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A rapid procedure for the detection and isolation of enterohaemorrhagic Escherichia coli (EHEC) serogroup O26, O103, O111, O118, O121, O145 and O157 strains and the aggregative EHEC O104:H4 strain from ready-to-eat vegetables.

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Abstract

Human infections with Enterohaemorrhagic Escherichia coli strains (EHEC) as agents of Haemorrhagic Colitis (HC) and Haemolytic Uraemic Syndrome (HUS) are frequently associated with the consumption of EHEC contaminated foodstuffs of different origins. EHEC O26, O103, O111, O118, O121, O145 and O157 strains are responsible for the majority of HC and HUS cases worldwide. In May 2011, the emerging aggregative EHEC O104:H4 strain caused a large outbreak with high HUS incidence in northern Germany. Contaminated sprouted seeds were suspected to be the vehicles of transmission. The examination of vegetables retailed for raw consumption revealed low numbers of E. coli (<100 cfu/g) together with high titres of Enterobacteriaceae and Pseudomonas (approx. 5.6×10^7 cfu/g). Specific methods of EHEC detection adapted to vegetables are not yet published. Therefore, we have developed a rapid and sensitive method for detecting low EHEC contamination in vegetables (1-10 cfu/25 g) with artificially EHEC contaminated ready-to-eat salads. A 6-hour enrichment period in BRILA-broth was sufficient to detect 1-10 EHEC from spiked samples after plating 0.1 ml portions of enrichment culture on selective TBX-agar and CHROMagar STEC plates that were incubated at 44 °C overnight. Unlike EHEC strains, the growth of bacteria of the plant flora was substantially inhibited at 44 °C. DNA for real-time PCR detection of EHEC characteristic genes (stx(1), stx(2), eae, ehxA, and O-antigen associated) was prepared with bacteria grown on TBX-agar plates. The storage of EHEC inoculated salad samples for 72 h at 6 °C resulted in a significant reduction (mean value 14.6%) of detectable EHEC, suggesting interference of EHEC with the resident plant microflora. CHROMagar STEC was evaluated as a selective medium for the detection of EHEC strains. Growth on CHROMagar STEC was closely associated with EHEC O26:[H11], O111:[H8], O118:H16, O121:[H19], O145:[H28], O157:[H7] and aggregative EHEC O104:H4 strains and with the presence of the terB gene (tellurite resistance). TerB sequences were found in 87.2% of 235 EHEC but only in only 12.5% of 567 non-EHEC strains. EHEC strains which did not grow on CHROMagar STEC were negative for terB as frequently observed with EHEC O103:H2 (52.9%) and sorbitol-fermenting O157:NM strains (100%). The enrichment and detection method was applied in the examination of sprouted seeds incriminated as vehicles in the EHEC O104:H4 outbreak in Germany.

Aggregative EHEC O104:H4 could be detected and isolated from a sample of sprouted seeds which was suspected as vector of transmission of EHEC O104 to humans.

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