Molecular Analysis of three Aeromonas hydrophila AH-3 (serotype O34) Lipopolysaccharide Core Biosynthesis Gene Clusters

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By the isolation of three different Aeromonas hydrophila strain AH-3 (serotype O34) mutants with an altered lipopolysaccharide (LPS) migration in gels, three genomic regions encoding LPS core biosynthetic genes were identified and characterized. Mutants were constructed when possible in each gene from the three regions, containing seven, four, and two genes (regions 1-3, respectively). The mutant LPS core structures were elucidated using mass spectrometry, methylation analysis and comparison with the full core structure of an O-antigen-lacking AH-3 mutant established by us earlier. Combining the gene sequence and complementation test data with the structural data and phenotypic characterization of the mutant LPSs enabled a presumptive assignment of all LPS core biosynthesis gene functions in A. hydrophila AH-3. The three regions and the genes contained are in complete agreement with the recently sequenced genome of A. hydrophila ATCC7966. The function of A. hydrophila waaC in region 3 and waaF in region 2 was completely established allowing the genome annotation of these two heptosyl transferases not previously assigned. Having functions of all genes involved with the LPS core biosynthesis and most corresponding single-gene mutants allows now experimental work on the role of the LPS core in virulence of A. hydrophila.