Abstract

Bacteria of the genus *Aeromonas* are the etiological agent of MAI/MAS diseases causing mass infections and deaths of farmed fish. Currently, in order to limit the scale of the use of antibiotic therapy in bacterial infections in aquaculture, activities are taken to increase the specific immunity of fish through the use of (auto)vaccines, which are developed on the basis of strains with an antigenic profile characteristic of serogroups among which pathogenic species are identified most often and dedicated to specific farms. The aim of this doctoral dissertation was to carry out immunochemical (structural and serological) studies of O-specific polysaccharides (OPS) of *Aeromonas* sp. strains, serogroup PGO1, which is important in the pathogenicity of this genus for fish intended for consumption, and to determine the organization of the region encoding the O antigen synthesis (OGC). The research material consisted of three isolates: *Aeromonas hydrophila* Pt679 (trout), *A. popoffii* A4 (carp), and *A. sobria* K928 (carp). The agglutination test, Western blotting experiment, and ELISA confirmed the classification of the strains into the PGO1 serogroup. The LPS preparations extracted from the outer membrane were hydrolyzed, and the O-specific polysaccharides were obtained after GPC and subjected to chemical and structural studies by GC-MS and ^{1}H and ^{13}C NMR spectroscopy. It was shown that the O-antigens of A4, K928, and Pt679 are composed of branched pentasaccharide repeating units that have a common linear tetrasaccharide consisting of three L-rhamnose residues and amino-6-deoxyhexose (D-FucN or D-QuiN). In turn, 3-amino-D-fucose was identified as the terminal sugar containing an amide-linked 3-hydroxybutyric group (Fuc3N*R*Hb) in A4 and K928 and an N-acetyl group in Pt679. It was found that the serological specificity of the immunotypes is determined by 3-amino-D-fucose residues with non-carbohydrate substituents; on this basis, the PGO1 serogroup was divided into two subgroups: PGO1a for serotypes having a terminal α-D-Fuc3N*R*Hb (A4 and K928) as the immunodominant epitope in the O antigens and the PGO1b subgroup, with α-D-Fuc3NAc (Pt679). The bioinformatics analyses of OGC showed the presence of key genes for the synthesis of O-units and their polymerization, confirming the phenotypic studies of O-antigens, and additional genes differentiating the regions. The analyses also facilitated the identification of genes as potential markers for molecular serotyping of the PGO1 serogroup and both subgroups PGO1a and PGO1b.

key words: *Aeromonas* sp., lipopolysaccharide (LPS), O-antigen gene cluster (OGC), serogroup PGO1, GC-MS, NMR

Maria Lukasiewicz-Mikołajczyk