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Monoclonal Antibodies for Use in Detection of *Bacillus* and *Clostridium* Spores†

J. J. QUINLAN‡ AND P. M. FOEGEDING*

Department of Food Science, North Carolina State University and Southeast Dairy Foods Research Center, Raleigh, North Carolina 27695-7624

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Five monoclonal antibodies against bacterial spores of *Bacillus cereus* T and *Clostridium sporogenes* PA3679 were developed. Two antibodies (B48 and B183) were selected for their reactivity with *B. cereus* T spores, two (C33 and C225) were selected for their reactivity with *C. sporogenes* spores, and one (D89) was selected for its reactivity with both *B. cereus* and *C. sporogenes* spores. The isotypes of the antibodies were determined to be immunoglobulin G2a (IgG2a) (B48), IgG1 (B183), and IgM (C33, C225, and D89). The antibodies reacted with spores of *B. cereus* T, *Bacillus subtilis* subsp. *globigii*, *Bacillus megaterium*, *Bacillus stearothermophilus*, *C. sporogenes*, *Clostridium perfringens*, and *Desulfotomaculum nigrificans*. Antibody D89 also reacted with vegetative cells of *B. cereus* and *C. sporogenes*. Analysis of *B. cereus* spore extracts showed that two of the antigens with which the anti-*Bacillus* antibodies reacted had molecular masses of 76 kDa and approximately 250 kDa. Immunocytochemical localization indicated that antigens with which B48, B183, and D89 react are on the exosporium of the *B. cereus* T spore. Antibody D89 reacted with the exosporium and outer cortex of *C. sporogenes* spores in immunocytochemical localization studies but did not react with extracts of *C. sporogenes* or *B. cereus* spores in Western blotting. Some *C. sporogenes* antigens were not stable during long-term storage at −20°C. Antibodies B48, B183, and D89 should prove to be useful tools for developing immunological methods for the detection of bacterial spores.

Spore-forming bacteria are responsible for a variety of food spoilage and food-borne illness problems. As the production of minimally processed refrigerated products becomes more efficient and aseptic, background microflora are eliminated, and it is the spore-forming organism which eventually may limit the shelf-lives of these products. Sporeformers have been responsible for the spoilage of canned foods, bread (2), vacuum-packed meats (16, 17, 21), pasteurized dairy products (13, 24), and fruit juices (23). The presence of spores at high levels in ingredients going into any of these types of products may increase the potential for spoilage of the finished product. The availability of a rapid method to detect the total spore load in raw ingredients would allow selection of the highest-quality ingredients for use in foods which have potential for spoilage by sporeformers.

Enzyme-linked immunosorbent assays (ELISAs) and other immunologically based detection systems are widely used in the food industry for quality control purposes, including detection of pathogens and toxins. The current technology for spore detection, however, still relies on a variety of cultural procedures for detection of the different classes of sporeformers (20, 26, 32).

Previous research has demonstrated the feasibility of detecting a broad range of bacterial spores by using a polyclonal antibody-based ELISA (11). Broad-range detection was possible because of cross-reactivity between species (25) and because of the use of a polyclonal antibody. In order to be practical, such an ELISA would require monoclonal antibodies, since they are less variable and are available in an unlimited supply through cell culture techniques.

In the study reported here, monoclonal antibodies against both *Bacillus* and *Clostridium* spores were developed. These antibodies were selected on the basis of having a broad range of reactivity with spores of a variety of species within and across genera. The reactivities of antibodies against both vegetative cells and spores of *Bacillus cereus* and *Clostridium sporogenes* were determined in order to identify antibodies which were spore specific.

MATERIALS AND METHODS

Bacterial strains and sporulation. Spore stocks were prepared as indicated in Table 1. Vegetative cell cultures of *B. cereus* T, *Bacillus subtilis* A, *B. subtilis* subsp. *globigii*, and *Bacillus megaterium* were grown in nutrient broth at 30°C. *Bacillus stearothermophilus* was grown in nutrient broth at 55°C, and *Bacillus coagulans* was grown in Trypticase soy broth at 43°C with shaking. *C. sporogenes* cells were grown in cooked meat medium at 35°C, *Clostridium perfringens* cells were grown in fluid thioglycolate medium at 37°C under anaerobic conditions, and *Desulfotomaculum nigrificans* cells were grown in BETI (22) at 55°C under anaerobic conditions.

Antigen preparation. Antigen was prepared by formalin inactivation (28) of *B. cereus* T and *C. sporogenes* PA3679 spore stocks which were free of vegetative cell material as observed by phase-contrast microscopy. A total of 2×10^7 inactivated bacterial spores (1×10^7 *B. cereus* spores and 1×10^7 *C. sporogenes* spores) were used as the antigen.

Monoclonal antibody production. Monoclonal antibody production was done according to the procedures of Kohler and Milstein (18). Hybridomas were screened for reactivity with spores of *B. cereus* T and *C. sporogenes*. Hybridomas found to be reactive with either spore type were screened for reactivity with the vegetative cells of that spore type.

Isotyping of monoclonal antibodies. Isotyping of monoclonal antibodies was performed by using a dot blot immunoassay format. Spores (10^7 of the spores indicated as antigens in Table 2) were applied to an Immobilon-P membrane (Millipore Corp., Bedford, Mass.) in a dot blot apparatus. The spores were then incubated with 100 μl of the appropriate tissue culture supernatants. Monoclonal antibody bound to spores was isotyped by using rabbit anti-mouse subclass-specific antiserum obtained from Bio-Rad (Hercules, Calif.).

Reactivities of monoclonal antibodies with bacterial spores and vegetative cells. Antibodies were screened for reactivity with spores from a range of *Bacillus* and *Clostridium* species. Spores (10^7 of the spores indicated in Table 3) were applied to an Immobilon-P membrane, followed by incubation with the indicated antibodies. Similarly, 10^6 of the indicated vegetative cells were applied to the membrane, followed by incubation with the indicated antibodies. Reactivity was

* Corresponding author. Phone: (919) 515-2971.

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‡ Present address: North Carolina Department of Agriculture, Food & Drug Protection Division, Raleigh, NC 27520.

TABLE 1. Bacterial strains used for production of spore crops

Organism	Sporulation medium[a] (reference for method)	Temp (°C)	Time (days)	Culture source[b]
B. cereus T	FNA (15)	35	2	NCSU
B. subtilis A	FNA (15)	30	2	NCSU
B. subtilis subsp. *globigii*	FNA (15)	30	2	AMSCO
B. megaterium 12872	FNA (15)	30	2	NCSU
B. stearothermophilus 7953	Supplemented nutrient agar (6)	55	3	ATCC
B. coagulans 56177	BHIA (19)	43	21	ATCC
C. sporogenes PA 3679	TSP (34)	35	4	NCSU
C. perfringens 3624	Duncan-Strong (10)	37	1	NCSU
D. nigrificans 7946	Mushroom compost (8)	55	14	F. Busta, University of Minnesota

[a] FNA, fortified nutrient agar; BHIA, brain heart infusion agar; TSP, Trypticase-sodium chloride-peptone medium.
[b] NCSU, North Carolina State University; AMSCO, American Sterilizer Company, Apex, N.C.; ATCC, American Type Culture Collection, Rockville, Md.

detected with a goat anti-mouse immunoglobulin G (IgG)–horseradish peroxidase conjugate (Sigma A4416) or a goat anti-mouse IgM–horseradish peroxidase conjugate (Sigma A8786). Color development was with the insoluble substrate 3-amino-9-ethyl-carbazole (Biomeda Corp.).

Adsorption of monoclonal antibodies. Culture supernatants were diluted 1:32 and 1:128 with Tris-buffered saline (TBS) (0.01 M Tris, 0.15 M NaCl, pH 7.4). Aliquots of diluted culture supernatants were mixed with an equal volume of the spore antigen (4×10^9 spores/ml) by gentle inversion overnight at 4°C. Diluted culture supernatants mixed with sterile distilled water served as a positive control. Antibodies adsorbed to spores were removed by centrifugation (ca. 4,000 × *g*, 10 min). The supernatant fluid was used in immunoblot assays as detailed above. The antigens used in the immunoblot assays included each spore with which the nonadsorbed antibody showed reactivity.

Purification and biotinylation of monoclonal antibodies. Antibodies were concentrated by ammonium sulfate precipitation of tissue culture supernatants (14). The concentrated protein was biotinylated by incubation with *N*-hydroxysuccinimidobiotin (Sigma Chemical Co.) (ca. 100 μg/mg of antibody in the sample) at room temperature for 4 h. One molar ammonium chloride (20 μl/250 μg of biotin) was added to stop the reaction, and the biotinylated protein was dialyzed extensively against phosphate-buffered saline (0.1 M sodium phosphate, 0.8% NaCl) (PBS), pH 7.0.

For immunocytochemical localization studies, IgG antibodies were purified out of tissue culture supernatants by using a protein A column (Pierce Chemical Co., Rockford, Ill.) according to the manufacturer's directions. IgM antibodies were purified from the culture supernatant by using a goat anti-mouse IgM antibody column (Pierce Chemical Co.). The tissue culture supernatant was diluted 1:1 in PBS. This dilution was applied to the goat anti-mouse IgM column, which had been equilibrated with PBS. Washing and elution of antibody off the column were according to the manufacturer's directions. Fractions containing protein were pooled and concentrated with a Centricon 10 microconcentrator (Amicon, Danvers, Mass.) and centrifugation at 3,000 × *g* for 30 min.

Antigen characterization. Spores were extracted by the method of Aronson and Pandey (1) to remove spore coat and exosporium components. Spores of *B. cereus* T (2×10^{10}) or *C. sporogenes* (1.1×10^9) were extracted in 5 mM cyclohexylaminoethane sulfonic acid–8 M urea–50 mM β-mercaptoethanol–0.8% sodium dodecyl sulfate (SDS) at pH 9.8 for 90 min at 37°C. Spores were centrifuged at 12,000 × *g* for 10 min, and the supernatant extract was boiled for 5 min. Extracts were applied directly to SDS-polyacrylamide gels containing 6 M urea. For molecular weight determination, gels were transferred to 0.2-μm-pore-size nitrocellulose. Blots were blocked with 3% bovine serum albumin in TBS, pH 7.4. The blots were then incubated with the appropriate biotinylated antibody (B48, B183, or D89 for *B. cereus* spore extracts and C33, C225, or D89 for *C. sporogenes* extracts) diluted 1:1,000 in TBS containing 0.05% Tween 20. Avidin-labeled peroxidase (Sigma A7419) diluted 1:1,000 in TBS–0.05% Tween 20 was used to detect the antigens with the insoluble substrate 4-naphthol (Bio-Rad). Glycoprotein determination involved periodic acid-Schiff staining (30). The gels were fixed overnight and treated with 0.7% periodic acid for 2 h and then with 0.2% metabisulfite for 2 h. Gels were then exposed to Schiff reagent (basic fuchsin, sodium metabisulfite, and hydrochloric acid), purchased from Fisher Scientific, Pittsburgh, Pa. The mobilities of the proteins which stained positive as glycoproteins from the spore extracts were determined and compared to the mobilities of the antigens determined by Western blotting.

Immunocytochemical localization. Ultrathin (ca. 100-μm) cryosections of spores for immunological labeling with colloidal gold were prepared according to the method of Chang and Foegeding (5). *B. cereus* T and *C. sporogenes* PA3679 spores from the same stock as had been used for the antigen were used for these experiments. The cryosections were floated on PBS containing 5% fetal calf serum, followed in sequence by PBS, PBS containing 0.1 M ammonium chloride, and PBS, each for 5 to 10 min at room temperature. Sections were floated on drops of primary reagent (the appropriate monoclonal antibody diluted 1:100) for 1 to 2 h, followed by washing in PBS six times for 5 min each. Sections were then floated on drops of secondary reagent for 20 to 30 min. The secondary reagent was goat anti-mouse IgG plus goat anti-mouse IgM conjugated to 10-nm-diameter colloidal gold used at a dilution of 1:10. Sections were again washed six times in PBS, followed by three washes with distilled water. They were stained and embedded in methylcellulose by floating them on drops of 2.3 M methylcellulose containing about 20% (vol/vol) saturated aqueous uranyl acetate. Grids were picked up in loops, drained, and air dried. They were viewed in a Philips EM 300 electron microscope. Negative controls were spores treated with monoclonal antibodies of the IgG1 and IgM isotypes which did not react with bacterial spores.

Removal of exosporia. Exosporia were removed from *B. cereus* T spores essentially by the procedure of Du and Nickerson (9), except that homogenization in a Braun homogenizer was for 30-s intervals up to a total of 150 s, with 120 to 150 s required for optimal removal of exosporia as determined by crystal violet staining and light microscopy (9).

RESULTS

Following immunization of mice with a 1:1 mixture of *B. cereus* T spores and *C. sporogenes* PA3679 spores, 397 hybridomas were screened for their reactivities with 10^7 *B. cereus* spores and for their reactivities with 10^7 *C. sporogenes* spores. Nine hybridomas produced antibodies which reacted with *B. cereus* T spores but not with either *C. sporogenes* spores or *B. cereus* vegetative cells. Fifteen hybridomas produced antibodies which reacted with *C. sporogenes* spores but not with either *B. cereus* T spores or *C. sporogenes* vegetative cells. Seven hybridomas produced antibodies which reacted with both *B. cereus* T and *C. sporogenes* spores. Of these seven, one hybridoma which reacted with both types of spores was subcloned and was also found to react with both types of vegetative cells. This antibody was designated D89.

Two of the hybridomas producing antibodies which reacted only with *B. cereus* T spores were subcloned successfully, and the resulting antibodies are referred to as B183 and B48. Two of the hybridomas producing antibodies which reacted only with *C. sporogenes* spores also were subcloned successfully, and the resulting antibodies were designated C33 and C225. The antibodies were isotyped, and the two antibodies which were reactive with *B. cereus* spores, B183 and B48, were found to be IgG1 and IgG2a, respectively. Both antibodies (C33 and C225) which were reactive only with *C. sporogenes* spores were IgM isotypes. The antibody which reacted with both types of spores and both types of vegetative cells, D89, was also of the IgM isotype (Table 2). Light chains of all five antibodies were kappa chains.

The antibodies were examined qualitatively (visually) for their reactivities with a range of *Bacillus*, *Clostridium*, and *Desulfotomaculum* spores by using a dot blot format (Table 3). Monoclonal antibody B183 reacted very strongly with *B. cereus* T spores and reacted weakly with *B. megaterium* and *B. subtilis* subsp. *globigii* spores. Antibody B48 reacted strongly with *B.*

TABLE 2. Monoclonal antibodies against bacterial spores

Antibody	Isotype	Antigen
B183	IgG1	*B. cereus* T spores
B48	IgG2a	*B. cereus* T spores
C33	IgM	*C. sporogenes* (PA 3679) spores
C225	IgM	*C. sporogenes* (PA 3679) spores
D89	IgM	Antigen common to both *Bacillus* and *Clostridium* spores and vegetative cells

cereus T and *B. megaterium* spores and weakly with *B. subtilis* subsp. *globigii* spores. C33 reacted strongly with *C. sporogenes* spores and weakly with *C. perfringens* spores. The weak reactivity of C33 and C225 with *D. nigrificans* spores was questionable because the spore suspension was dark and appeared to mask a weak positive development of color. Antibody C225 reacted weakly with *B. megaterium* spores and strongly with spores of *B. stearothermophilus*, *C. perfringens*, and *C. sporogenes*. D89 reacted with *D. nigrificans* spores in addition to spores of *Bacillus* and *Clostridium*. D89 also reacted with vegetative cells of *B. cereus* T and *C. sporogenes.*

To validate that the antibodies were monoclonal yet indeed showed cross-reactivity, tissue culture supernatants were mixed with the antigen to adsorb the antibody. The fraction which did not adsorb was tested to determine if there was reactivity with other organisms; reactivity would indicate that the tissue culture supernatant contained a mixture of monoclonal antibodies rather than a single antibody. For example, B183 was adsorbed with *B. cereus* T spores and then tested for reactivity with *B. megaterium* and *B. subtilis* subsp. *globigii* spores. In each case, residual activity was not detected (data not shown), confirming that the antibodies were indeed monoclonal yet cross-reacted with different organisms.

Western blots of *B. cereus* T spore extracts (Fig. 1) showed that the antigen with which B183 reacts has a molecular mass of approximately 250 kDa. Antibody B48 reacted with a 76-kDa protein. The molecular mass of the antigen with which D89 reacted could not be determined by the spore extraction procedure of Aronson and Pandey (1). Periodic acid-Schiff staining indicated that the proteins which react with B183 and B48 may be glycoproteins. The presence of glycoproteins in bacilli is controversial, and it has been demonstrated that some covalently attached sugars on *Bacillus* proteins are a result of

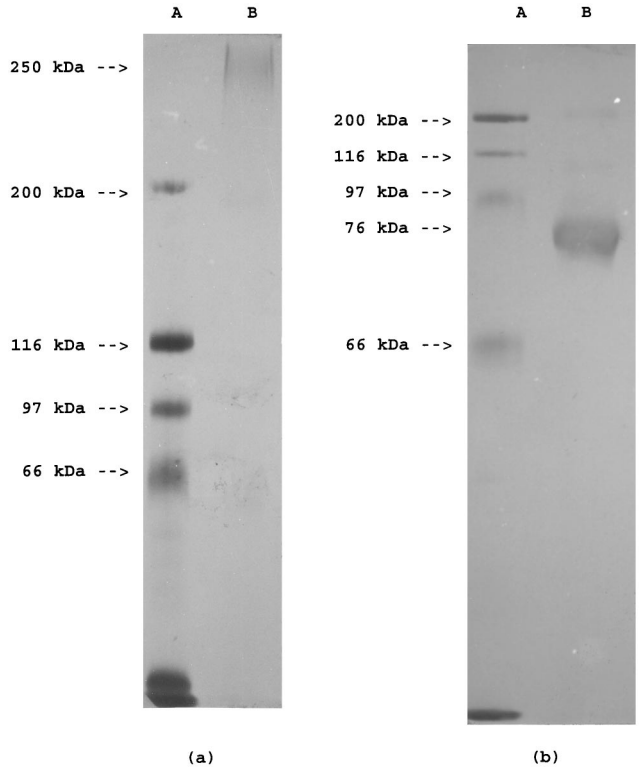

FIG. 1. Determination of molecular masses of antigens. (a) *B. cereus* T spore extracts were subjected to SDS–7.0% PAGE, transferred to nitrocellulose, and blotted with B183 as described in the text. Lane A, molecular mass standards; lane B, 250-kDa band which reacted with MAb B183. (b) *B. cereus* T spore extracts were subjected to SDS–12.5% PAGE, transferred to nitrocellulose, and blotted with B48. Lane A, molecular mass standards; lane B, 76-kDa band which reacted with MAb B48.

TABLE 3. Reactivities of monoclonal antibodies with bacterial spores and vegetative cells[a]

Spores or cells	Reactivity[b] of monoclonal antibody:				
	B183	B48	C33	C225	D89
B. cereus T spores	+++	++	−	−	+
B. megaterium spores	+	++	−	+	+
B. stearothermophilus spores	−	−	−	++	+++
B. subtilis A spores	−	−	−	−	−
B. subtilis subsp. *globigii* spores	+	+	−	−	−
C. perfringens spores	−	−	+	++	++
C. sporogenes PA 3679 spores	−	−	++	++	++
D. nigrificans spores	−	−	?	?	+
B. cereus T vegetative cells	−	−	−	−	++
C. sporogenes vegetative cells	−	−	−	−	+

[a] Reactivities of antibodies were determined by applying 10^7 of the indicated spores or 10^6 of the indicated cells on Immobilon-P membranes and determining detection by using the dot blot procedure detailed in the text.

[b] −, no reactivity; +++, strong reactivity; ?, reactivity uncertain due to spore pigments.

nonenzymatic glycosylation (4). It is not known whether the presence of sugars on the antigens arises from enzymatic glycosylation or is a result of environmental factors. It has been observed in our lab, however, that the antibodies react with *B. cereus* spores sporulated at different times and of various ages. Therefore, it appears that even if the glycosylation of the antigens is a result of environmental factors, it does not affect the ability of the antibody to detect the antigens. Immunocytochemical localization indicated that the antigens with which B48, B183, and D89 react are each located in the exosporium (Fig. 2). This reactivity was very specific, because none of the antibodies demonstrated cross-reactivity with any other components of the spore when they were used to probe cross sections of the spores. The location of the antigens in the exosporium was confirmed by conducting dot blot immunoassays on spores from which the exosporium had been removed with a cell homogenizer. The exosporium was nearly completely removed after 120 to 150 s of homogenization, and these spores did not react with the antibody (data not shown).

Western blotting of *C. sporogenes* spore extracts was performed with antibodies D89, C33, and C225 exactly as for *Bacillus* spore extracts. Coomassie blue staining of the extracts showed that proteins were present, but none reacted with the anti-*Clostridium* antibodies in Western blotting. Monoclonal antibodies C33 and C225 did not react with *C. sporogenes* spores in immunocytochemical localization studies. Monoclonal antibody D89 did react with *C. sporogenes* spores in immunocytochemical localization, and the antibody appeared to be

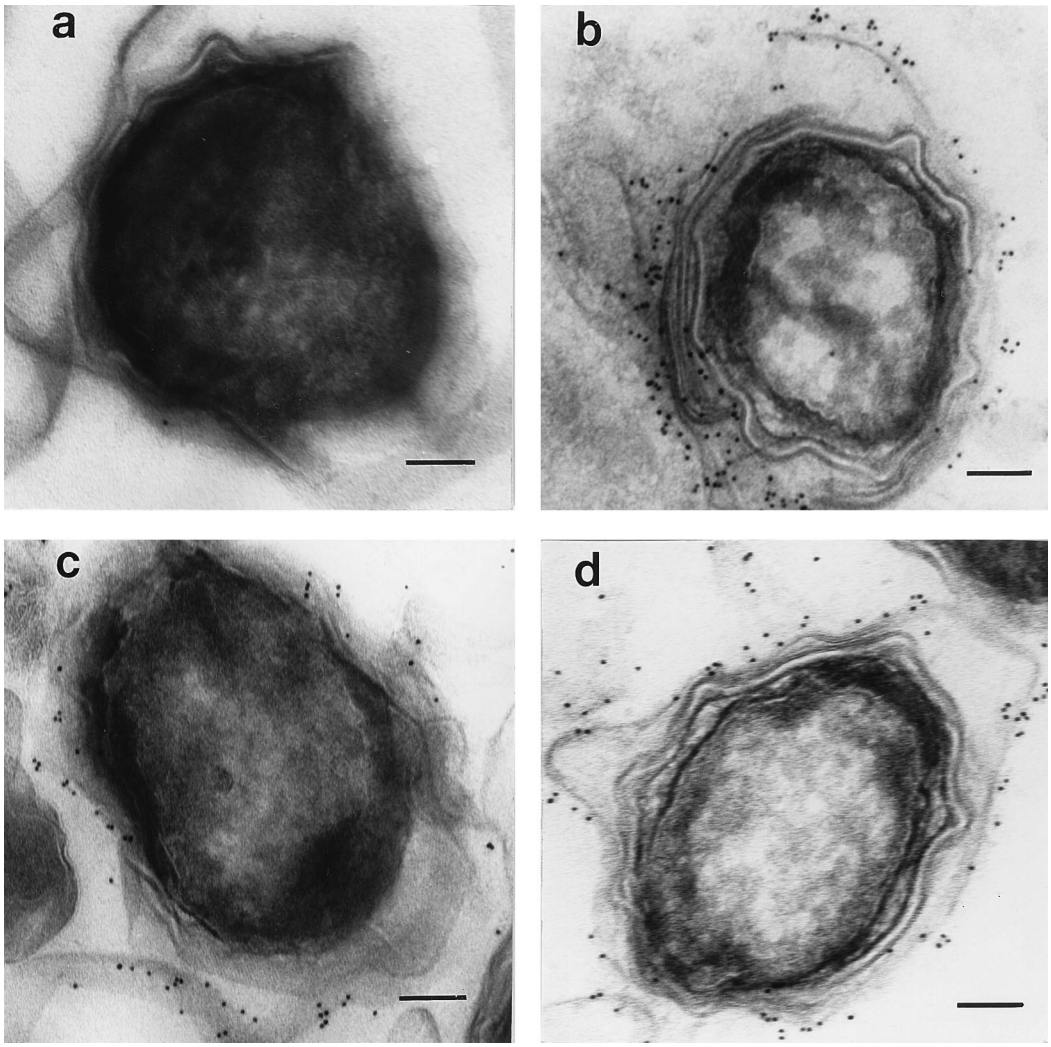

FIG. 2. Immunocytochemical localization of *B. cereus* T spore antigens. Ultrathin cryosections of *B. cereus* T spores were incubated with a negative primary antibody control (a), B48 (b), B183 (c), or D89 (d) and then with a colloidal gold-labeled secondary antibody. Bars, 0.1 μm.

specific for the exosporium and the outer regions of the spore cortex (Fig. 3).

DISCUSSION

The two monoclonal antibodies which react with *B. cereus* T spores, B48 and B183, appear to react with different antigens on the spore surface. Both antigens are located predominantly on the exosporium of the spore. Immunoblotting experiments indicate that the antigens are specific to the spore, as the antibodies did not react with vegetative cells of *B. cereus* T. The presence of spore-specific proteins has been demonstrated in the model system, *B. subtilis*, where spore coat proteins are known to be synthesized in a defined temporal order (31). We were primarily interested in reactivity with spores and not with vegetative cells, as the latter are likely to be eliminated with mild heat treatment. Their specificity for spore antigens should make these antibodies valuable for capturing spores but not vegetative cells in an immunoassay. B48 and B183 were also found to cross-react with spores of *B. megaterium* and *B. subtilis* subsp. *globigii* with slightly different affinities.

The exosporium of the spore is composed primarily of protein, with carbohydrate and lipid present (33). It is divided into a basal layer, an intermediate layer, and an outer layer of hair-like projections (12). Previous research with *B. cereus* spores has shown the exosporium to contain spore-specific antigens which are not present on the cells (7). Polyclonal antibodies made against *B. cereus* spores which reacted with a range of *Bacillus* spores, but not vegetative cells, were found to be located primarily on the exosporium (5). *B. cereus* and *B. megaterium* spores have similar hexagonally ordered lattice structures in the basal layers of their exosporia (3). Similar tertiary structures of the proteins which make up this basal layer may contribute to the cross-reactivity of the exosporium-reacting antibodies with *B. megaterium* spores.

The hybridoma producing monoclonal antibody D89 was subcloned because it produced the only antibody which reacted with both spores and vegetative cells of both the *Bacillus* and *Clostridium* genera. Such an antibody could be useful in an immunoassay because it could help to eliminate the need for an expansive cocktail of monoclonal antibodies to detect a broad range of spores. It is also of interest because it may prove to be a useful tool in studying an antigen which is common to two different genera of sporeformers and present on

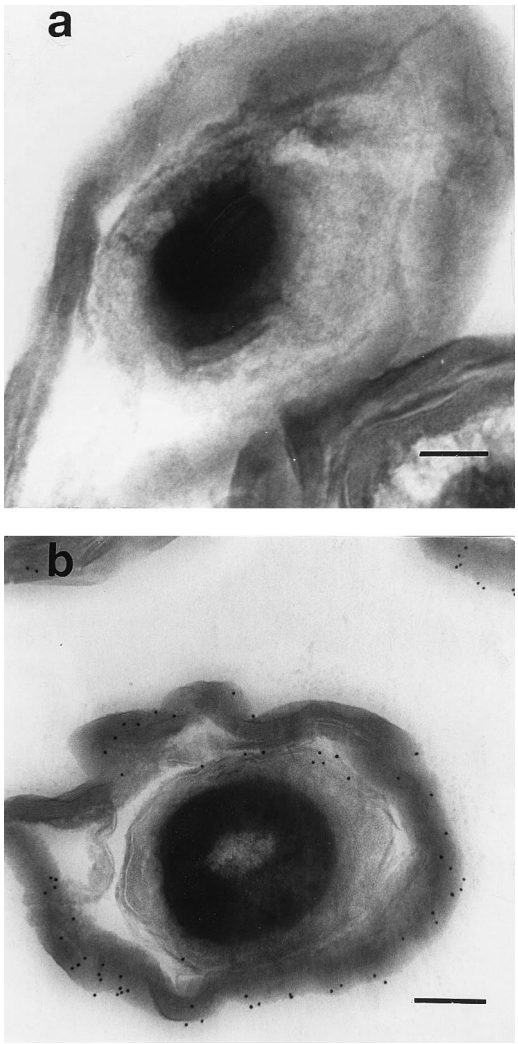

FIG. 3. Immunocytochemical localization of *C. sporogenes* PA3679 spore antigens. Ultrathin cryosections of *C. sporogenes* spores were incubated with a negative primary antibody control (a) or D89 (b) and then with a colloidal gold-labeled secondary antibody. Bars, 0.1 μm.

both of their vegetative cells and spores. Immunocytochemical localization of this antigen in *B. cereus* spores indicates that the antigen is present on the exosporium of the spore. Immunocytochemical localization of the antigen in *C. sporogenes* indicates that it is present on the exosporium and the outer cortex region of the spore. The antigen was not detectable by Western blotting of spore extracts of either species. This may be due to insufficient quantities of the antigen being extracted, alteration of the antigen upon extraction, or destruction of the epitope recognized by the antibody by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Alternatively, this lack of detection may be an indication that the antigen is not proteinaceous in nature.

While less information is available about *C. sporogenes* spore antigens, it has been demonstrated that specific spore antigens which are distinct from vegetative cell antigens exist (25, 29). It has also been shown in two different studies with polyclonal antibodies that *Clostridium* spore antigens which are common to more than one species of *Clostridium* exist (11, 25).

The current research indicates that some *C. sporogenes* spore antigenic determinants may be lost or altered over long-term storage at −20°C. *C. sporogenes* spores were reactive with antibodies C33, C225, and D89 in dot blot assays up to 18 months after the original preparation of the spore stock. Between 18 and 24 months of storage at −20°C, it was observed that the antibodies were not reactive in Western blots of *C. sporogenes* spore extracts. It was unclear whether the problem was with insufficient extraction of the antigens, alteration of the antigens upon extraction, or loss of the antigens in the stored spores. After approximately 24 months of storage of the spores, immunocytochemical localization of the antigens was performed to identify where on the spores the antigens were located. The antigens with which C33 and C225 reacted were no longer reactive when cross-sections of *Clostridium* spores were probed with the antibodies C33 and C225. After the same period of storage, the antigen which reacted with D89 was still reactive in immunocytochemical localization studies. Two new spore stocks were prepared and held at 4°C for <6 months. Spores of each of these stocks reacted with C33, C225, and D89 in dot blot immunoassays. These spores could also be detected by C33 in an ELISA at levels of $\geq 10^7$ spores/ml when spores of the original spore stock could not be detected even at levels of 10^9 spores/ml (data not shown). These results indicated that the antibody was still able to react with antigen, but they led us to conclude that some antigens on spores from the original spore stock were altered such that they were no longer recognized by the antibodies C33 and D225. The loss of bacterial spore antigen over long-term refrigerated storage of spores has previously been reported for *Bacillus anthracis* (27).

Together, three of these antibodies (B48, B183, and C33) are able to detect spores of *B. cereus*, *B. megaterium*, *B. subtilis* subsp. *globigii*, *B. stearothermophilus*, *C. perfringens*, and *D. nigrificans*. Future research will focus on the use of these antibodies, along with antibody D89, to develop immunoassays which will detect a range of bacterial spores of significance in food products and ingredients. Our results indicate that broad-range detection of spores with a monoclonal antibody-based detection system would most likely require a cocktail of monoclonal antibodies, as no one antibody was found to react with the entire range of spores examined. One antibody found to react with both *Bacillus* and *Clostridium* spores also reacted with vegetative cells of both genera. Such an antibody may be useful as a detector antibody once spores are isolated from a food or ingredient by using a more specific capture antibody.

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