

## ABSTRACT

The Habilitation Thesis "New Insights into Bacterial Infections - A Novel Concept of Attenuated Virulence" is based on research conducted over the past eight years. The thesis contains five chapters that describe a new concept that has not been published to date with regard to how intestinal epithelial cells do not kill pathogens but attenuates their virulence following the release hydrogen peroxide. The concept that hydrogen peroxide does not always kill pathogens is new and it was not previously described.

The story of this line of research began with the publication in 2009 of a study describing how intestinal pathogens are de-capsulated in the presence of human epithelial cells. Also, in this study we described the fact that the encapsulation does not rely on physical interaction between pathogen and epithelial cells but on host released signaling factors. This study is described in Chapter 2 and represents the basis of research for the next chapters. Also this thesis (Chapter 1) refers to the discovery of a novel tyrosine kinase from *Campylobacter jejuni*, an enzyme that has proved to be crucial in regards to how the pathogen responds to the hydrogen peroxide produced by intestinal epithelial cells. This concept, introduced in this chapter, refers to the fact that hydrogen peroxide produced by host cells inactivates a tyrosine kinase external bacterial membrane. This kinase is responsible for the phosphorylation of a set of outer membrane proteins that have been shown to be important in the generation and transmission of bacterial polysaccharides on the bacterial surface. The absence of this kinase leads to loss of the ability of pathogenic bacteria to produce capsular polysaccharide and therefore reduces its virulence.

Another important part of this thesis refers to the manner in which the probiotic bacteria prevent or inhibits colonization of intestinal mucosa by pathogenic bacteria. It is important to note that this part of my research led to winning in 2013 a research contract funded by the Romanian Government. This grant aims to identify combinations of probiotics, most effective to prevent pathogen contamination of broilers with *Campylobacter jejuni*. Our early research (and published in prestigious journals in the field of Microbiology - American Society of Microbiology) shows that probiotic bacteria have the ability to prevent infections with *Campylobacter jejuni* of human epithelial cells. This is due to the fact that probiotic bacteria compete with pathogenic bacteria for adhesion sites to the human or animal tissue. These studies are presented in Chapter 3.

Another area that I'm very interested in, presented in Chapters 4 and 5 relates to the characterization of bacterial cytochromes P450s and their potential to be used in creating new drugs that can be used successfully in the treatment of gastrointestinal disorders. The results presented in these two chapters describe how this enzyme has been cloned for large-scale production in *E. coli*. After purification the enzyme was characterized spectroscopically which showed its characteristic cytochrome P450 functionality. To prove the role of this enzyme in pathogenicity we have created a mutant strain by replacing the gene that produces this protein in *Campylobacter jejuni* with a gene conferring resistance to chloramphenicol. The resulting mutant was found to be significantly impaired in its ability to infect cells. Phenotypical characterizations performed brought up that this enzyme is involved in the production of capsular polysaccharides. These capsular polysaccharides are well known virulence factors in pathogenic bacteria. All these guiding results are consistent with the genomic location of the genes in a region that is involved in the production of these polysaccharides.

The last chapter of this thesis refers broadly to the future development plans that are based solely on previous research and are presented in detail in previous chapters. The material presented in this chapter forms the base for new research projects that I intend to submit for funding. Research in this area is very poor in Romania, and therefore a research direction in this area is absolutely necessary especially because the elimination of food pathogens is one of the main priorities of the European Commission.