The relationship between the O-specific region length of lipopolysaccharide and the virulence of bacteria from the genus of *Salmonella*

Infections caused by non-typhoidal *Salmonella* strains are, along with *Campylobacter* infections, the main cause of food poisoning in the European Union and are a serious public health problem. In addition to the typical course of salmonellosis, an infection with *Salmonella* bacteria can often lead to a parenteral infections and sepsis, which are particularly dangerous for the health and life of children, the elderly and the people with immunodeficiency. Bacterial resistance to serum is a key virulence factor for the development of systemic infections. An important element of *Salmonella* virulence is the ability to change the structures of the outer envelopes, what allows for the survival and multiplication of bacteria in an extremely adverse environment. In the lipopolysaccharide (LPS) of some *Salmonella* strains three fractions, differing by the number of repeating subunits of the O-specific polysaccharide, can be clearly distinguished: : low molecular weight LPS (LMW-OAg, consisting of up to 15 subunits), long LPS (L-OAg, 16-35 subunits) and very long LPS (VL- OAg, over 100 subunits). The number of subunits of the O-antigen is regulated by the *wzz* and wzz_{fepE} (*fepE*) genes. Bacteria of the *Salmonella* genus are able to modulate the synthesis and structure of LPS to protect the cell from the lytic action of the complement system. In order to fully understand the mechanisms of bacterial pathogenicity, it seems important to characterize the interactions occurring between the components of the bacterial cell envelopes and components of the host's immune system.

This dissertation analyzes the effect of the distribution of LPS molecules with different length located on the surface of *Salmonella* cells on its ability to evade complement response of the host. It was investigated how passaging in human serum influences the bacterial survival rate, the length of the produced lipopolysaccharide and the proteome composition of the outer membrane of *Salmonella* O48. In order to determine the role of different LPS types in the mechanisms of avoiding the host immune response by *S.* Enteritidis, a panel of chromosomal mutants with different length of the O-specific LPS part was constructed. The collection of chromosomal mutants included 4 strains producing, respectively: one O-specific subunit (Δ*wzy*), LMW-OAg (Δ*fepE* Δ*wzz*), LMW-OAg and L-OAg (Δ*fepE*), LMW-OAg and VL-OAg (Δ*wzz*). In order to better characterize the different types of LPS present on the surface of the bacterial cell, and to measure the average length of the O-specific part, a method using gas-liquid chromatography was developed. The method is based on the analysis of the proportion between the amount of the sugar component present in the repeating subunits of the O-specific part and one of the components of the LPS core. Using the panel

of *S*. Enteritidis mutants, the effect of the LPS length on bacterial pathogenicity was tested *in vitro* (determination of the interaction with complement components and the degree of bacteria uptake by mammalian cells) and *in vivo* (determination of the pathogenicity level using *Galleria mellonella* larvae model). Additionally, the study also determined the influence of the length of the O-specific part of LPS on the composition of the outer membrane proteome of the created *S*. Enteritidis mutants.

The conducted experiments have shown that multiple passages of *Salmonella* O48 in serum lead to an increase in the survival rate of the bacteria and changes in their cell envelopes. Among the proteins present in greater quantity after nine-fold passaging in serum, proteins related to the response to environmental stress and proteins related to the biosynthesis of fatty acids were identified. On the other hand, the changes in the LPS profiles were small and concerned mainly L-OAg LPS. The performed *in vitro* tests with the created *S*. Enteritidis mutants showed the key role of L-OAg LPS in the protection of *S*. Enteritidis bacteria against the lytic action of serum. The relationship between the length of the O-specific portion of LPS and the proteolytic activity of the PgtE protein has been demonstrated. A relationship between the O-antigen length and the degree of bacterial uptake by mouse macrophages was also demonstrated. *In vivo* studies using the *Galleria mellonella* larvae model showed that *S*. Enteritidis mutants differ in their pathogenicity. The proteome analysis of the outer membrane proteome of the created *S*. Enteritidis mutants showed that there is an increase in the amount of some proteins produced in the tested strains compared to the wild type strain. Proteins associated with the biosynthesis of flagella and proteins of two-component regulatory systems can be distinguished among the proteins present in greater amounts in the studied *S*. Enteritidis mutants.

In summary, the research described in this doctoral dissertation provided a number of important information on the role of the O-specific chain length of LPS in the pathogenesis of *Salmonella* bacteria. Moreover, the study characterized the effect of lipopolysaccharide length on the composition of the outer membrane proteome.