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Identification and Characterization of Polysaccharide Biosynthesis Protein (CAPD) of Enterococcus faecium

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Abstract : Enterococcus faecium is an emerging multidrug-resistant nosocomial pathogen increased dramatically worldwide and causing bacteremia, endocarditis, urinary tract and surgical site infections in immunocomprised patients. The capsular polysaccharides that contribute to pathogenesis through evasion of the host innate immune system are also involved in hindering leukocyte killing of enterococci. The gene cluster (enterococcal polysaccharide antigen) of E. faecalis encoding homologues of many genes involved in polysaccharide biosynthesis. We identified two putative loci with 22 kb and 19 kb which contained 11 genes encoding for glycosyltransferases (GTFs); this was confirmed by using genome comparison of already sequenced strains that has no homology to known capsule genes and the epa-locus. The polysaccharide-conjugate vaccines have rapidly emerged as a suitable strategy to combat different pathogenic bacteria, therefore, we investigated a polysaccharide biosynthesis CapD protein in E. faecium contains 336 amino acids and had putative function for N-linked glycosylation. The deletion/knock-out capD mutant was constructed and complemented by homologues recombination method and confirmed by using PCR and sequencing. For further characterization and functional analysis, in-vitro cell culture and in-vivo a mouse infection models were used. Our ΔcapD mutant shows a strong hydrophobicity and all strains exhibited biofilm production. Subsequently, the opsonic activity was tested in an opsonophagocytic assay which shows increased in mutant compared complemented and wild type strains but more than two fold decreased in colonization and adherence was seen on surface of uroepithelial cells. However, a significant higher bacterial colonialization was observed in capD mutant during animal bacteremia infection. Unlike other polysaccharides biosynthesis proteins, CapD does not seems to be a major virulence factor in enterococci but further experiments and attention is needed to clarify its function, exact mechanism and involvement in pathogenesis of enteroccocal nosocomial infections eventually to develop a vaccine/ or targeted therapy.

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