

Title: Role of pili in enterococcal virulence

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Abstract

Nosocomial infections by multiple antibiotic resistant bacteria are especially difficult to cure, pose a significant health risk to humans and place an enormous burden on the economy. A leading cause of such nosocomial infections is the Gram-positive bacterium *Enterococcus faecalis*. Although enterococci are a part of the commensal flora of the gastrointestinal tract in humans, they are opportunistic pathogens with the propensity to cause serious disease. Adhesive interactions of bacteria with host cells can lead to the establishment of commensal or pathogenic relationships. Adhesion of bacteria to host cells or tissues involves several bacterial surface organelles including pili or fimbriae. Pili have been characterized extensively in Gram-negative bacteria such as *E. coli* and are important virulence factors especially in uropathogenic strains. Only recently have pili been identified in Gram-positive bacteria such as *Corynebacterium diphtheriae*, *Streptococcus agalactiae* and *Streptococcus pyogenes*. The assembly of pili in Gram-positive bacteria occurs through a mechanism distinctly different from those involved in the assembly of Gram-negative pili. Pilus assembly in Gram-positive bacteria requires sortase, a specific transpeptidase that can mediate the ordered assembly of pilin subunits at the bacterial cell surface. Moreover, the genes encoding the pilin subunits and cognate sortase(s) are frequently found in close proximity on the genome of these organisms.

We have recently identified in *E. faecalis*, a gene cluster which closely resembles the gene clusters involved in pilus biogenesis in *C. diphtheria* and other pathogenic streptococci. The newly identified gene cluster, termed the bee locus, consists of three genes encoding putative surface proteins and two putative sortase genes in tandem. The specific aims of this proposal are designed to answer the following questions: (1) Does the bee locus encode pilin subunits that can be assembled into a pilus structure at the cell surface? (2) What role(s) do each of the sortase enzymes encoded on this locus play in pilus biogenesis? (3) Does the pilus structure encoded by the bee locus play a role in the pathogenesis of enterococcal urinary tract infection? We propose to initially generate the immunological tools to detect the pilin subunits in bacterial cell surface extracts from wild type and mutant strains, follow this up with identifying the pili at the cell surface using a combination of specific antibodies and electron microscopy and then examine how the ordered assembly of pilus subunits occurs at the cell surface. Lastly, we will examine the contribution of these pili to the persistence of *E. faecalis* in a well established animal model of urinary tract infection. These studies will be the first-of-its-kind and novel because virtually nothing is known in enterococci about pili, assembly of pilus subunits or their role in virulence. In the long run, the results of the proposed work will significantly enhance our understanding of enterococcal biology and pathogenesis, potentially leading to new anti-infectives (pilicides) to control severe enterococcal infections.