BIOLOGICAL AND FUNCTIONAL ACTIVITY OF AZOSPIRILLUM BRASILENSE LIPOPOLYSACCHARIDES

Bulyhina T.

D.K. Zabolotny Institute of Microbiology and Virology of the NAS of Ukraine, Department of microorganisms biochemistry

e-mail: tati20@ukr.net

Introduction. Azospirillum brasilense is a Gram-negative, nitrogen-fixing bacterium that colonizes the rhizosphere of various grasses and cereals. Azospirils can stimulate plant growth, its productivity and organic nitrogen content under certain environmental and soil conditions. Lipopolysaccharides (LPS) are a class of complex glycolipids present in the cell membrane of Gram-negative bacteria that mediate plant-bacteria interactions. Although the effects of LPS of pathogenic plant bacteria in the induction of plant defense mechanisms have been characterized, the role of LPS of beneficial rhizobacteria on plant growth is less clear. Therefore, a very important point is the study of the chemical characteristics, biological and functional activities of A. brasilense LPS, which was the aim of this work. Methods. A. brasilense LPS were isolated from dry bacterial mass by phenol-water method. The carbohydrates were analyzed by Dubois method, nucleic acids - by Spirin, protein content - by Lowry and 2-keto-3-deoxyoctonic acid (KDO) - by Osborn. Pyrogenicity of LPS were tested observing the rules of bioethics in mice and rabbits. Serological studies were performed by Ouchterlony method. The identification of monosaccharides and fatty acids in LPS preparations was carried out on an Agilent 6890N/5973 inert chromatography-mass spectrometry system. Results and discussions. LPS of 3 strains of A. brasilense were isolated from dry bacterial mass and purified from nucleic acids by ultracentrifugation. The purified LPS were characterized by different relative yields from 2.44% to 4.75%, which is slightly higher than in other strains of the A. brasilense species (1-3%). The studied preparations were characterized by a rather high content of carbohydrates from 50.1% to 72.1% in comparison with the literature data for other strains of A. brasilense (21% - 53%). The protein content ranged from 3% to 9.39%; nucleic acids - from 3.52 to 4.34%. All LPS molecules contained up to 0.17% KDO, which is a specific component of the LPS of gram-negative bacteria. Heptoses were not detected. Analysis of the monosaccharide composition indicates that the LPSs of the studied A. brasilense strains turned out to be heterogeneous. All three strains had differences in the monosaccharide composition of LPS. At the same time, such monosaccharides as mannose, galactose, glucose and heptose were recorded in LPS of all studied strains. The study of the fatty acid composition of LPS showed the presence of fatty acids containing from 14 to 18 carbon atoms. Hydroxylated, saturated, monounsaturated acids and their cis isomers were found. Typical for most A. brasilense strains is the presence of such fatty acids as 14:0(3-OH), 16:0 and 16:0(3-OH). In the studied LPS, the dominant fatty acids were 16:0, 18:1, 14:0(3-OH), and 16:0(3-OH), which coincides with the literature data. The study of the pyrogenic effect of LPS of A. brasilense strains showed that LPS solutions are not pyrogenic, since no temperature rise above the physiological norm of healthy animals was observed. The double immunodiffusion reaction in Ouchterlon agar showed that all tested LPS in homologous systems exhibited antigenic activity. Serological cross-reactions can be used as an approach in classifying bacteria. Thus, we found that antisera to A. brasilense 18-2 and 61 react with all LPS molecules of the studied strains. That may indicate the presence of common antigenic determinants in the strains and allows us to assign them to the same serogroup. Conclusions. The results received during biological-functional studies of three strains of A. brasilense LPS contribute to the biological characteristics of this species.