Rapid isolation of *Escherichia coli* O157:H7 from enrichment cultures of foods using an immunomagnetic separation method

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Received 23 August 1991

A method employing immunomagnetic beads was developed for the isolation of Escherichia coli *O157:H7. Magnetic beads precoated with sheep anti-rabbit IgG were coated with rabbit antiserum against* E. coli *O157. The level of bacteria, amount of beads, amount of antibody coating the beads and incubation time were varied to determine their effects on recovery of the organism. The sensitivity of the test for recovery of* E. coli *O157:H7 was* 1×10^1 *cfu* ml^{-1}. *The organism was selectively recovered from 24 h enrichment cultures of meat products in which the initial inoculum level was 1 bacterium* g^{-1}. *Sorbitol negative colonies were selected from Sorbitol MacConkey agar and were positively identified as* E. coli *using a direct fluorescent antibody procedure. The results indicate that immunomagnetic separation is a convenient and rapid method for the isolation of* E. coli *O157:H7 from foods.*

Introduction

Escherichia coli serotype O157:H7 has emerged as a new foodborne enteric pathogen responsible for outbreaks of hemorrhagic colitis and hemolytic uremic syndrome. Undercooked ground meat has been most frequently implicated in documented outbreaks of hemorrhagic colitis (Uyeyama et al. 1982, Riley et al. 1983, Wells et al. 1983, Ryan et al. 1986, MacDonald et al. 1988) with additional cases being associated with the consumption of raw milk (Martin et al. 1986, MacDonald et al. 1988). Doyle and Schoeni (1987) isolated *E. coli* O157:H7 from 3·7% (6/164) of market samples of fresh beef, and also recovered the organism from a significant percentage of other fresh meat products such as pork (1·5%), poultry (1·5%) and lamb (2·0%). The organism appears to be a relatively common contaminant of food products of animal origin.

Several methods have been developed for the detection of *E. coli* O157:H7 in foods and other samples (Szabo et al. 1986, Soncini et al. 1988, March and Ratnam 1989, Okrend et al. 1990b, Samadpour et al. 1990, Szabo et al. 1990). Many of these methods either involve the use of radioisotope-labeled probes or are cumbersome and time consuming when testing large numbers of samples. Furthermore, the reliability of a number of these tests has not been evaluated. For example, it has been reported that commercial latex agglutina-

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tion screening tests for *E. coli* O157:H7 had a significant rate of false positives (Borcyzk et al. 1990).

The method of cell separation employing immunomagnetic polymer beads, developed originally for the isolation of blood cells (Lea et al. 1985, Gaudernack et al. 1986), has recently been used successfully for recovery of various target bacteria from samples such as food, milk and lake water (Lund et al. 1988, Johne et al. 1989, Skjerve et al. 1990, Cudjoe et al. 1991, Morgan et al. 1991). This simple and rapid technique allows for convenient screening of large numbers of samples. The present paper describes the application of immunomagnetic separation for the detection of *E. coli* O157:H7 in foods.

Materials and Methods

Bacterial strains and growth conditions

The *Escherichia coli* O157:H7 strains used in this study were: strain B1409, a Shiga-like toxin II producer, obtained from CDC; strains 933 and 45753-35 obtained from the Food Safety and Inspection Service; strain C984, from CDC, produces SLT-I; and strain B1375GSC15 was from CDC and is a SLT-II producer. *Escherichia coli* 11602 serotype O159:H34 was obtained from the Central Public Health Laboratory, London and *Salmonella typhimurium* was ATCC strain 14028. For all experiments the bacteria were grown in BHI medium for 18 h with aeration at 37°C. A viable cell number (cfu ml^{-1}) was determined by surface plating onto BHIA using a spiral plater (Spiral Systems, Model D, Bethesda, MD, USA). The plates were incubated aerobically at 37°C for 18 h and colonies were counted using a laser counting system (Spiral Systems).

Coating procedure

Dynabeads M-450 (diameter 4·5 μm) (P & S Biochemicals, Inc., Gaithersburg, MD, USA) monosized supermagnetic poly-styrene beads coated with affinity purified sheep anti-rabbit IgG (approximately 100 μg IgG per $2{\cdot}7 \times 10^8$ beads) or Dynabeads M-450 coated with goat anti-mouse IgG were used. The beads were coated with secondary antibody according to the instructions provided by the manufacturer. Briefly, a portion of the Dynabeads M-450 suspension ($2{\cdot}7 \times 10^8$ beads) was incubated at 4°C with either 50 of 160 μg (IgG) of rabbit antiserum against *E. coli* O157 (Difco Laboratories, Detroit, MI, USA) or with 160 μg of M Ab 8–9H (IgG2a) for 4 h with even rotation. The monoclonal antibody was kindly provided by Dr Rebecca Durham at Organon Teknika, Rockville, MD, USA Washing was performed by collecting the beads using a Dynal magnetic particle concentrator (Dynal MPC-1, P & S Biochemicals, Inc.). The supernatant was discarded and the beads were washed four times for 30 min at 4°C in 1X phosphate-buffered saline Dulbecco's formulation (ICN Biomedicals, Inc., Costa Mesa, CA, USA) containing 1% fetal bovine serum (ICN Biomedicals, Inc.) (PBS-FBS). The Dynabeads M-450 coated with secondary antibody (Difco antiserum against *E. coli* O157) (DB-O157) and Dynabeads coated with M Ab 8–9H were stored in PBS-FBS at 4°C as instructed by the manufacturer.

Detection of E. coli *O157:H7 using DB-O157*

Levels of *E. coli* O157:H7 strain B1409 ranging from 10^1 to 5×10^4 cfu were suspended in 1 ml of PBS-FBS in a 1·5 ml microcentrifuge tube and incubated with either DB-O157 coated with 50 μg of O157 antiserum or coated with 160 μg antiserum (5 μl, 2×10^6 beads). The samples were incubated at room temperature with even rotation for either 15 or 60 min. The effect of varying the amount of DB-O157 was determined by incubating *E. coli* B1409 ($1{\cdot}5 \times 10^3$ cfu for 15 or 60 min with amounts of DB-O157 ranging from 5×10^5 to 4×10^6 beads. The beads were washed four times with PBS-FBS using the MPC-1 magnet and resuspended in PBS-FBS. A viable count was determined by surface plating the DB-O157 onto BHI agar plates using sterile bent glass rods. The colonies were counted after 18 h at 37°C. To determine if *E. coli* B1409 could be recovered from a heterogenous suspension of bacteria, the organism (10^2 cfu) was incubated with either *E. coli* 11602 or *S. typhimurium* at different concentrations in either 1 ml of PBS-FBS (*E. coli* 11602 and *S. typhimurium*), lauryl tryptose broth (LST) (Difco) or EC broth (Difco) containing 20 mg l^{-1} novobiocin

(Sigma) (EC+n) (*S. typhimurium*). The beads were washed four times and a portion was plated onto Sorbitol MacConkey agar (SMA). SMA is MacConkey agar with the lactose replaced with sorbitol. Sorbitol negative colonies are white, while sorbitol positive bacteria form pink colonies.

Recovery of E. coli *O157:H7 in foods*

The ability of DB-O157 to recover *E. coli* O157:H7 from artificially inoculated meat products was tested in conjunction with a three-tube MPN (1·0, 0·1 and 0·01 g) (Mehlman et al. 1984) using both the BAM presumptive coliform assay and the EC broth + novobiocin enrichment technique of Okrend et al. (1990b). Canned minced beef dog food (a sterile product) was used as a model system for cooked meat products, in order to determine whether the meat itself may hinder recovery of the *E. coli*. The organism was introduced at levels of 1 and 100 bacteria g^{-1} meat. The number of bacteria in the original bacterial suspension used to inoculate the meat was verified by the standard plate count. The samples were incubated for 24 h in the two enrichment media, LST and EC+n. Ten microliters of DB-O157 (4×10^6 beads) was added to 1 ml portions of each MPN tube, and the samples were incubated for 60 min at room temperature with even rotation. The DB-O157 were washed four times and part of the bead suspension was surface plated onto SMA. The plates were incubated for 18 h at 37°C and examined for sorbitol negative colonies. The identity of representative colonies was confirmed by immunofluorescence microscopy (see below) and using API 20E kits (API Analytab Products, Plainview, NY, USA). The same procedure was employed to assess the detection of the organism from artificially inoculated raw ground beef. In total, five batches of ground beef were tested and greater than one replicate experiment per batch was performed for a majority of the batches. The background level of coliforms in the ground beef was determined by performing a duplicate MPN assay using uninoculated meat.

Fluorescence microscopy

To visualize *E. coli* O157:H7 bound to DB-O157, various concentrations of bacteria were incubated with DB-O157 (2×10^6 beads) for 60 min. The beads were washed once and incubated with FITC-labeled polyclonal antiserum against *E. coli* O157:H7 (1:20 dilution; Kirkegaard and Perry Laboratories, Inc., Gaithersburg, MD, USA) for 30 min. The beads were then washed three times, placed on glass slides and the slides were examined by fluorescence microscopy. For confirmation of the organism from the meat samples, sorbitol negative colonies were selected from SMA plates and incubated with the labeled antiserum either in microcentrifuge tubes or on glass slides for 30 min. The samples were washed and examined by fluorescence microscopy.

Results

Evaluation of DB-O157 for detection of E. coli *O157:H7*

With an increase in the level of *E. coli* B1409 in the sample, there was also an increase in the number of organisms recovered when the amount of beads added to each sample was kept constant [Figs 1(a) and 1(b)]. There was a higher degree of binding when the bacteria were incubated for 60 min with DB-O157 compared to the 15 min incubation. This difference was more evident when the bacteria were incubated with the DB-O157 coated with 50 μg of antiserum. There was a considerable increased in recovery following the 60 min incubation. Although the actual number of cfu recovered increased, the efficiency of recovery decreased somewhat with increasing levels of initial cfu ml^{-1}. For example, when DB-O157 coated with 50 μg of antibody [Fig. 1(a)] were incubated for 60 min with 1×10^2 cfu, there was recovery of 60% of the bacteria. However, when the beads were incubated with 5×10^4 cfu, recovery was only 36%.

With an increase in the amount of DB-O157 and the level of cfu ml^{-1} kept constant, there also was an increase in the number of cfu recovered [Figs 2(a) and 2(b)]. However, with the samples incubated for 60 min, there was not a significant increase in the number of cfu recovered when the amount of beads

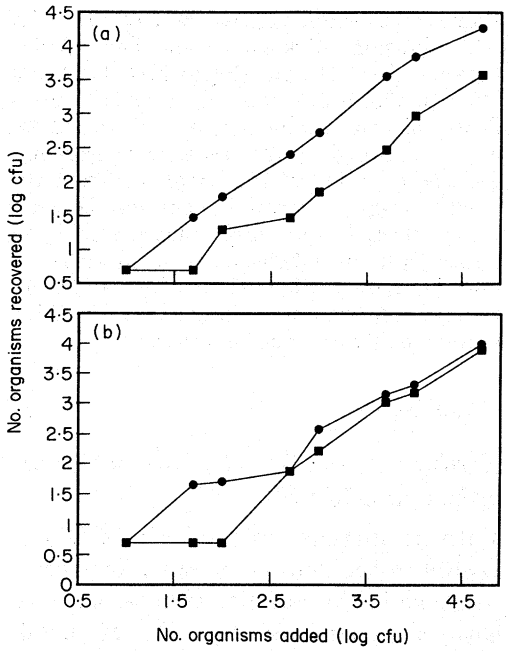

Fig. 1. Recovery of *E. coli* O157:H7 B1409 using DB-O157 (2×10^6 beads per sample) with different levels of cfu ml^{-1} added. DB-O157 were coated with 50 μg antiserum (a) and DB-O157 were coated with 160 μg antiserum (b). The data were obtained by averaging the results of two separate experiments. The results were similar in both experiments. (■) 15 min; (●) 60 min.

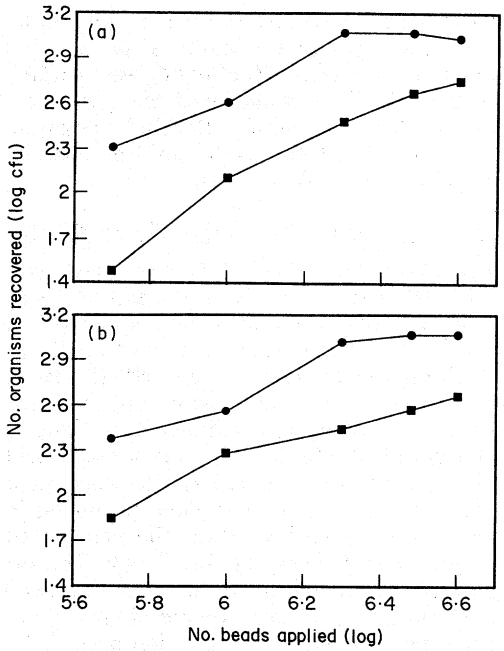

Fig. 2. Recovery of *E. coli* B1409 ($1{\cdot}5 \times 10^3$ cfu per sample) varying the amount of DB-O157 added. DB-O157 were coated with 50 μg antiserum (a) and DB-O157 were coated with 160 μg antiserum (b). The data were obtained by averaging the results of two separate experiments. The results were similar in both experiments. (■) 15 min; (●) 60 min.

added was greater than 2×10^6 (log 6·3). This occurred because a maximum level of bacteria was bound to the DB-O157. Using the beads coated with 50 μg of antiserum there was a slight decrease in recovery of cfu when the amount of DB-O157 was greater than 2×10^6. The percentage of bacteria recovered increased with increasing amounts of beads. The maximum percent recovery obtained when the samples were incubated for 15 min was approximately 40% and for the 60 min incubation, the maximum percent cfu recovered was 78%. As a control testing for the possible physical trapping of bacteria to the Dynabeads M-450, beads which were not coated with the O157 antiserum were incubated with different levels of *E. coli* B1409. Results showed that bacteria did not bind non-specifically to uncoated beads. When *E. coli* B1409 was incubated with an equal number of *E. coli* 11602 or *S. typhimurium* and the DB-O157, only *E. coli* B1409 was recovered. There was some non-specific binding of *E. coli* 11602 and *S. typhimurium* when they were added at levels 50 times greater than the level of *E. coli* B1409; however, this did not interfere with recovery of *E. coli* B1409. When *E. coli* B1409 and *S. typhimurium* were incubated in LST or in EC+n instead of in PBS-FBS, non-specific binding of *S. typhimurium* to the DB-O157 was considerably less. The decreased level of non-specific binding may have been due to

the presence of surface active agents in the two media, sodium lauryl sulfate in LST and bile salts in EC+n.

Recovery of E. coli *O157:H7 from foods after enrichment*

Following a 24 h enrichment in LST or EC+n medium, a 1 ml portion from each tube was incubated with 4×10^6 DB-O157 for 1 h. *E. coli* B1409 was detected in enrichment cultures of artificially inoculated dog food when the initial inoculum level was 1 bacterium g^{-1} meat. The only colonies which formed on SMA were sorbitol negative.

E. coli strains B1409, 933, 45753-35, C984 and B1375GSC15 were also isolated from enrichment cultures of raw ground beef when the initial inoculum level was 1 cfu g^{-1}. Using the MPN method of estimation, the total coliform count in the uninoculated beef samples ranged from 15 to greater than 240 bacteria g^{-1} meat. A high percentage of the coliforms were identified as *E. coli* strains. A high initial level of bacteria in the meat resulted in a considerable amount of non-specific binding. However, this did not interfere with the selection of sorbitol negative colonies on SMA. To determine if the length of incubation with DB-O157 had an effect on the level of non-specific binding, 1 ml portions of the ground beef enrichment cultures were incubated with the beads for 15 or 30 min instead of 60 min. With the shorter incubation times, there appeared to be approximately 50% less non-specific binding. Monoclonal antibody-coated beads were also used in the procedure for recovery of *E. coli* O157:H7 from raw ground beef. M Ab 8–9H is highly specific for the O157 polysaccharide antigen. Two 1 ml aliquots of the enrichment cultures were removed and 1 ml was incubated with the monoclonal antibody-coated beads while the other was incubated with DB-O157. There was approximately the same level of non-specific binding when monoclonal antibody-coated beads were used as when DB-O157 were used.

Fluorescence microscopy

Isolated colonies of bacteria, either one large white colony or a mixture of several white colonies, were selected from SMA and incubated with FITC-labeled antiserum against *E. coli* O157. Only the *E. coli* O157 strains reacted with the antiserum and were visible as bright green bacteria. Sorbitol negative colonies which were not *E. coli* O157 did not react with the antiserum. Forty-five bacterial strains were screened by immunofluorescence to determine if they would cross-react with the FITC-labeled antibody. The strains included *Listeria* spp., *Staphylococcus aureus*, *Salmonella* spp., *Bacillus cereus*, *Aeromonas hydrophila*, *Shigella* spp., *Yersinia enterocolitica*, *Pseudomonas* spp., *Escherichia coli* of serotypes other than O157 and *E. coli* O157:NM (non-motile) strains. Only the *E. coli* O157:NM strains reacted with the antiserum. DB-O157 bound to *E. coli* B1409 were then reacted with FITC-labeled antiserum (Fig. 3). The bacteria appear to be firmly attached to the DB-O157.

Discussion

The present paper demonstrates that immunomagnetic separation is a simple and rapid technique and can be used for the selective recovery of *E. coli* O157:H7 from foods. The sensitivity of the assay was 10^1 *E. coli* ml^{-1} using an incubation time of 15 min (Fig. 1). Fifty percent of the bacteria were recovered when the incubation time was extended to 60 min. Pure cultures of *E. coli* B1409 were recovered on SMA when the organism was mixed with equal levels of *E. coli* 11602 or *S. typhimurium*, both of which fer-

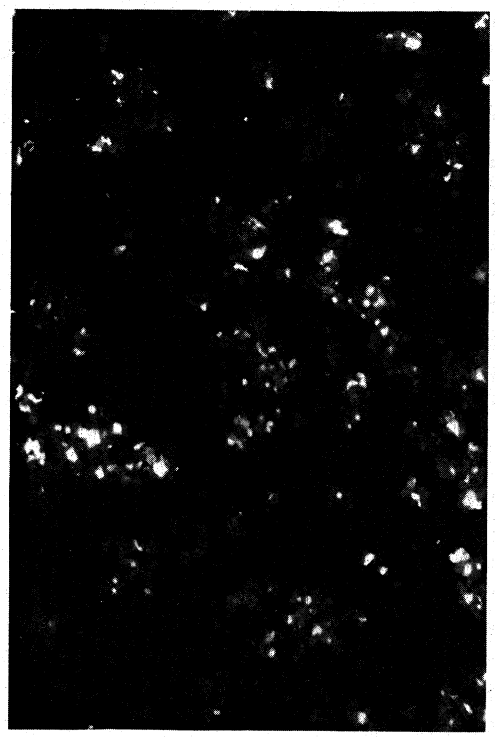

Fig. 3. Fluorescence microscopy of *E. coli* bound to DB-O157 and incubated with FITC-labeled antiserum against *E. coli* O157:H7. 1×10^8 ml^{-1} were incubated with 2×10^6 DB-O157 for 60 min.

ment sorbitol and appear as pink colonies on SMA. When considerably higher levels of these bacteria were mixed with *E. coli* B1409, there was non-specific binding to DB-O157, although recovery of *E. coli* B1409 was not hindered. When detergents are present in the incubation mixture, non-specific binding appears to be decreased. Morgan et al. (1991) showed that non-specific attachment of *Pseudomonas putida* to monoclonal antibody-coated magnetic beads could be prevented by incorporating detergents in their isolation method.

Approximately the same level of non-specific binding occurred with the use of the monoclonal antibody-coated beads as when the DB-O157 were used for recovery of *E. coli* O157:H7 from raw ground beef. Since the objective of the present study was to enhance for the presence of *E. coli* O157:H7 and not to obtain pure cultures, the level of non-specific binding did not present a problem. Sorbitol negative colonies were easily identifiable. Since there was also non-specific binding using the beads coated with a highly specific monoclonal antibody, it is possible that some of the background bacteria were attaching to the sample tube surface or were trapped by meat particles instead of actually binding to the antibody.

In general, an incubation time of 60 min allowed for increased recovery of *E. coli* B1409 when it was the only organism present. However, when a high level of other bacterial strains was present in the mixture, such as in the ground beef enrichment cultures, it was preferable to use shorter incubation times to reduce the level of non-specific binding.

Dynabeads M-450 (4·5 μm in diameter), which were used in the present study, have also been used successfully by other investigators who have employed the immunomagnetic separation technique for isolating bacteria (Lund et al. 1988, Johne et al. 1989, Morgan et al. 1991). Smaller sized beads, Dynabeads M-280 (2·8 μm in diameter), that were introduced recently may enhance microbiological and immunological applications.

The level of *E. coli* O157:H7 was amplified equally well in both of the enrichment media used allowing for the isolation of the organism when the initial inoculum level was as low as 1 cfu g^{-1} meat. SMA allowed for the selection of *E. coli* O157:H7 strains since bacteria belonging to this serotype do not ferment sorbitol. However, organisms in the meat samples which did not ferment

sorbitol and gave the same appearance as *E. coli* O157:H7 were detected on the SMA plates. These colonies were presumably due to a low level of non-specific binding since none of the isolates could be confirmed as *E. coli* O157 by fluorescence microscopy. An alternative to SMA which may be used for the selection of *E. coli* O157:H7 is SMA containing BCIG (5-bromo-4-chloro-3-indoxyl-β-D-glucuronide) (Okrend et al. 1990a). SMA-BCIG differentiates colonies which are sorbitol negative and β-glucuronidase negative (e.g. *E. coli* O157:H7) from those which are sorbitol negative and β-glucuronidase positive. Preliminary studies have indicated that streaking the beads on this medium provides enhanced differentiation.

It has been demonstrated that the FITC-labeled antiserum to *E. coli* O157:H7 was highly specific for the identification of the organism isolated from fecal specimens (Tilson 1990). The present study also demonstrates the usefulness of this antiserum for simple and rapid identification of *E. coli* O157:H7 colonies selected from SMA. Frequently, several white colonies were selected, mixed in one tube and incubated with the antiserum. When present, only the *E. coli* O157:H7 were fluorescent; other bacterial strains were negative. The antiserum also reacted with three strains of *E. coli* O157:NM. These strains were also sorbitol negative. It has been reported that verotoxigenic non-motile O157 strains which were similar to typical O157:H7 strains by plasmid profile and negative or slow sorbitol fermentation were also enterohemorrhagic (Bopp et al. 1987, Ostroff et al. 1991). Therefore, detection of these strains would also be possible using the FITC-labeled antibody to *E. coli* O157: H7. For serologic confirmation of the H7 antigen, a second separate immunofluorescence assay using FITC-labeled antiserum specific for the H7 antigen or H7 antiserum followed by a secondary FITC-labeled antibody may also be performed simultaneously. Furthermore, biochemical tests such as cellobiose fermentation, yellow pigmentation on tryptose agar or assays for the Shiga-like toxins may also be performed.

The method of immunomagnetic separation using DB-O157 described in this paper should be applicable for the detection of *E. coli* O157:H7 in other types of food and dairy products or fecal specimens. The equivalent results achieved with LST and EC+n enrichments indicate that this method can be incorporated directly into the standard presumptive coliform assay thereby eliminating the need for a separate enrichment protocol for the detection of *E. coli* O157:H7. Using this protocol, it should be possible to confirm the presence or absence of the pathogen within 48 h.

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