Structural peculiarities of biopolymers produced by diazotrophic endobiont *Herbaspirillum* spp.

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Key message. Diazotrophic endobionts *Herbaspirillum* spp. were studied in respect to the structural peculiarities of the lipopolysaccharides (LPS), O-specific polysaccharides (OPS) structure, antigenic composition and genetics.

Keywords: *Herbaspirillum*, lipopolysaccharide, bacterial polysaccharide structure; O-specific polysaccharide; O antigen

Herbaspirillum spp. is a member of the family Oxalobacteraceae of the phylum β -Proteobacteria harbors mostly diazotrophic bacteria with the potential of endophytic and systemic colonization of a variety of plants. *Herbaspirillum* is capable of promoting the growth of economically relevant plants through biological nitrogen fixation and phytohormone production or protecting the host against pathogenic microorganisms. More recently, some *Herbaspirillum* species have transitioned from environment to human hosts, mostly as opportunistic bacteria. *Herbaspirillum* species are close phylogenetic and phenotypic resemblance to several γ -Proteobacteria species and can be misidentified by phenotypic identification methods used in the clinical laboratory. Endophyte-plant interactions initiates with the attachment to the root surface, preferentially at the intercellular junctions of root epidemics tissue. After this step, cortical intercellular infection occurs, and the bacteria invade the stele and xylem. The molecular basis of suppression the immune system of host is still unclear. Ubiquitously present in the outer membrane, LPS form a protective wall between the bacterial cell and the environment and thus could play an essential role in the process of molecular bacteria-host communication. The array of carbohydrate structures carried on LPS confer resistance to plant defense mechanisms and may serve as signals that trigger the host to allow the infection to proceed. LPS has two potential ways to act as barrier: the packed saccharide portion of the molecule that forms a hydrophilic wall, and the fatty acid portion of the lipid-A that constitutes a hydrophobic wall. Structural and genetic studies of these biopolymers can provide insight into the understanding of possible mechanisms of formation and functioning of host-microbial interactions. To prevent the LPS from being contaminated capsular polysaccharides were removed by repeated resuspending in 0.15 M NaCl. The LPS were isolated by modified phenol extraction of delipidated, dried and dispersed bacterial cells and cleaved by mild acid hydrolysis to yield the OPS, which was studied by sugar analysis along with ¹H and ¹³C NMR spectroscopy, including ¹H, ¹H COSY, TOCSY, ROESY, and ¹H, ¹³C HSQC and HMBC experiments. LPS fatty acids were determined as methyl esters by GLC. The LPS from various strains were quantified, analyzed by polyacrylamide gel electrophoresis (SDS-PAGE), ELISA, and by immunoblotting. The *Herbaspirillum* produce different LPS forms, which has O-polysaccharides, and which has none. The LPS contained different carbohydrate fraction depending on the strain, including Kdo, and phosphates. The LPS's lipid A contained 3-hydroxydecanoic, 3-hydroxydodecanoic, dodecanoic, tetradecanoic, and hexadecanoic acids. The structures of the OPS from two strains of *Herbaspirillum* were established for the first time. To our knowledge, the OPS structures established are unique among known bacterial polysaccharide structures one consisting of two types repeating units, and another consist of tetrasaccharide repeating units. Both characterized by the presence of components, rarely found in Gram-negative bacteria. Analysis of the *Herbaspirillum* genome showed a set of sequentially arranged operons (presumably a cluster of genes) associated with the OPS. Genes responsible for the biosynthesis of OPS are dispersed in the genome and found to be in agreement with the OPS structure established. Amino acid sequences analysis carried out in BLAST demonstrated the specificity of this putative cluster for *Herbaspirillum* spp.