1. State the objective of the project

The main objective of the project is to understand the biological function of the molecular chaperone ClpB from the pathogenic bacterium *Leptospira interrogans* ($ClpB_{Li}$). *L. interrogans* is responsible for causing a serious disease in both humans and animals called leptospirosis. The project aims to address biochemical characterization of $ClpB_{Li}$, its specific role in survival of pathogenic bacteria under environmental stress conditions, and also during infection of the host. We intend to prove that a specific role of $ClpB_{Li}$ during infection is linked with its chaperone function and therefore with the involvement of ClpB in disaggregation and reactivation of aggregated proteins.

2. Basic research to be carried out during the implementation of the project

To show involvement of the $ClpB_{Li}$ function during infections caused by pathogenic *Leptospira* spp., we plan to examine whether the level of $clpB_{Li}$ gene expression is elevated in kidney tissues of experimental animals during infection. We also intend to examine whether $ClpB_{Li}$ is able to activate the host immune system. Next, we will perform biochemical studies using purified $ClpB_{Li}$. It is known that biochemical properties and a specific structure define a protein's function in the cell. We will compare properties of $ClpB_{Li}$ to the well-known $ClpB_{E.coli}$ and ClpB from the saprophytic species of *Leptospira* (i.e. *L. biflexa*). Comparison of biochemical properties of the ClpB chaperones from different bacteria seems to be a particularly interesting aspect of the project, because it may disclose the virulent properties of $ClpB_{Li}$. Furthermore, we will attempt to identify *Leptospira* proteins interacting with $ClpB_{Li}$. Identification of such proteins will help to reveal the underlying mechanism by which ClpB influences virulence traits in *L. interrogans*.

Our studies will set the stage for assessing whether the function of ClpB chaperone is essential for *L. interrogans* persistence and rapid adaptation to intracellular life conditions during the infectious process.

3. Present reasons for choosing the research topic

The data accumulated over the last few years regarding several bacterial pathogens (including *Leptospira*) suggest that ClpB chaperone may play an important role in their virulence (Kannan et al., 2008; Capestany et al., 2008; Chastanet et al., 2004; Meibom et al., 2008; Lourdault et al., 2011). It was demonstrated that the loss of ClpB function in *L. interrogans* has resulted in bacterial growth defects under stress conditions and complete loss of bacterial virulence (Lourdault et al., 2011). These observations form the basic principles for our assumptions and research. The role of stress and stress response in host-pathogen interactions, and also the participation of ClpB in pathogen-stress response designate a new field of research on molecular mechanisms of leptospirosis. Leptospirosis is considered to be the most widespread bacterial zoonosis of worldwide importance (Adler et al., 2011). Sources of this pathogen are mainly infected and sick animals (also carriers), which excrete the leptospires with urine into the environment. Thus, the environment i.e. water and soil contaminated with infected urine may also facilitate the spread of pathogenic *Leptospira* spp. In countries, that have a temperate climate (like Poland), the environment is a strong risk factor for Leptospira infections. More than 1 million cases of severe leptospirosis occur worldwide each year, with a mortality rate of 5% to 20% (Adler et al., 2011). It is worth noting that leptospirosis is also a serious economic problem in many countries, including the EU countries. There are huge economic losses due to reproductive disorders in cattle, sheep, pigs and horses each year. The course of disease in these species has often a latent, chronic nature. The reproductive disorders and ocular inflammation in horses are the only symptoms of the disease, which generates huge economic losses. Many serological and microbiological studies indicate a high rate of infections in domestic animals (Ryan et al., 2012; Arent, Kędzierska-Mieszkowska, 2013). Despite severity of leptospirosis and global importance, the molecular mechanisms of leptospiral pathogenesis remain largely unknown. Identification of *Leptospira* virulence factors and understanding their properties is crucial to understand the mechanisms of the disease induction. ClpB chaperone is among leptospiral virulence factors. In light of these data, the purpose of the project, i.e. to study the specific role of $ClpB_{Li}$ and molecular mechanism of its action, seems legitimate. We believe that the obtained data may provide insights for understanding the role of ClpB chaperone in the stress response of *L. interrogans* and also in pathogenesis of leptospirosis. Since ClpB does not exist in animal cells, it might be a new therapeutic target for treatment of infections caused by pathogenic *Leptopsira* spp. Interfering with the function of *clpB* could provide a new direction in the treatment of leptospirosis. New antibacterial therapies may be more effective than current antibiotics. In addition, immunological aspect of our research may have practical significance and may contribute in the future to construction of a diagnostic test that will allow a serological differentiation between vaccinated animals and natural infections. The research planned will also contribute to complementing present knowledge about the biological function of chaperone proteins in pathogenic bacteria and their role during infection of the host.

4. References

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