“Effect of naturally occuring isothiocyanates on the virulence of *Vibrio cholerae*”
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Vibrio cholerae is a Gram-negative pathogen that lives in the aquatic environment or the animal digestive tract. Infections of *V. cholerae* lead to gastrointestinal disease occurring mainly in developing countries. The main virulence factor of pathogen, responsible for the symptoms of the disease, is the cholera toxin. Commonly accepted treatment for *V. cholerae* infections is oral hydratation, however it does not eliminate the pathogen from the organism. For this purpose, antibiotics are used, but increasing antibiotic resistance of pathogenic strains makes treatment difficult. Thus, it is of great importance to search for new antimicrobial compounds. A vast majority of potential antibacterial substances originates from plants.
The example of such compounds are glucosinolates, thioglycosides present in cruciferous plants. The hydrolysis of glucosinolates by the enzyme myrosinase produces isothiocyanates (ITC), substances with chemopreventive, antibacterial and anti-inflammatory properties.

The main goal of my dissertation was to investigate the influence of selected isothiocyanates on the growth and virulence of *V. cholerae.* In my work I studied two aromatic ITCs, *i.e.*, benzyl (BITC) and phenethyl (PEITC) and sulphoraphane (SFN) which is an aliphatic isothiocyanate.

First step of my work was to assess the impact of ITCs on the viability of bacteria. Based on the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values, I determined that each of the ITCs has an antibacterial effect, with BITC being the most effective (MIC 0.5 mM) and PEITC the weakest (MIC 2 mM) against *V. cholerae.* Moreover, the inhibition of bacterial growth was dependent on the concentrations of compound. Importantly, I showed also that ITCs reduced the ability of *V. cholerae* to form biofilm and limited the metabolic activity of cells.

The ITCs led to a reduction in both DNA and RNA synthesis in *V. cholerae* cells. The phenomenon of rapid inhibition of RNA synthesis in bacterial cells can be a result of the induction of a stringent response, one of the key mechanisms that adapts the bacterial cell to stressful conditions. Two main proteins participate in the stringent response in *V. cholerae* and numerous Proteobacteria: RelA synthetase and the bifunctional protein SpoT with the properties of the synthetase and hydrolase of guanosine tetra- and

pentaphosphates ((p)ppGpp). These proteins lead to the synthesis and accumulation of (p)ppGpp due to unfavorable environmental conditions, such as amino acid starvation, limitation of carbon, phosphorus, or iron source. This alarmon directly and indirectly regulates the transcription from many genes. In my work I showed that each of the tested ITCs led to the accumulation of (p)ppGpp alarmons. Moreover, in the presence of excess of the specific amino acids I observed a suppression of the effect of stringent response induction by isothiocyanates and the inhibition of accumulation of (p)ppGpp alarmon. It may indicate the influence of ITC on amino acid metabolism or the translation process.

Based on the qPCR analysis, I determined that treatment of *V. cholerae* culture with PEITC and SFN led to an effective repression of genes encoding pathogen's virulence factors. Moreover, the inhibition of transcription of these genes by amino acid starvation, was like this observed for ITC. The results thus suggested that ITCs exert their function by the induction of the stringent response.

Moreover, the Western blot analysis confirmed that PEITC and SFN decreased the level of synthesis of the β-subunit forms of the cholera toxin. Importantly, ITC significantly reduced the toxicity of bacterial lysates against HeLa and Vero cell lines.

In summary, the tested ITCs inhibit the growth of *V. cholerae* and reduce the mass and viability of cells in biofilms. Moreover, the ITCs lead to impair the expression of genes involved in the virulence of the pathogen and directly to inhibit the synthesis of cholera toxin subunits.

The proposed mechanism of action of the compounds involves the induction of a stringent response and the accumulation of (p)ppGpp in cells, effectively inhibiting the expression of virulence genes and reducing the toxicity of *V. cholerae.*