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Biodiversity and bacterial pathogenicity

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The aim of this contribution is to illustrate how intraspecific as well as interspecific biodiversity plays a key role in the complex event of bacterial pathogenesis. *Helicobacter pylori* and *Escherichia coli* were chosen as examples for bacterial species with heterogeneous (*H. pylori*) and conserved (*E. coli*) genomic structures.

PATHOGENICITY OF *HELICOBACTER PYLORI*

Helicobacter pylori, first described by WARREN & MARSCHALL in 1983, is known to be involved in the pathogenesis of upper gastrointestinal diseases. *H. pylori* establishes a long-term colonization of the mucosa of the human stomach (BLASER, 1997). The chronic infection often occurs without symptoms, but some individuals develop severe features such as peptic ulcer disease (lifetime risk 15%), gastric adenocarcinoma, and mucosa associated lymphoid tissue lymphoma (lifetime risk 0.1%) (FENNERTY, 1996). The development of the disease is influenced by the virulence of the infecting bacterial strain, the genetic susceptibility of the host and environmental co-factors.

In the 1990s with the aid of powerful molecular techniques, our knownledges of the genetic of *H.pylori* genome has expanded exponentially. Physical and genetic maps of the genome have been constructed, physiologically and pathologically important genes have been cloned and characterized. The complete genome sequence of *H. pylori* strain 26695 has been unmasked (TOMB *et al.,* 1997) and published on the internet (http://www.tigr.org). The genetic heterogeneity among different isolates is high and can be assessed by various methods, such as restriction fragment length polymorphism analysis and PCR fingerprinting (TAYLOR *et al.,* 1992, KANSAU *et al.,* 1996, GIBSON *et al.,* 1998). The genetic variation of *H.pylori* is greater than that of other bacteria that have been studied. Comparison of the genetic maps of five *H. pylori* strains demonstrated that there is no characteristic arrangement of 17 known genes on the chromosome conserved by these strains (macrodiversity) (JOANG *et al.,* 1996). Mechanisms causing genomic diversity are poorly understood at present. For macrodiversity, one hypotesis suggests that this diversity reflects a long evolutionary association with the human host. Different strains remain within each individual human for many generations and may indipendently undergo evolution. Alternatively, the diversity could result from gen-rearrangement within the chromosome caused by transposon-mediated gene mobility and recombination between repeated sequences (GE & TAYLOR, 1998). Analysis of this overall genomic variability is useful for epidemiological fingerprinting, but this genomic variability is not related to the pathogenicity of *H.pylori* strains.

Virulence genes of *H. pylori*

Recently, specific bacterial genes that are associated with phenotypic strain virulence have been described. Approximately 50 to 60% of the *H.pylori* strains contain the cytotoxin-associated *(cagA)* gene and consequently produce the 128-kDa cagA protein. CagA was first described as a protein which was expressed more commonly by toxigenic than non-toxigenic strains. The presence of cagA is associated with duodenal ulceration, gastric mucosal atrophy, and gastric cancer. *CagA* is part of a larger genomic entity, designated the pathogenicity (cag) island of about 40 kbp, which contains a collection of about 40 genes (CENSINI *et al.*, 1996). This multiple genes are related to the virulence and the pathogenicity of the strain, for exemple, by inducing cytokine production by the host, and are associated with higher levels of inflammation. Therefore the presence of *cagA* can be considered a marker for this genomic pathogenicity (RUDI *et al.*, 1998). The *cagA* region has much in common with pathogenicity islands in other bacteria: it appeared to be involved in virulence, has a different nucleotide composition to other *H. pylori* genes, is flanked by direct nucleotide repeats, and is relatively genetical instable. Like other bacterial pathogenicity islands, it is thought to have been acquired relatively late in the evolution from an external source, perhaps a bacteriophage or a plasmid. In many strains it is present in his enterely, but in others it is re-organized or even partially deleted following interruption by a novel insertion sequence which is though to be involved in chromosomal reorganisation.

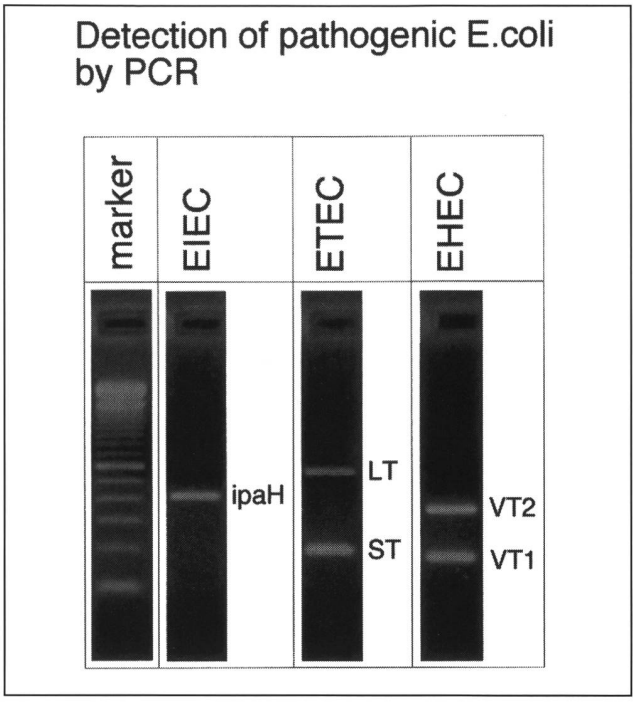
Another virulence factor, produced by approximately 50% of the *H. pylori* strains, is a cytotoxin that induces the formation of vacuoles in mammalian cells in vitro

and that leads to cell death. This toxin is encoded by the *vacA* gene. Although *vacA* is present in all *H. pylori* strains, it contains at least two variable parts. Recently different allelic variants in these two parts has been described (VAN DOORN *et al.*, 1998). The VacA protein has a short N-terminal signal peptide (or signal sequence) which is recognized and cleaved during export of the toxin across the bacterial cytoplasmic membrane. Further cleavage of the C-terminal third of the toxin precursor occurs during passage across the outer membrane to leave an 87 kDa secreted polypeptide. The N-terminal signal(s) region occurs as either an s1a, s1b, or s2 allele. Recently, a novel subtype, designated s1c, was found (STROBEL *et al.*, 1998). However this subtype was observed exclusively in isolates from East Asia and appears to be the major s1 allele in that part of the world. The middle (m) region is present as an m1 or an m2 allele. The mosaic structure of the *vacA* gene accounts for the differences in the cytotoxin production between strains. The particular *vacA* s and m genotype is a marker of the pathogenity of an individual strain, since in vitro production of the cytotoxin, in vivo epithelial damage, and peptic ulcer disease are all related to the vacA genotype (DONATI *et al.*, 1999). In a study group of 106 patients with duodenal ulceration *H.pylori* of genotype s1 was isolated from 96% , whereas genotype s2 was only present in 4% indicating a strong correlation between the *vacA* genotype and peptic ulceration. Particular midregion genotypes were not associated with clinical manifestations.

PATHOGENICITY OF *ESCHERICHIA COLI*

Escherichia coli is the predominant facultative anaerobe of the human colonic flora. *E.coli* usually remain harmlessly confined to the intestinal lumen; however, in the debilitated or immunosupressed host, or when gastrointestinal barrieres are violated, even normal *E.coli* strains can cause infection. Three general clinical syndromes result from infections with pathogenic *E.coli* strains: (i) urinary tract infections, (ii) sepsis/menengitis; (iii) enteric diarrheal disease. This article will focuse on the diarrheagenic *E.coli* strains in particular on their differentiation on the basis of pathogenic features.

Identification of diarrheagenic *E.coli* strains requires that these organisms be differentiated from nonpathogenic members of the normal flora. Serotypic markers correlate, sometime very closely, with specific categories of diarrheagenic *E.coli*; however, these markers are rarely sufficient to reliably identify a strain as diarrheagenic. Those *E.coli* strains were among the first pathogens for which molecular diagnostic methods were developed. Indeed, molecular methods remain the most popular reliable techniques for differentiating diarrheagenic strains from nonpathogenic members of the stool flora, and distinguishing one category from another. PCR is a major advance in molecular diagnostics of pathogenic microorganisms and has been successfully performed for several diarrheagenic *E.coli.*



E. coli virulence

The most highly conserved feature of diarrheagenic *E.coli* strains is their ability to colonize the intestinal mucosal surface. Once colonization is established, the pathogenic strategies exhibit remarkable variety. Three general paradigms have been described by which *E.coli* may cause diarrhea: (i) enterotoxin production (ETEC and EAEC), (ii) invasion (EIEC), and/or (iii) intimate adherence with membrane signalling (EPEC, EHEC). The versatility of the *E.coli* genome is conferred mainly by two genetic configurations: virulence related plasmids and chromosomal pathogenicity islands. Such islands have been described for uropathogenic *E.coli* strains (DONNENBERG & WELCH, 1996) and systemic *E.coli* strains as well (BLOCH & RODE, 1996) and may represent a common way in which the genomes of pathogenic and nonpathogenic *E.coli* strains diverge genetically.

In the following sections the pathogenicity of three categories of diarrheagenic *E.coli strains* (ETEC, EIEC, and EHEC) will be presented.

Enterotoxinogenic *E. coli*

ETEC is defined as containing the *E.coli* strains that produce at least one member of the two defined groups of enterotoxins: ST (heat-stable toxins) and LT (heat-labile toxins) (LEVINE, 1987). The LTs of *E.coli* are large, oligomeric and closely related in structure and function to the cholera enterotoxin (CT) expressed by *Vibrio cholerae.* In contrast, the STs are small, monomeric and contain multiple cysteine residues, whose disulfide bonds account for the heat stability of these toxins.

ETEC strains are associated with two major clinical syndromes: diarrhea among childrens in the developing countries, and traveller's diarrhea. Epidemiologic investi-

gations have implicated contaminated food and water as the most common vehicles for ETEC infections. The diarrhea is watery, usually without blood, mucus or pus; fever and vomiting are present in a minority of patients. ETEC diarrhea may be mild, brief, and self-limiting or may be severe and similar to that seen in *V. cholerae* infections.

Enteroinvasive *E. coli*

EIEC strains are biochemically, genetically, and pathogenetically related closely to *Shigella* spp.; like Shigella spp. EIEC strains are generally lysine decarboxylase negative, nonmotile, and lactose negative (BRENNER *et al.*, 1973). Genes necessary for invasiveness are carried on a 120-MDa plasmid in *Shigella sonnei* and a 140-MDa plasmid in other *Shigella* serotypes and in EIEC. The invasion related plasmid has been designated pInv. EIEC strains can be difficult to distinguish from *Shigella* spp. and from other *E.coli* strains, including nonpathogenic strains. Molecular techniques such as PCR with specific primers overcome this problem. It should be noted that EIEC strains may lose all or part of the pInv plasmid on in vitro passage or storage. Therefore PCR assay with primers derived from genes which are located on the plasmid as well as on the chromosom may increase the detection level. The *ipaH* (multicopy invasion plasmid antigen gene) gene is unique in that five complete or partial copies are present on the invasion plasmid of the various *S. flexneri* serotypes and multiple copies are also found on the invasion plasmids of other *Sighella* species and EIEC but not on the chromosome of non pathogenic *E. coli* strains. EIEC infections present most commonly as watery diarrhea, which can be indistinguishable from the secretory diarrhea seen with ETEC.

Enterohemorrhagic *E. coli*

E. coli strains, causative agens of bloody diarrhea in humans associated with hemorrhagic colitis, hemolytic uremic syndrome, produce one or more toxins of the Stx (Shiga-like toxin) family. The Stx family contains two major, immunologically non-cross-reactive groups called Stx1 and Stx2. A single EHEC strain may express Stx1 only, Stx2 only or both toxins. In recent years, there have been significant advances in our understanding of the pathogenesis of these *E.coli* infections, and these are contributing to the development of improved diagnostic methods, as well as to the development of therapeutic and preventive strategies. The alternative nomenclature «verotoxigenic *E. coli*» or «Verotoxin producing *E. coli*» was derived from the observation that these strains produced a toxin that was cytotoxic for Vero cells. Representative for this group is serotype O157:H7 isolated from large and severe outbreaks occured after ingestion of undercooked hamburgers at a fast food restaurant chain (RILEY *et al.*, 1983). In conventional diagnostic to trace for Verotoxin producing *E. coli* O157:H7 MacConkey medium supplemented with sorbitol was used. More recently other methods such ELISA and immunomagnetic separation were also used to detect O157:H7. However, serological cross reaction with other enterobacteriaceae has been observed. MacConkey Agar Sorbitol is not enough discriminating because part of the O157:H7 can ferment sorbitol and it has been demonstrated that other serotypes can cause diarrhea. Virolence test on HeLA cells or Verocells can not be easely performed in a routine laboratory. Large scale of meat favorizes the spread of EHEC contaminations. In the USA serotype O157:H7 plays a dominant role. In beef from the Swiss Market VT producing serotypes other than O157:H7 must be expected. Therefore the diagnosis with high specific molecular techniques such as PCR is highly recommended.

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