



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

Natalia Jimenez<sup>1</sup>, Rocío Canals<sup>1</sup>, Anna Lacasta<sup>1</sup>, Anna N. Kondakova<sup>2,3</sup>, Buko Lindner<sup>3</sup>, Yuriy A. Knirel<sup>2</sup>, Susana Merino<sup>1</sup>, Miguel Regué<sup>1</sup>, and Juan M. Tomás<sup>1\*</sup>

<sup>1</sup> Departamento de Microbiología, Facultad de Biología, Universidad de Barcelona, Diagonal 645, 08071 Barcelona, Spain.

<sup>2</sup> N.D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Moscow, Russia

<sup>3</sup> Forschungszentrum Borstel, Leibniz-Zentrum für Medizin und Biowissenschaften, 23845 Borstel, Germany

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*.