CORRESPONDENCE

First isolation outside Europe of sorbitol-fermenting verocytotoxigenic *Escherichia coli* (VTEC) belonging to O group O157

Shortly after the first descriptions of cases of human infection with verocytotoxigenic *Escherichia coli* (VTEC) belonging to O group O157 [1] it was realised that they do not ferment sorbitol, unlike most other types of *E. coli* [2]. This led to the development of sorbitol-MacConkey (SMAC) agar, which has been very useful for the detection of strains belonging to the VTEC O157 clone throughout the world. However, over the last decade VTEC strains of serotype O157:H–, which do ferment sorbitol, have been isolated increasingly in continental Europe from cases of human disease [3]. These types would not be detected with SMAC agar. Because of the importance in Australia of the non-O157 VTEC in human infections [4–6], there has been far less reliance on the use of SMAC agar for the isolation of VTEC. The realisation that the majority of VTEC, regardless of serotype, produce enterohaemolysin (Ehly), which can be detected easily [7, 8], has greatly enhanced the isolation rate of VTEC in Australia [6]. The ability to screen rapidly and specifically for VTEC colonies from a primary isolation plate by means of the adaptation of the VTEC-Screen 'Seiken' (DSA; Denka Seiken, Tokyo, Japan), regardless of their production of Ehly or fermentation reactions [9], has greatly facilitated the isolation of VTEC from human infections.

Two specimens of very watery faeces submitted from an adult patient clinically diagnosed with haemolytic uraemic syndrome (HUS) were spread on MacConkey (MAC), SMAC, sheep blood (SBA) and washed sheep blood (WSBA) agars. After overnight incubation there was a mixture of colonial types on the MAC and SMAC, while the SBA and WSBA were unreadable because of overgrowth by swarming *Proteus*. The mass growth from the MAC plate from each specimen was tested by PCR based on the method of Paton *et al.* [10] for the presence of DNA sequences specific for the VT genes and material from one specimen was strongly positive, whereas that from the other was weakly positive. The sweeps from both plates as well as a typical lactose-fermenting (LF) colony were negative by the DSA test.

The original mass growth from the PCR-positive MAC plates was also inoculated into two *E. coli* (EC) broths [11] and one each was incubated at 37°C and 44°C. Subculture after 24 h on MAC agar gave many colonies but none was positive by the DSA test. After 48 h the EC broths were again spread onto MAC plates and incubated overnight at 37°C. One distinct colony originating from the 37°C EC-broth and two similar

colonies from the 44°C EC-broth were positive in the DSA test, as was the mass growth from the 44°C EC broth. None of the colonies or the mass growth from either EC broth of the specimen, which originally gave a weak PCR reaction, was positive.

The three DSA-positive colonies were cultured on MAC, SMAC, SBA and WSBA and nutrient agar (NA). The three DSA test-positive colonies gave typical Ehly-producing colonies for VTEC, they fermented lactose on MAC and sorbitol on SMAC and were characterised as *E. coli* by standard methodology. The strains were O serogrouped [12] and found to be O157. They were all non-motile following repeated subculture through semi-solid agar. Two separate multiplex PCRs were used for the detection of genes for Shiga toxins 1 and 2 (*stx*₁/*stx*₂) and intimin and Ehly (*eaeA*/*ehxA*) [13], respectively (R. Robins-Browne, personal communication). The strains were found to be positive for *stx*₂, *eaeA* and *ehxA* and negative for *stx*₁.

Having identified the strains as *E. coli* O157:H–, we were interested to examine the presence of several plasmid (pO157)-encoded genes (*katP*, *espP* and *sfp*) known to differentiate non-sorbitol-fermenting (NSF)O157 and sorbitol-fermenting (SF)O157 isolates [3, 14]. Unlike NSFO157 strains, SFO157 isolates typically lack genes for catalase (*katP*) and the extracellular serine protease (*espP*). Furthermore, a cluster of 6 genes (*sfpA*, *sfpH*, *sfpC*, *sfpD*, *sfpJ* and *sfpG*) which mediate mannose-resistant haemagglutination and the expression of fimbriae were shown to reside in SFO157 in a location where *katP* and *espP* normally reside in plasmid pO157 [14]. Consequently, SFO157 strains typically do not possess *katP* and *espP* but uniquely possess *sfp* genes. Table 1 summarises the results of comparing virulence gene characteristics of the Australian SFO157 isolate with a typical Australian NSFO157. With primers ESPPa and ESPPb [15] we were unable to amplify a 301-bp fragment of the *espP* gene. However, essentially the entire *katP* gene was amplified with primers wKAT-B and wKAT-F [16]. We were also unable to amplify a 440-bp fragment of *sfpA* with primers sfpA-U and sfpA-L [14].

In summary, our results indicate that the Australian SFO157 VTEC possess different plasmid gene markers compared with those reported in European isolates of SFO157. The absence of *sfpA* is particularly striking as this gene was shown to be present in all 14 European SFO157 isolates [14]. The pattern of plasmid gene

Table 1. Virulence factor profiles of sorbitol-fermenting *E. coli* isolate

Serotype	<i>stx</i> ₂ subtype	Intimim subtype	Multiplex PCR results				Plasmid genes		
			<i>stx</i> ₁	<i>stx</i> ₂	<i>eaeA</i>	<i>ehxA</i>	<i>katP</i>	<i>espP</i>	<i>sfpA</i>
SFO157:H–	<i>stx</i> ₂ _{vha}	γ	–	+	+	+	+	–	–
NSFO157:H7	Not done	γ	+	+	+	+	+	+	–

markers (*ehxA*⁺, *katP*⁺, *espA*[–]) identified among the Australian SFO157 isolates examined resembles those described for O103:H2 and O111:H–/H2 isolates in studies published by Brunder *et al.* [16, 17]. Unlike European isolates of SFO157, which do not express the *ehxA* gene, the three Australian isolates described in this study generate typical enterohaemolytic colonies when grown on WSBA. Further molecular studies are needed to examine the phylogenetic relationship between Australian and European isolates of SFO157. This study clearly demonstrates the usefulness of the various techniques employed to isolate VTEC, especially sorbitol-fermenting VTEC, and the fact that often they may be present in only very low numbers.

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