparison of the Tecra VIA kit, Oxoid BCET-RPLA kit and CHO cell culture assay for the detection of *Bacillus cereus* diarrhoeal enterotoxin. Lett. Appl. Microbiol. **19:**353–356.
7. **Christiansson, A.** 1993. Enterotoxin production in milk by *Bacillus cereus*: a comparison of methods for toxin detection. Neth. Milk Dairy J. **47:**79–87.
8. **Christiansson, A., A. S. Naidu, I. Nilsson, T. Waldstrom, and H. E. Petterson.** 1989. Toxin production by *Bacillus cereus* dairy isolates in milk at low temperatures. Appl. Environ. Microbiol. **55:**2595–2600.
9. **Day, T. L., S. R. Tatini, S. Notermans, and R. W. Bennett.** 1994. A comparison of ELISA and RPLA for detection of *Bacillus cereus* diarrhoeal enterotoxin. J. Appl. Bacteriol. **77:**9–13.
10. **Faille, C., V. Lebret, F. Gavini, and J. F. Maingonnat.** 1997. Injury and lethality of heat treatment of *Bacillus cereus* spores suspended in buffer and in poultry meat. J. Food Prot. **60:**544–547.
11. **Foegeding, P. M., and Y. H. Chang.** 1993. Polyclonal antibodies in an enzyme-linked immunosorbent assay (ELISA) to detect bacterial spores. J. Rapid Methods Automat. Microbiol. **2:**135–150.
12. **Galfre, G., and C. Milstein.** 1981. Preparation of monoclonal antibodies: strategies and procedures. Methods Enzymol. **73:**3–46.
13. **Granum, P. E.** 1997. *Bacillus cereus*, p. 327–336. *In* M. P. Doyle, L. R. Beuchat, and T. J. Montville (ed.), Food microbiology: fundamentals and frontiers. American Society for Microbiology, Washington, D.C.
14. **Granum, P. E., and T. Lund.** 1997. *Bacillus cereus* and its food poisoning toxins. FEMS Microbiol. Lett. **157:**223–228.
15. **Granum, P. E., S. Brynestad, and J. M. Kramer.** 1993. Analysis of enterotoxin production by *Bacillus cereus* from dairy products, food poisoning incidents and non-gastrointestinal infections. Int. J. Food Microbiol. **17:**269–279.
16. **Granum, P. E., S. Brynestad, O. Sullivan, and H. Nissen.** 1993. Enterotoxin from *Bacillus cereus*: production and biochemical characterization. Neth. Milk Dairy J. **47:**63–70.
17. **Griffiths, M. N.** 1990. Toxin production by psychrotrophic *Bacillus* spp. present in milk. J. Food Prot. **53:**790–792.
18. **Harlow, E., and D. Lane (ed.).** 1988. Antibodies: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
19. **Harmon, S. M., and D. A. Kautter.** 1991. Incidence and growth of *Bacillus cereus* in ready-to-serve foods. J. Food Prot. **54:**372–374.
20. **Hartman, P. A.** 1992. Rapid methods and automation, p. 665–746. *In* C. Vanderzantz and D. R. Splittstoesser (ed.), Compendium of methods for the microbiological examination of foods. American Public Health Association, Washington, D.C.
21. **Holbrook, R., and J. M. Andersson.** 1980. An improved selective and diagnostic medium for the isolation and enumeration of *Bacillus cereus* in foods. Can. J. Microbiol. **26:**753–759.
22. **Jackson, S. G.** 1993. Rapid screening test for enterotoxin-producing *Bacillus cereus*. J. Clin. Microbiol. **31:**972–974.

23. **Jonhson, K. M.** 1984. *Bacillus cereus* foodborne illness—an update. J. Food Prot. **47:**145–153.
24. **Kim, H. U., and J. M. Goepfert.** 1971. Enumeration and identification of *Bacillus cereus* in foods. I. 24-hour presumptive test medium. Appl. Microbiol. **22:**581–587.
25. **Konuma, H., K. Shinagawa, M. Tokumaru, Y. Onoue, S. Konno, N. Fujino, T. Shigehisa, H. Kurata, Y. Kuwabara, and C. A. M. Lopes.** 1988. Occurrence of *Bacillus cereus* in meat products, raw meat and meat product additives. J. Food Prot. **51:**324–326.
26. **Kramer, J. M., and R. J. Gilbert.** 1989. *Bacillus cereus* and other *Bacillus* species, p. 21–70. *In* M. P. Doyle (ed.), Foodborne bacterial pathogens. Marcel Dekker, New York, N.Y.
27. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
28. **Lund, B. M.** 1990. Foodborne disease due to *Bacillus* and *Clostridium* species. Lancet **336:**982–986.
29. **Mäntynen, V., and K. Lindström.** 1998. A rapid PCR-based DNA test for enterotoxic *Bacillus cereus*. Appl. Environ. Microbiol. **64:**1634–1639.
30. **Matar, G. M., T. A. Slieman, and N. H. Nabbut.** 1996. Subtyping of *Bacillus cereus* by total cell protein patterns and arbitrary primer polymerase chain reaction. Eur. J. Epidemiol. **12:**309–314.
31. **Meer, R. R., J. Baker, F. W. Bodyfelt, and M. W. Griffiths.** 1991. Psychrotrophic *Bacillus* spp. in fluid milk products: a review. J. Food Prot. **54:**969–979.
32. **Meira de Vasconcellos, F. J., and L. Rabinovitch.** 1995. A new formula for an alternative culture medium, without antibiotics, for isolation and presumptive quantification of *Bacillus cereus* in foods. J. Food Prot. **58:**235–238.
33. **Mossel, D. A. A., M. J. Koopman, and E. Jongerius.** 1967. Enumeration of *Bacillus cereus* in foods. Appl. Microbiol. **15:**650–653.
34. **Norris, J. R., and J. Wolf.** 1961. A study of antigens of the aerobic spore-forming bacteria. J. Appl. Bacteriol. **24:**42–56.
35. **Overcast, W. W., and K. Atmaram.** 1974. The role of *Bacillus cereus* in sweet curdling of fluid milk. J. Milk Food Technol. **37:**233–236.
36. **Quinlan, J. J., and P. M. Foegeding.** 1997. Monoclonal antibodies for use in detection of *Bacillus* and *Clostridium* spores. Appl. Environ. Microbiol. **63:**482–487.
37. **Quinlan, J. J., and P. M. Foegeding.** 1998. Monoclonal antibody-based ELISAs for the detection of bacterial spores. J. Rapid Methods Automat. Microbiol. **6:**1–16.
38. **Segrest, J. P., and R. L. Jackson.** 1972. Molecular weight determination of glycoproteins by polyacrylamide gel electrophoresis in sodium dodecyl sulfate. Methods Enzymol. **28:**54–62.
39. **Szabo, R. A., E. C. D. Todd, and M. K. Rayman.** 1984. Twenty-four hour isolation and confirmation of *Bacillus cereus* in foods. J. Food Prot. **47:**856–860.
40. **te Giffel, M. C., R. R. Beumer, S. Leigendekkers, and F. M. Rombouts.** 1996. Incidence of *Bacillus cereus* and *Bacillus subtilis* in foods in the Netherlands. Food Microbiol. **13:**53–58.
41. **Väisänen, O. M., N. J. Mwaisumo, and M. S. Salkinoja-Salonen.** 1991. Differentiation of dairy strains of the *Bacillus cereus* group by phage typing, minimum growth temperature, and fatty acid analysis. J. Appl. Bacteriol. **70:**315–324.
42. **van Netten, P., A. van de Moosdijk, P. van Hoensel, D. A. A. Mossel, and I. Perales.** 1990. Psychrotrophic strains of *Bacillus cereus* producing enterotoxin. J. Appl. Bacteriol. **69:**73–79.