Detection of VT genes by PCR

An *Escherichia coli* strain can be identified as a VTEC by detecting the presence of the verocytotoxin (VT)-coding genes. There are two major types of VTs, encoded by *vtx1* and *vtx2* genes, located on lambdoid phages integrated in the bacterial chromosome. While VT1 doesn’t show significant variation in sequence, several variants of VT2 have been described so far, and such toxins have been named VT2c (Schmitt et al. 1991), VT2d (Pierard et al. 1998), VT2e (Weinstein et al. 1988) and VT2f (Schmidt et al. 2000). VT2, VT2c and VT2d are produced by VTEC strains isolated from human infections, VT2e is mainly found in VTEC causing oedema disease in pigs and VT2f appears to be closely associated with VTEC of avian origin. A VT2g variant has been recently described in strains of bovine origin and in animal wastewater samples (Garcia-Aljaro et al. 2006; Leung et al. 2003).

A PCR method for VT-coding genes detection will take into account the existence of these variants and of their importance for human disease. Epidemiological studies have revealed that strains associated with severe human disease, like bloody diarrhoea and HUS produce VT2 more frequently than VT1 (Boerlin et al. 1999; Friedrich et al. 2002; Persson et al. 2007).

However, the different *vtx2* genotypes seem to be associated with different clinical manifestations:

- *vtx2* and *vtx2c* are the most frequently found genotypes in human infections and are usually associated with HUS (Caprioli et al. 1995; Eklund et al. 2002; Friederich et al. 2002; Pierard et al. 1998)
- *vtx2d* is also isolated from human infections (Jenkins et al. 2003; Persson et al. 2007; Pierard et al. 1998), but has been mainly found in cases of non bloody diarrhoea and asymptomatic carriers (Friedrich et al. 2002). Association with HUS has been reported in a few cases (Jenkins et al. 2003; Pradel et al. 2001).
- *vtx2e* is rare in human infections (Friedrich et al. 2002; Jenkins et al. 2003; Persson et al. 2007)
- *vtx2f* has been reported only in a single human case (Gannon et al. 1990)
- *vtx2g* variant has been described only in two asymptomatic carriers (Persson et al. 2007).
**Primers available**

A number of primer sets able to detect vtx genes has been developed during the years. The primer pair (KS7 and KS8, table 1) described by Russmann et al (1995) can be used to identify vtx1. The choice of primer sets to detect the variants of vtx2 genes may depend on the purposes of the laboratory involved. Primers GK3/GK4 (Russmann et al. 1995, table 1) only recognize vtx2 and vtx2c genes, which on the other hand represent the most common variants in human isolates and in particular in bloody diarrhoea and HUS. Primer pair LP43 e LP44 (Cebula et al. 1995, table 1) recognizes a region of the A subunit coding gene and react with a wider range of variants: vtx2, vtx2c, vtx2d and vtx2e. A further characterization of the vtx variants can be carried out by using the specific primer pairs listed in table 1, together with the amplification conditions. Template DNA can be constituted by 10 μl of a bacterial suspension (10^8 cells/ml) incubated for 10 min at 95°C. PCR products are detected by 1.5% agarose gel electrophoresis.

**Additional virulence factors**

The production of VT appears to be essential but not solely responsible for the pathogenic effects of VTEC infections. VTEC associated with severe human disease are usually capable of colonizing the intestinal mucosa with a characteristic “attaching and effacing” mechanism, while this property is significantly less common among VTEC strains isolated from healthy cattle (Caprioli et al. 2005). An adhesin, termed intimin, mediates the intimate attachment of VTEC and also of enteropathogenic E.coli (EPEC) to the host cell. The intimin-coding genes (eae) present high sequence conservation in the 5’ terminal region and variability in the 3’ terminal region. Based on the sequence and antigenic differences in this C-terminal cell-binding domain, several distinct intimin types have been identified and classified with a nomenclature system based on the Greek alphabet (Oswald et al. 2000; Zhang et al. 2002). PCR primers designed in the 5’ conserved region are used as eae universal primers for the identification of eae-positive strains. Primers designed in the 3’ variable region can be used for the determination of the different intimin types (Zhang et al. 2002).
Primer pair SK1/SK2 (Karch et al. 1993, table 1) can be effectively used in screening strains for the presence of the eae gene.

References


