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Immunomagnetic separation of *Escherichia coli* O26, O111 and O157 from vegetables

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Aims: Raw fruits and vegetables have been increasingly associated with human infections caused by Shiga toxin-producing *Escherichia coli*. This study evaluates the isolation and detection of *E. coli* O26, O111 and O157 from vegetable samples using immunomagnetic particles.

Methods and Results: Standard cultivation and immunomagnetic separation (IMS) procedures were compared. It was found that immunomagnetic particles could efficiently concentrate *E. coli* cells, detecting significantly more bacteria than with standard cultivation procedures.

Conclusions: Bacteria were detected in 93–100% of the inoculated samples using the IMS procedure, but only 36–93% samples tested by standard cultivation procedures were found to be positive.

Significance and Impact of the Study: The results indicate that *E. coli* O26, O111 and O157 immunomagnetic particles can be a very useful and efficient tool for the detection of *E. coli* strains in raw vegetables, and could probably be used with samples of animal origin.

INTRODUCTION

Shiga toxin-producing *Escherichia coli* (STEC) are closely associated with gastrointestinal diseases and post-infectious severe illnesses with high mortality rates, especially in children and the elderly. The most common serotype associated with such diseases is *E. coli* O157:H7, but infections involving various non-O157 serotypes are being found with increasing frequency (Bielaszewska *et al.* 1996).

In most cases, STEC infections are connected with the consumption of contaminated, undercooked ground-beef products (Feng 1995). In the past decade, however, the number of outbreaks of human illnesses associated with the consumption of raw vegetables and fruits has increased. *Escherichia coli* O157:H7, and possibly other Shiga-toxin-producing *E. coli* strains, can contaminate fresh produce in different ways, for example, contaminated manure, irrigation or wash water, contact with infected animals and humans (Beuchat and Ryu 1997). Outbreaks of diseases caused by STEC have been associated with the consumption of leaf lettuce (Ackers *et al.* 1998), potatoes (Chapman *et al.* 1997), radish sprouts (Michino *et al.* 1999; Watanabe *et al.* 1999), alfalfa sprouts (Anon. 1997; Taormina *et al.* 1999) and raw vegetables (Pebody *et al.* 1999). Fruit-related outbreaks have also been caused by the consumption of fresh-pressed apple juice (Besser *et al.* 1993; Cody *et al.* 1999; Tamblyn *et al.* 1999). Food-borne outbreaks caused by STEC can affect large numbers of people and cause serious morbidity, making STEC one of the most important emerging pathogens. There is an urgent need for rapid, sensitive and simple procedures for the detection of the pathogens both in human samples and in foods.

The potential low infective dose of these *E. coli* serotypes necessitates the ability to detect low numbers in foods. The insensitivity of direct plating has led to the development of more sensitive methods (ELISA, immunomagnetic separation) and selective enrichment culture media. Immunomagnetic separation (IMS) has been used widely for the detection of various microbial pathogens (Olsvik *et al.* 1994; Šafařík *et al.* 1995; Šafařík and Šafaříková 1999).

This procedure, which is based on the specific capture of target cells on magnetic particles directly from

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pre-enrichment media, reduces the bacteria detection time by eliminating the selective enrichment stage. It also allows increasing sensitivity in detection due to the selective pre-concentration effect. IMS has been used to detect *E. coli* O157 especially from ground beef and bovine faeces, cider, human stool samples, etc. The increased rate of STEC infections caused by strains other then *E. coli* O157 has led to the development of immunomagnetic particles for non-O157 strains of STEC. Recently, immunomagnetic particles for the separation of *E. coli* O26 and O111 have become commercially available (Denka Seiken, Japan). The aim of this study was to test the suitability of these particles for the detection of selected STEC in food samples. Vegetables were chosen as samples because of the increasing number of outbreaks associated with the consumption of raw vegetables or their unpasteurized products.

Table 1 Detection of *Escherichia coli* O26, O111 and O157 using direct plating and immunomagnetic separation (IMS). Up to five presumptive colonies were checked until the first agglutination-positive colony was found

		E. coli O111				*E. coli* O26				*E. coli* O157			
Vegetable sample		Inoculum (no. of cells)	Direct plating (loop)*	Direct plating (100 μl)*	IMS*	Inoculum (no. of cells)	Direct plating (loop)*	Direct plating (100 μl)*	IMS*	Inoculum (no. of cells)	Direct plating (loop)*	Direct plating (100 μl)*	IMS*
Cabbage	1	12	2	N	1	12	1	3	1	10	1	N	1
	2	12	N	N	1	12	2	1	1	10	N	N	1
	3	12	N	N	1	12	1	1	1	10	N	4	1
Cabbage	1	6	N	N	1	6	1	2	1	5	N	N	1
	2	6	N	N	2	6	1	2	1	5	N	N	1
	3	6	N	N	2	6	4	3	1	5	1	4	1
Leaf lettuce	1	12	N	N	1	12	1	2	1	10	N	N	2
	2	12	N	2	1	12	1	2	1	10	N	5	1
	3	12	3	N	3	12	3	1	1	10	N	N	1
Leaf lettuce	1	6	N	N	N	6	1	2	1	5	N	N	3
	2	6	N	2	N	6	1	5	1	5	N	N	2
	3	6	N	N	1	6	1	1	1	5	N	N	4
Potato peels	1	12	1	N	1	12	1	1	1	10	4	N	1
	2	12	2	N	3	12	1	1	1	10	1	N	1
	3	12	N	1	4	12	N	3	1	10	N	3	1
Potato peels	1	6	4	N	2	6	1	4	1	5	2	N	1
	2	6	N	N	3	6	2	2	1	5	2	2	1
	3	6	N	N	1	6	3	1	1	5	5	N	1
Radish	1	12	3	1	1	12	1	1	1	10	1	1	1
	2	12	1	2	1	12	1	1	1	10	1	1	1
	3	12	1	1	1	12	1	1	1	10	4	4	1
Radish	1	6	N	1	1	6	1	3	1	5	2	2	1
	2	6	N	N	1	6	1	1	1	5	1	1	1
	3	6	3	4	1	6	3	1	1	5	1	1	1
Carrot	1	12	1	2	2	12	1	1	1	10	1	1	2
	2	12	2	2	1	12	1	1	1	10	1	1	1
	3	12	4	4	1	12	2	1	1	10	1	2	1
Carrot	1	6	N	N	1	6	1	N	1	5	N	1	1
	2	6	N	N	1	6	1	N	1	5	N	3	1
	3	6	N	N	1	6	N	4	1	5	N	N	1

*Numbers indicate the order of the first presumptive colony confirmed as positive.
N, five presumptive colonies not confirmed as positive.

MATERIALS AND METHODS

Micro-organisms

Escherichia coli O26 (strain 2663/90, non-fermenting rhamnose), *E. coli* O111 (strain 2231/81, non-fermenting sorbitol) and *E. coli* O157 (strain 1715/95, non-fermenting sorbitol) were isolated from humans. The strains used did not produce Shiga-like toxins. The strains were obtained from the Institute of Medical Microbiology, Charles University (Prague, Czech Republic).

Preparation of vegetable samples

Five types of vegetables (cabbage, leaf lettuce, radish, carrot and potatoes) were selected as food matrices. Vegetables (slightly washed cabbage, leaf lettuce, white radish and carrot; non-washed potato skins) were grated or cut into small pieces. The samples were checked for the absence of the test micro-organisms.

Inoculation of vegetable samples

Vegetable samples (25 g) were added to 225 ml buffered peptone water and inoculated with higher and lower numbers of *E. coli* cells (see Table 1). Inoculum levels were confirmed using plate counts on Nutrient Agar (Imuna, Slovak Republic). Samples were enriched for 18 h at 37°C. All experiments were performed in triplicate. Uninoculated control samples were included in all experiments.

Detection procedures

Escherichia coli O111 and *E. coli* O157 (both non-fermenting sorbitol) were cultivated on sorbitol MacConkey agar (SMAC), and *E. coli* O26 (non-fermenting rhamnose) was cultivated on rhamnose MacConkey agar (RMAC). RMAC was prepared by addition of 1% rhamnose to MacConkey Agar Base (Difco). One loop or 100 μl of enriched nutrient medium was streaked on the appropriate nutrient agar in the standard way. Plates were cultivated for 18–24 h at 37°C.

Immunomagnetic separation was performed with *E. coli* O26, O111 and O157 IMS 'Seiken' particles (Denka Seiken, Japan). The enriched samples were mixed, then 1 ml aliquots were added to 1·5 ml Eppendorf tubes containing 1 drop (about 25 μl) of the immunomagnetic bead suspension and incubated for 30 min at room temperature. Following the standard IMS procedure, bacteria were attached to magnetic beads and washed twice with PBS/Tween buffer (pH 7·4) using a magnetic separator MPC-M (Dynal, Oslo, Norway). Finally, the beads were suspended in 100 μl buffer and 50 μl suspension were inoculated on two agar plates (SMAC or RMAC). Plates were incubated for 18–24 h at 37°C.

Confirmation of suspected colonies was performed by agglutination with specific antibodies (Imuna, Slovak Republic). Up to five presumptive colonies were checked until the first agglutination-positive colony was found.

RESULTS AND DISCUSSION

The aim of the work was to test new types of specific immunomagnetic particles for the selective enrichment of *E. coli* O26, O111 and O157 from vegetable samples. In order to determine the sensitivity of the detection method used, the samples were inoculated with very low numbers (5–12) of bacterial cells. Vegetables were either slightly washed or unwashed to prevent the lowering of surface microbial load. Up to five presumptive colonies from each plate were taken for confirmation. All control samples (i.e. vegetables without inoculation) were found to be negative for the micro-organisms tested.

The results are summarized in Table 1. In general, IMS was very effective in concentrating the target *E. coli* cells for plating onto nutrient agar. The number of presumptive colonies (i.e. those non-fermenting sorbitol or rhamnose) on the plates inoculated after IMS was significantly higher when compared with direct plating. In most cases (82%), the first colony taken for agglutination after IMS was found to be positive, compared with 39% for agglutination after direct plating and 29% for direct 100 μl plating. In only two cases (leaf lettuce), the IMS procedure failed to detect the target strain (probably because of higher sample contamination), while in one case, the standard procedure showed a positive result.

In the case of direct inoculations (both direct loop and direct 100 μl plating) of *E. coli* O111 and O157, the numbers of presumptive colonies on the plates were usually very low and in some cases, not a single typical presumptive colony could be detected. After IMS of *E. coli* O111, positive colonies could easily be detected (93% samples) compared with standard procedures (36–40% positive samples). A similar result was observed for the detection of *E. coli* O157, where all samples (100%) were found to be positive after IMS, in comparison with 53% positive samples after direct plating. In the case of *E. coli* O26, the situation was different. The number of presumptive colonies was sufficiently higher on all plates with nutrient agar (RMAC). Nevertheless, on the plates after IMS, the number of presumptive colonies was significantly higher. Although 93% samples were observed to be positive after direct plating, after IMS (100% positive samples) the first presumptive colony taken was positive in all cases.

Although only a limited number of experiments could be performed, the results indicated that *E. coli* O26, O111 and O157 IMS 'Seiken' immunomagnetic particles are very

useful and efficient tools for the detection of *E. coli* strains in raw vegetables, and could probably be used with samples of animal origin.

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