The aim of the project is to determine the role of the components of *Agrobacterium tumefaciens* outer membrane in the process of tumor formation (crown gall) on the stems of crop plants.

The main components of the outer membrane (OM) surrounding bacterial cells of the *Agrobacterium tumefaciens* species are: lipopolysaccharide, phospholipids and alternative lipids (i.e. those lipids that bacteria synthesize while living under the conditions of non-availability of soluble phosphorus compounds) and proteins, both peripheral and transmembrane. Lipopolysaccharide forms the outer layer of OM bilayer, occupying at least 75-80% of its surface. The remaining part is filled with proteins, among which the porins dominate. There is little or no room for free lipids (phospholipids). These compounds dominate on the inner level of the outer membrane, constituting it. Agrobacteria, living in a medium (environment) rich in nutrients and containing soluble phosphorus compounds, synthesize mainly phospholipids. They include: PE (~ 30%), PG (~ 12%), CL (~ 15%), PC (~ 24%) and MMPE (~ 15%) and DMPE (~ 4%). Sometimes small amounts of PS and PI are found, as well as lipids containing ornithine or lysine. Under conditions of phosphorus limitation, phospholipids are actively degraded and replaced by a wide range of lipophilic compounds lacking phosphorus. Under such circumstances ornithine lipids, glyceroglycolipids or betaine lipids predominate. Rhizobia, including also *Agrobacterium* as well as many intracellular pathogens (e.g. *Brucella* or *Legionella*) have the ability to synthesize and incorporate into lipid A (lipophilic part of LPS) long-chain fatty acids type (ω-1) (VLCFA). The presence of VLCFA makes OM more dense and the bacteria surrounded by it are more resistant to changed environmental conditions. *Agrobacterium tumefaciens* is a dangerous pathogen of dicotyledonous plants. By genetically colonizing plant tissue, bacteria causes its excessive growth and forces cells of this tissue (genetically modifying them) to synthesis and secretion of opines. Opines are a particularly good source of carbon and nitrogen for this pathogen. The expanded plant tissue becomes a safe ecological niche for agrobacteria. Interacting with plant cells, bacteria are in direct contact with the environment prevailing in intercellular spaces. Safe contact with such micro-world is ensured by appropriate cell wall architecture, and especially appropriate OM spatial organization, which is the first and the outermost line of defense for bacteria. It has been proven that severe deficiencies of phosphatidylcholine in membranes (OM and IM) prevent *Agrobacterium tumefaciens* from transforming plant cells. Moreover, such bacteria become much more susceptible to stressors (detergents or elevated temperature). In turn, the inability to synthesize ornithine lipids by bacteria increases the invasiveness of *A. tumefaciens*. By constructing mutants defective in PE synthesis, we want to check how these strains will behave. These bacteria as a result of compensatory actions (synthesis of additional PG portions) should modify their OM. Synthesis of increased amounts of PG should change the surface charge of OM, which may result in the lack of proper folding of membrane proteins. We also expect that the pathogenic properties of *A. tumefaciens* mutants in the synthesis and incorporation of long-chain fatty acids into lipid A will be reduced. Weakened in this way, and therefore a more permeable structure of OM, may cause mutants to develop more slowly within the forming tumorous tissue. We assume that *Agrobacterium* will be the most weakened by mutating the *msbB* gene encoding a specific acyltransferase. The lack of this enzyme will prevent the bacteria from attaching to lipid A any acyl substituent as a replacement for VLCFA. Analogous mutations in rhizobia caused significant impairment of symbiotic systems.

We predict that the agrobacteria weakened by the planned mutations will behave differently in the tissues of infected plants. To confirm our assumptions, we intend to follow the development of infection, tumor tissue growth and the spread of bacteria in plant tissues using a variety of imaging techniques: confocal microscopy, FTIR microscopy, Raman imaging and Mass Spectrometry Imaging (MSI MALDI-TOF). The MSI technique will also allow precise localization of secondary metabolites secreted by a tumor tissue.