Production of Monoclonal Antibodies against the Major Capsid Protein of the Lactococcus Bacteriophage u136 and Development of an Enzyme-Linked Immunosorbent Assay for Direct Phage Detection in Whey and Milk

SYLVAIN MOINEAU,1,2 DENIS BERNIER,3 MARIE JOBIN,3 JACQUES HÉBERT,3 TODD R. KLAENHAMMER,2 AND SITIHNAN PANĐIAN4*

Centre de Recherche STELA and Département de Sciences et Technologie des Aliments, Université Laval, Pavillon Comtois, Sainte-Foy, Québec, Canada G1K 7P4; Unité de Recherche Inflammation et Immunologie-Rhumatologie, Centre Hospitalier de l’Université Laval, Sainte-Foy, Québec, Canada G1V 4G2; and Southeast Dairy Foods Research Center and Department of Food Science, North Carolina State University, Raleigh, North Carolina 27695-76242

Received 1 January 1993/Accepted 22 April 1993

The only major structural protein (35 kDa) of the lactococcal small isometric-headed bacteriophage u136, a member of the P335 species, was isolated from a preparatory sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. Monoclonal antibodies (MAbs) were raised against the denatured 35-kDa protein. Six MAbs were selected and characterized. Western blots (immunobLOTS) showed that all MAbs recognized the 35 kDa but also a 45 kDa that is in lower concentration in the phage structure. Binding inhibition assays identified five families of MAbs that recognized nonoverlapping epitopes of the 35- and 45-kDa proteins. Immunoelectron microscopy showed that these two proteins are localized within the phage head, therefore indicating that the 35 kDa is a major capsid protein of u136 and that the 45 kDa is a minor capsid protein. With two MAbs, a sandwich enzyme-linked immunosorbent assay (ELISA) was developed for direct detection of lactococcal phages in whey and milk samples. Whey and milk components, however, interfered with the conduct of the assay. Partial denaturation of milk samples by heat treatment in the presence of SDS and β-mercaptoethanol removed the masking effect and increased the sensitivity of the assay by 100-fold. With the method used here, 10^7 PFU/ml were detected by the ELISA within 2 h without any steps to enrich or isolate bacteriophages.

Despite improvements in starter culture technology during the last decade, perturbation of milk fermentsations due to bacteriophage attack is still a major concern for the dairy industry (9). Cheesemaking is a nonsterile process, and phages are virtually always present in the plant (10). If a susceptible host is provided, the phage population will increase very quickly because of short latent periods and large burst sizes (23). Phage population level of 10^2 to 10^7/ml in starter media or milk is an indication of a potential problem and is likely to retard milk acidification (30). In fact, in defined starter systems, phage-sensitive strains are normally replaced when phage populations reach that level in wheys (30).

Lactococcal phages can be detected in various ways: spot test (12), starter activity test (11, 24), indicator test (29, 32), ATP measurement (5), and electrical impedance (34). However, these assays have several disadvantages. They are time-consuming, especially when large numbers of samples are tested. Most of them rely on the availability of single indicator strains, which is not possible when mixed strain starters are used in cheese manufacture. Most importantly, methods currently available are indirect because they evaluate the effects of a sample (suspected to contain phages) on the bacterial metabolic activity and not the presence of the phage itself. In this case, a slow metabolic activity of the starter could be improperly attributed to phage contaminations (false positive). A direct method to rapidly detect phage contamination is definitely needed in the cheese industry so that precautionary measures could be taken to minimize or prevent economic losses (20, 28).

Direct detection of lactococcal phages in various media by DNA probes was reported recently (20). The detection limit of the assay was between 10^6 and 10^7 PFU/ml. Although many samples could be processed simultaneously by a dot blot apparatus, the time required for performing the assay was still too long for industrial purposes. To be efficient in industrial conditions, phage detection tests must be fast, easy to handle, and very sensitive (28). Recently, it was suggested that immunological detection could be used to achieve these objectives and detect phages in the dairy industry (28, 30). Polyclonal and monoclonal antibodies (MAbs) could serve as a tool in a direct detection system (4).

Detailed information on a large number of lactococcal phages is now available. These data have led to a new classification of lactococcal phages into 12 species (15). However, most phages belong to three species composed of two small isometric-headed species (936 and P335) and a prolate-headed species (c2). Phages from the same species have nearly the same morphology, share strong DNA homology, and exhibit similar structural protein profiles (15). Therefore, because of the presence of proteins in their structure, lactococcal phages should be detectable by assays based on antibodies. However, to our knowledge, only one study addressed that possibility over 10 years ago (17). In that case, polyclonal antibodies were raised against intact lactococcal phages (c2 and 936 species). The detection limit was 10^7 PFU/ml of whey, and the results were obtained within 3 h. No attempt to detect phages in milk was reported.
Other serological analyses have shown that polyclonal antibodies raised against lactococcal phages will bind only to phages from the same species (13, 14). Polyclonal antibodies raised against β-galactosidase fused to lactococcal phage structural protein (c2 species) was recently reported (28). However, no utilization of these antibodies for phage detection purposes was reported.

For most of the small isometric-headed phages studied thus far, only one major structural protein has been observed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) (2, 19, 25, 26), whereas prolate-headed phages and large isometric-headed phages have at least three major structural proteins (2, 19, 25, 26). They are designated major because of their high concentration in the phage structure compared with minor proteins that are much more numerous but lower in concentration. Chung et al. (6) have estimated that the major capsid protein of the lactococcal small isometric-headed phage P4-1 represents nearly 80% of the total phage structural proteins. Interestingly, it has been reported recently that major capsid proteins of lactococcal phages are produced in excess during infection (18). Thus, because of their high structural concentration and excess production during infection, major capsid proteins could be a suitable target for phage detection by immunological assays.

In this study, MAbs were raised against the major capsid protein of the lactococcal small isometric-headed phage u136, a member of the P335 species. An enzyme-linked immunosorbent assay (ELISA) which allowed direct detection of lactococcal phages in whey or milk without purification or an enrichment step was then developed. To our knowledge, this is the first report on production of MAbs against a lactococcal phage capsid protein.

MATERIALS AND METHODS

Bacterial strains, phage, and medium. Lactococcus lactis subsp. cremoris U18 and phage u136 were isolated from Québec cheese plants (19). Phage u136 is a member of the lactococcal phage species P335 (15). L. lactis U18 was propagated in M17 broth containing 0.5% lactose (31). Phage u136 was produced and concentrated to 10^{12} PFU/ml in a CsCl gradient as described by Jarvis (14).

Purification of the 35-kDa major structural protein. The concentrated phage sample was dialyzed twice against distilled water for 24 h at 4°C, and its protein profile was examined by preparative 12% acrylamide SDS-PAGE by the method of Laemmli (16). After completion of electrophoresis, the portion of the gel containing one sample lane next to molecular weight markers was sliced off, fixed, and stained with Coomassie blue. The remaining unstained portion of the gel was stored at −80°C to avoid diffusion of the protein bands. The region in the unstained gel corresponding to the only major structural protein (35 kDa) of u136 was sliced out. Three ml of phosphate buffer (10 mM, pH 7.0) was added to the gel slice, which was homogenized in an 18-gauge needle on a 5-ml syringe. The protein diffused into the buffer over a 16-h incubation at room temperature. The extraction was repeated twice. The protein purity was confirmed by analyzing a small aliquot by SDS-PAGE. Extracts were pooled and concentrated in a Speed Vac (Savant, Farmingdale, N.Y.) to 1 mg of protein per ml. The protein concentration was estimated by the Bradford assay (Bio-Rad Laboratories, Richmond, Calif.).

Production and characterization of MAbs. Female BALB/c mice were immunized subcutaneously at days 1, 14, 28, and 42 with 150 μg of the 35-kDa protein emulsified in complete Freund's adjuvant (day 1) or incomplete Freund's adjuvant (days 14, 28, and 42). A final booster injection was administered intravenously 5 days before fusion. Spleen cells were fused with nonsecreting SP2/0 myeloma cells in the presence of polyethylene glycol as described previously (22). Hybrids were grown in hypoxanthine-aminopterin-thymidine selective medium for 10 days. The resulting hybridoma cells were screened for their ability to produce antibody against the 35-kDa protein determinants, by a direct-binding ELISA. Flat-bottom polyvinyl chloride plates (Dynatech Laboratories, McLean, Va.) were coated overnight at 4°C with the 35-kDa protein (200 ng per well) in carbonate buffer (50 mM, pH 9.6). Wells were saturated with 3% bovine serum albumin (BSA) in TBS (50 mM Tris, 200 mM NaCl [pH 7.5]) containing 0.1% Tween 20 for 2 h at 37°C. After three washes with TBS-Tween, the culture supernatant (100 μl) was incubated for 3 h at 37°C. Following three additional washes, peroxidase-labeled goat anti-mouse immunoglobulin G (dilution, 1:1,000) was added and incubated for 2 h at 37°C. After three washes, the substrate (0.4 mg of 0-phenylenediamine per ml in 50 mM citrate buffer [pH 4.5] and 0.006% hydrogen peroxide) was added and the reaction was stopped by addition of 0.1 M H_{2}SO_{4}. The A_{492} was read with a Titertek automatic ELISA reader. Hybrid cells secreting MAb against the 35-kDa protein were cloned twice by limiting dilution before propagation in culture. MAbs were isolated from culture supernatant by using AFI-gel protein A (Bio-Rad) according to the manufacturer's protocol.

Purification of MAbs from ascitic fluid. When high concentrations of a particular MAb were needed, hybrid cells (5 × 10^{6}) were propagated in the peritoneal cavity of BALB/c female mice. The ascitic fluid of the mice was harvested 10 to 14 days later, and antibodies were purified through a protein A-Sepharose 4B column. MAbs were eluted (0.1 M citrate buffer [pH 5.5]) and stored at −80°C until use.

Isotyping of MAbs. Isotyping was performed with the Immuno Select kit (MAb-based isotyping system for mouse immunoglobulins) according to the manufacturer's directions (GIBCO/BRL, Gaithersburg, Md.).

Immunoblotting analysis. To determine the specificity of the MAbs, immunoblotting experiments were conducted. Denatured phage u136 was electrophoresed on a 12% acrylamide SDS-polyacrylamide gel and transferred onto nitrocellulose by using LKB 2117 Multiphor II and according to manufacturer’s protocol (Pharmacia, Piscataway, N.J.). The membrane was cut into strips corresponding to the sample lanes and saturated with 3% BSA diluted in TBS. The strips were then incubated overnight at 25°C with MAbs. The bound MAbs were detected by autoradiography after binding of 125I-labeled goat anti-mouse immunoglobulin G (GIBCO/BRL).

Competitive inhibition radioimmunoassay. To determine if MAbs recognized nonoverlapping epitopes of the 35-kDa protein, a binding inhibition radioimmunoassay was developed. In this assay, cyanogen-activated cellulose discs were coated with the 35-kDa protein from u136 (100 ng per disc) and incubated overnight at 4°C. After washes, discs were saturated with 3% BSA for 2 h at 37°C and incubated overnight at 4°C with an unlabeled MAb. After washes, a 125I-labeled MAb was added and incubated at 37°C for 3 h. Following washes, the radioactivity fixed on the solid phase was counted in a LKB gamma counter (Pharmacia). The percent inhibition was calculated according to the following formula: [([cpm]_t - [cpm]_i)/[cpm]_i] × 100, where [cpm]_i is the radioactivity of bound 125I-MAb without prior incubation of
unlabeled MAb (positive control) and cpm\textsubscript{2} is the radioactivity of the bound \textsuperscript{125}I-Mab with prior incubation of unlabeled MAb.

**Radioiodination.** Protein A-purified MAb was iodinated with \textsuperscript{125}I by using Iodo-Gen (Pierce Chemical Co., Rockford, Ill.). Free \textsuperscript{125}I was separated from \textsuperscript{125}I-labeled MAb by passage through an Econo-Pac 10DG desalting column (Bio-Rad).

**Electron microscopy and gold labeling.** Immunoelectronmicroscopy analysis was conducted to identify the MAb binding site on the native lactococcal phage ul36 and localize the 35-kDa protein. Preparation of phages for electron microscopy was performed as described previously (19). Next, 25 µl of prepared phage was mixed with 25 µl of MAb (7H5) diluted 1:100 in 50 mM TBS [pH 7.5]-0.1% Tween 20-0.5% BSA and incubated for 1 h at 37°C. Finally, 25 µl of gold-labeled protein A (5 or 10 nm; Sigma Chemical Co., St. Louis, Mo.) diluted 1:10 in the above buffer was added to the phage-antibody solution and incubated for 1 h at 37°C. A drop of the phage-antibody-gold solution was transferred onto a Formvar grid. Excess liquid was withdrawn with a filter paper. The preparation was stained with 2% phosphotungstate (pH 7.2) and examined under the electron microscope (Phillips 410).

**ELISA.** A sandwich ELISA was developed for detection of lactococcal phages in whey and nonfat dry milk. Phage ul36 was diluted to various concentrations in whey (13% solids; Sigma) and in nonfat dry milk (11% solids; ICN, Cleveland, Ohio). Flat-bottom polyvinyl chloride plates (Immulon-IV; Dynatech) were coated overnight at 4°C with the MAb 7H5 (200 ng per well) in carbonate buffer (50 mM, pH 9.6). After four washes with TBS buffer, wells were saturated with 1% nonfat dry milk incubated at 37°C for 2 h. After two washes, plates were stored at 4°C (up to 1 week) until use. BSA (1 and 3%) was also tested to saturate nonspecific sites but gave a higher background. Generally, casein has high affinity with plastics and low affinity with other proteins, and it was reported to be the most effective material for plate postcoating (33). Following two washes, 250 µl of test sample (in triplicate) was added and incubated for 30 min at 37°C. After four washes, the biotin-labeled antibody (200 µl of biotinylated MAb 6C1 diluted 1:1,000 in 1% TBS-BSA) was added and incubated for 30 min at 37°C. After washes, 50 µl of streptavidin-alkaline phosphatase (GIBCO-BRL, Grand Island, N.Y.) (diluted 1:1,000 in 1% TBS-BSA) was added, and the plates were incubated at 37°C for 30 min. After the final four washes, 100 µl of substrate (2 mg of \textit{p}-nitrophenyl phosphate \textit{p}NP, GIBCO-BRL) was added and incubated at 37°C for 30 min. The reaction was stopped by adding 50 µl of 3 N NaOH. The \textit{A}_{405} was read with a Dynatek MR5000 automatic ELISA reader. The concentration of MAb, streptavidin-alkaline phosphatase, and the substrate pNP were optimized to lower the background. To increase the sensitivity of the assay, samples were denatured prior to conducting ELISA. Different microliter quantities of denaturation buffer (125 mM Tris, 4% SDS, 10% \textit{β}-mercaptoethanol) were added to 1-ml samples (M17, whey, and milk). The samples were heated in boiling water for 5 to 10 min and then assayed.

**Biotinylation of MAb.** The MAb 6C1 was biotinylated with the Immunopure NHS-LC-Biotinylation kit (Pierce). Briefly, 10 mg of 6C1 was diluted in 1 ml of phosphate buffer (0.1 M, pH 7.2) and added to 400 µg of NHS-LC-biotin. The mixture was incubated for 30 min at room temperature with continuous gentle stirring and then passed through a desalting column. The biotinylated MAb was recovered after elution of the column with PBS (0.01 M sodium phosphate, 0.15 M NaCl [pH 7.2]).

**RESULTS**

**Purification of the major structural protein of bacteriophage ul36.** The protein profile of phage ul36 (Fig. 1, lane 2) showed one major structural protein estimated at 35 kDa. This is in agreement with the profiles exhibited by other small isometric-headed phages (2, 19, 25). This major protein was successfully isolated following extraction from the preparative SDS-polyacrylamide gel (Fig. 1, lane 1).

**Characterization of MAbs.** By a direct-binding ELISA, six hybridoma clones (6C1, SD1, 9G4, 7H5, 7H7, and 7H9) were found to produce MAbs directed toward the 35-kDa major protein of ul36. The binding of the six MAbs increased with increasing 35-kDa protein concentration, and all six MAbs carried an immunoglobulin G2a heavy chain (data not shown).

**Immunoblotting analysis.** The specificity of each MAb was tested by Western blot (immunoblot) analysis (Fig. 2). The immunoblot revealed that all six MAbs recognized the 35-kDa protein, but also another ul36 structural protein estimated at 45 kDa. Extended heating in the presence of SDS (4%) and \textit{β}-mercaptoethanol (10%) did not remove the 45-kDa protein band. No trace of the 45-kDa protein was found in the 35-kDa purified protein stock. Since this protein was not injected into the mice, it must, therefore, share common epitopes with the 35-kDa protein. Western blot analysis was also conducted with one phase each of the c2 and 936 species as well as with \textit{L. lactis} UL8 and \textit{Escherichia coli} DH5α. None of the MAbs reacted with these phages and bacterial strains (data not shown), indicating that the MAbs were specific to ul36 phage capsid proteins.

**Binding inhibition of MAbs to lactococcal phage ul36.** Table 1 shows competitive binding inhibition assays to the 35-kDa protein with different pair combinations of the six MAbs. The initial binding of a MAb to a ul36-coated well would normally inhibit a second binding with the same MAb, and the percentage inhibition should be high. As expected, for the MAbs 6C1, SD1, 9G4, 7H5, and 7H7, binding inhibition
35-kDa protein an ideal target for developing an ELISA to detect lactococcal phages in whey and milk. The MAb pair 6C1/7H5 was selected for the ELISA on the basis of the competition binding assays, nonoverlapping epitopes, high affinity with u136, and the high stability and production levels of the hybridoma cell lines. The sensitivity of the ELISA was 10^6 PFU/ml in M17 (Fig. 4A) and in whey (Fig. 4B) but 10^8 in milk (Fig. 4C). Since MAbs were produced against the denatured 35-kDa protein, attempts were made to improve the sensitivity level by treating samples with a denaturation buffer before their addition to the microtiter wells. The concentration of denaturing buffer was reduced to the extent possible to avoid modifying the antibodies coated in the wells. The optimum quantity of denaturing buffer added to 1-mL samples varied depending on the media in which the phage was diluted (Fig. 5). To obtain the highest sensitivity, 5, 55, and 80 μL of denaturing buffer were used for M17, whey, and milk, respectively. These buffer concentrations probably represent the best compromise to detect the phages without denaturing the antibodies. The increasing concentration of denaturing buffer correlated with the increasing protein content of the sample (data not shown). This suggests that milk proteins may be most responsible for interfering with phage detection by this ELISA. Treating the sample with the denaturing buffer increased the sensitivity of the ELISA. For whey, the detection limit was lowered from 10^6 to 10^3 PFU/ml and for milk from 10^8 to 10^5 PFU/ml. However, the sensitivity in M17 was not modified by providing denaturing conditions. Interestingly, the sensitivity level was higher in whey and milk than in M17. This could suggest the presence of an accelerator of the antibody reaction in whey and milk (27).

L. lactis or E. coli cells, phages lambda or T7, and 34 lactococcal phages of the 936 and c2 species tested (at concentration up to 10^10 PFU/ml) were not detected by the ELISA (data not shown). All the eight phages of the P335 species (like u136) tested were detected by the ELISA to various levels (10^7 to 10^10 PFU/ml).

### DISCUSSION

The small isometric-headed lactococcal phage u136 (P335 species) has one major structural protein, estimated at 35 kDa. The high concentration of the 35-kDa protein in the overall phage structure and its localization on the phage head demonstrated that the 35 kDa is the major capsid protein of the lactococcal phage u136.

MAbs were raised against the denatured form of the 35-kDa protein isolated by preparative SDS-PAGE. Western blots showed that all six MAbs we have obtained recognized the 35-kDa protein and also a minor protein of 45 kDa. A possible explanation for the cross-reactivity of the 35- and the 45-kDa proteins is provided through previous observations on bacteriophage T7 (8). This small isometric-headed phage from E. coli has also one major structural protein that corresponds to the major capsid protein (36.4 kDa). This protein is specified by gene 10 that also encodes a second 41.8-kDa head protein which is produced in smaller amounts. Translation of the two proteins begins at the same initiation site, but the 41.8-kDa protein appears to be produced because of a shift in the translational reading frame just ahead of the normal termination codon. Fifty-three amino acids are thereby added to the carboxy terminus of the 36.4-kDa major capsid protein (8). Therefore, the minor head protein (41.8 kDa) is derived from the gene for the major capsid protein (36.4 kDa), and the two share some
common epitopes. Although no DNA homology or immunological reactions were detected between T7 and u136 (data not shown), these observations prompt us to speculate that the lactococcal u136 45-kDa protein may be derived from the gene of the 35-kDa protein because of a genomic organization similar to that found in E. coli phage T7.

The high concentration of the major capsid protein in the phage structure makes it a preferred antigenic target for detection by immunological methods. Furthermore, we have reported previously that capsid proteins of lactococcal phages are produced in excess during infection of L. lactis (18). An ELISA for the detection of lactococcal phage in milk and whey using two MAb s recognizing nonoverlapping epitopes of the 35-kDa major capsid protein was successfully developed. Detection of microorganisms with polyclonal antibodies and MAb s raised against native or extracted cell wall components is well known, and many commercial kits are available (4). However, an enrichment step is required in most cases, because the target microorganism is initially present in low concentration. With an ELISA system, whole bacterial cells can be detected at levels as low as $10^3$ to $10^6$ CFU/ml of pure culture or enrichment broth (4).

Lytic lactococcal phages are commonly found in cheese wheys, and they are problematic when they reach high populations ($10^5$ to $10^7$ PFU/ml of whey). High phage concentration provokes fermentation failures by lysing a high percentage of starter cells (30). If phage build up could be detected rapidly, precautions could be taken to minimize any subsequent economic losses. In this study, we have detected phages directly in whey or milk within 2 h without an enrichment step or use of an indicator strain. The time for the assay may even be shortened by 30 min if the second antibody was conjugated with an enzyme rather than relying on the biotin-streptavidin system.

Lower sensitivity in the assay was obtained when phages were assayed in whey or milk than when the phages were in M17 broth. Numerous workers have also encountered difficulties detecting microorganisms directly in a food matrix without enrichment, especially with DNA probes and polymerase chain reaction technology (20, 35). Interestingly, the ELISA described here, combined with partial denaturation of the samples (by heat, SDS, and β-mercaptoethanol) has increased the sensitivity (up to 100-fold) and decreased the masking effects of milk proteins. The results were obtained in 2 h, and the sensitivity level was $10^7$ PFU/ml, although $10^6$...
LACTOCOCCAL PHAGE DETECTION IN WHEY AND MILK

The MAbs were raised against the capsid protein of u136, a member of the lactococcal P335 phage species. Attempts with these MAbs to detect lactococcal phages from the 936 and c2 species were unsuccessful. These agree with previous studies on the serology of lactococcal phages (13, 14). These results are also in agreement with DNA hybridization data in which no homology is found between phages of different species (2, 19, 25, 26). Interestingly, when other members of the P335 species were tested, the MAbs reacted at various levels but never as strongly as with u136. For example, in wheys with a phage population of 100 PFU/ml, the maximum absorbance was about 7.0 optical density units for phage u136 (Fig. 4B) but only an average of 2.0 units for the other P335 phages (data not shown). All P335 phages tested in this study shared partial DNA homology (1, 21), but they apparently have differences in the capsid protein epitopes which may be disadvantageous for ELISA techniques. However, recent data have suggested that the P335 species is much more diverse than the other two most commonly found lytic phage species, 936 and c2 (1, 21).

One technical problem that ELISA-based tests may face is the possibility of false-positive results due to the so-called own phages present in mixed strain starters (7, 10, 30). Some mixed strain starters, which are of undefined strain composition, are permanently infected with a low number of virulent phages (10). These phages propagate on a limited number of sensitive cells present in the culture, while the remaining cells are phage resistant. The culture’s own bacteriophages can reach population levels up to 108 PFU/ml without disturbing the activity of the culture (30). This pseudolysogeny has been poorly investigated, and these phages have rarely been characterized (7). Although small isometric phages have been isolated from mixed strain starters, their identity and relationship to disturbing lytic phages of the 936 and P335 species are unknown (17a). If they are found to be similar to common lytic lactococcal phages, undisturbing high level of own phages in mixed strain starters would be detected as false positive by an ELISA-based test. Since pseudolysogeny does not occur in defined starter systems (10), it is possible that the phage ELISA could be confined to such starter cultures. On the other hand, if they are distinct phages, ELISA methods with antibodies against the three most important lytic phage species (936, P335, and c2) would be devoid of any such false-positive results.

This study demonstrated the effectiveness of a rapid detection method for lactococcal phages present in milk or whey samples with MAbs directed against a denatured phage capsid protein. Furthermore, these results suggest that, when combined with partial denaturation of a food sample, MAbs raised against denatured proteins may provide a new approach to increase the level of sensitivity at which microorganisms can be detected in food matrices.

ACKNOWLEDGMENTS

This study was supported in part by Conseil de recherche en Pêches et Agro-Alimentaire du Québec, Centre de recherche STELA, and North Carolina Agricultural Research Service project NC01268. S.M. was supported by a FONDS FCAR graduate scholarship. We gratefully acknowledge Brendalyne Bradley and Michael J. Dykstra for helpful suggestions in the immunoglobulin labeling experiments. We also thank Yvan Boutin for assistance during mice immunizations and Sylvie Toussaint for assistance during biotinylation.

REFERENCES

14. Jarvis, A. W. 1978. Serological studies of a host range mutant of