

Identification of specific host virulence factors of enterohemorrhagic *Escherichia coli* strains of serogroup O26 by subtractive suppressive hybridization

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Enterohemorrhagic *Escherichia coli* strains (EHEC) are characterized by attaching and effacing lesions on epithelial cells of intestine, expression of verotoxins and production of hemorrhagic colitis (HC) and/or haemolytic uremic syndrome (HUS). They represent an important problem for public health in developed countries. Indeed, EHEC strains infect the man by vegetal and animal foods and cause intoxications with diarrhoeas. They are generally accompanied by hemorrhagic colitis with, in 10 % of the cases, apparition of renal sequelae that can lead to death. In veterinary field, several serogroups of EHEC strains (O26, O111, O118 for example) are directly associated with digestive disorders in two weeks to two months old calves. The consequences are economic losses due to delay of growth and weakness of calves.

Pathogenicity are divided in three stages: (1) colonisation of intestine by specific adhesins, (2) production of a signal by the bacteria that cause cytoskeleton's rearrangement in enterocyte and (3) intimate adhesion of bacteria with eukaryotes cells by specific proteins, the intimins. Factors implicated in host specificity have been identified for enteropathogenic *Escherichia coli* strains (EPEC) but not for enterohemorrhagic *Escherichia coli* strains (EHEC). Such factors could be based on proteins intervening in colonisation stage (adhesins for example). This kind of proteins is still unknown for EHEC strains.

Our aim is the determination of the factors implicated in initial attachment and in host specificity (man or cattle) of EHEC strains O26 and thus develop additional diagnostic tools.

This study is based on three different stages. First, technique of subtractive suppressive hybridisation (SSH) will allow comparing the genome of EHEC pathogenic bovine O26 strains with EHEC non-pathogenic bovine O26 strains and with EHEC pathogenic human O26 strains. Specific fragments found will be cloned and sequenced. After comparison of the sequences obtained with those available in the data bases, the candidates presenting similarities of sequence with potential and proven adhesins will be selected. The inactivation of these candidates by mutagenesis will allow evaluating their role in pathogenesis by comparison of a mutated strain with the parental strain in adhesion tests on cultured cells. Secondly, the technique of selective capture of transcribed sequences (SCOTS) will allow comparing the transcriptoms of EHEC bovine O26 strains afterwards, on the one hand, *in vitro* growth in liquid medium and, on the other hand, contact with eukaryotes cells in culture. Finally, the results so obtained will be applied to diagnostic by the development of fast and reliable tests of analysis.

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