Biolog identification of non-sorbitol fermenting bacteria isolated on *E. coli* O157 selective CT-SMAC agar

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Abstract

E. coli O157:H7 is recognised as an important human pathogen world-wide and has been associated with diseases such as haemorrhagic colitis (HC), haemolytic uraemic syndrome (HUS) and thrombotic thrombocytopaenic purpura (TTP). Accurate laboratory detection of *E. coli* O157:H7 is important for diagnostic purposes and to justify epidemiological data on *E. coli* O157:H7. A well-known phenotypic characteristic of *E. coli* O157:H7 bacteria is their inability to ferment sorbitol. This characteristic is often used to isolate these organisms from food and water using selective agar medium such as SMAC. However, the high number of false positive results obtained by a number of researchers when selectively screening for *E. coli* O157:H7 on CT-SMAC has prompted an investigation to determine which other sorbitol-negative bacteria also grow on CT-SMAC. The agar medium used for the investigation consisted of Sorbitol MacConkey agar (SMAC) supplemented with Cefixime-tellurite (CT). All sorbitol-negative colonies obtained from CT-SMAC, after selective enrichment and IMS were identified using the Biolog microbial identification system. The majority of sorbitol-negative isolates identified were *Burkholderia*, *Pseudomonas*, *Vibrio* and *Aeromonas* spp. Only two *E. coli* O157:H7 isolates were identified with Biolog and confirmed with a polymerase chain reaction (PCR) specific for the shiga toxin 1 (Stx1) genes and with O157 and H7 antisera. The inability of the CT-SMAC agar medium to specifically select for *E.coli* O157:H7 was confirmed by the results of this study. These observations call for further improvement of affordable methods for the selective isolation of *E. coli* O157:H7 in the presence of large numbers of interfering bacteria capable of growing on CT-SMAC.

Keywords: *Escherichia coli* O157:H7, Sorbitol-MacConkey agar, Biolog bacterial identification

Introduction

Escherichia coli (*E. coli*) is the species most commonly isolated from human faecal samples and is part of the normal intestinal flora of healthy individuals (Nataro and Kaper, 1998). *E. coli* O157:H7 is the classical serotype linked to serious outbreaks and sporadic cases of enterohaemorrhagic diseases such as haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS) (Nataro and Kaper, 1998; Verweyen et al., 2000). Animals such as cattle are the main reservoir for *E. coli* O157:H7 but they do occur in other animal species such as sheep, goats, pigs, cats, dogs, chickens and gulls (Johnson et al., 1990; Griffin and Tauxe, 1991; Beutin et al., 1994; Wallace et al., 1997). Water-borne transmission of *E. coli* O157:H7 has been well documented and reported from both recreational water and contaminated drinking water (Swerdlow et al., 1992; Keene et al., 1994; O'Connor, 2002). These strains of *E. coli* produce shiga toxins (Stx), which are potent cytotoxins that are similar to the toxin produced by *Shigella dysenteriae* Type 1 (Scotland et al., 1983; O'Brien et al., 1984).

Immunomagnetic separation (IMS) has been proposed as a sensitive method for the isolation of *E. coli* O157:H7 bacteria from clinical, food and environmental samples (Wright et al., 1994; Bennet et al., 1996; Chapman and Siddons, 1996; Cubbon et al., 1996; Vernozy-Rozand et al., 1997; Tomoyasu, 1998; Müller et al., 2003). A variety of selective and differential plating media have been developed for the isolation of *E. coli* O157:H7 (Nataro and Kaper, 1998). The most common *E. coli* O157 isolation media are Sorbitol MacConkey (SMAC) agar supplemented with Cefixime and potassium-tellurite (CT-SMAC) (March and Ratnam, 1986; Nataro and Kaper, 1998; Müller et al., 2001; Fujisawa et al., 2002; Müller et al., 2002). SMAC contains sorbitol, which replaces the lactose of the standard MacConkey agar medium (SMAC Oxoid product brochure, 2003). Unlike typical *E. coli*, *E. coli* O157:H7 do not ferment or produce acid from D-sorbitol within 24 h and lack glucoronidase activity (Manafi and Kremsmaier, 2001). *E. coli* O157:H7 bacteria present as colourless colonies on SMAC media (Manafi and Kremsmaier, 2001). SMAC agar is not suited for the isolation of non-O157 EHEC bacteria because there is no genetic link between Stx production and sorbitol fermentation (Nataro and Kaper, 1998). However, some non-O157 EHEC strains have proven to be sorbitol-negative (Ojeda et al., 1995). The addition of Cefixime and tellurite to SMAC agar permits the selective growth of *E. coli* O157:H7 and *Shigella sonnei* strains but inhibits the growth of most of the other *E. coli* strains (Zadik et al., 1993). *Pseudomonas* spp. do not ferment sorbitol and also present as colourless colonies on CT-SMAC (Cousin, 2000). Except for the addition of Cefixime and potassium-tellurite (CT) to SMAC (Zadik et al., 1993), researchers have modified SMAC agar by adding other agents to increase the selectivity of the media such as rhamnose and chromogenic enzyme substrates (Szabo et al., 1986; Chapman et al., 1991; Fujisawa et al., 2000; Okrend et al., 2000). Müller et al. (2001) found CT-SMAC to lack in selectivity and suggested further research to improve available methods for the selective cultivation of *E. coli* O157:H7 in the presence of large numbers of wild type *E. coli* and other bacteria capable of growing on the selective media. Resistance to the antibiotics used for the suppression of bacteria other than *E. coli* O157:H7 may largely be accountable for the problem. One solution may be to find an-

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