Abstract for PhD Thesis: "RepB proteins of *Rhizobium leguminosarum* and their role in active segregation of low-copy rhizobial plasmids"

Rhizobia are soil bacteria, which establish symbiosis with legumes, providing them with fixed nitrogen enabling their growth on nitrogen limited soils. Therefore, they act as an efficient and ecological bio-fertilizer. The significance of *Rhizobium*-legume symbiosis for sustainable agriculture results from the fact that many legumes are human food and animal feeding stuff. Thus, research of symbiosis, besides extending the basic knowledge, is also very important e.g. for agro-biotechnology, because may allow application of selected or modified rhizobial strains as bio-fertilizers.

Rhizobial genomes are usually composed of circular chromosome and several large plasmids, which are low-copy number and therefore require additional systems for stable maintenance. Rhizobial plasmids often relay on partitioning systems, distributing newly replicated plasmids to daughter cells.

Plasmid segregation machinery consists of three indispensable elements: a centromere-like DNA sequence and two proteins: a motor protein, generally an NTPase, and a centromere-binding protein (CBP). An NTPase is able to polymerize into filaments (ParA, RepA) and the second protein (ParB, RepB) binds both centromere-like sequence (*parS*) and the NTPase, acting as an adaptor between the plasmids and the filaments that are responsible for the segregation.

Rhizobial extrachromosomal replicons are equipped with *repABC* cassettes that control both their replication and partition process. *repA* and *repB* genes encode proteins involved in plasmid segregation, whereas *repC* encodes a replication initiation protein. Low-copy number and stability in population make rhizobial plasmids perfect candidates for molecular biotechnology tools e.g. as vectors useful in genetic engineering of rhizobial genomes. *Rhizobium leguminosarum* bv. *trifolii* TA1 (RtTA1) genome consists of chromosome and four large plasmids, each possessing *repABC* operon. The aim of the thesis was to characterize RepB centromere-binding proteins (CBP) originating from RtTA1 *repABC* plasmids and to define their role in active segregation of these replicons.

The conducted research allowed to confirm that all RtTA1 plasmids are equipped with functional *repABC* cassettes, which ensure their replication and stable maintenance in a population of dividing rhizobial cells. Individual *repABC* cassettes consist of clearly distinguishable partition and replication modules. The stability assays with recombinant plasmids containing fragments of RtTA1 *repABC* cassettes have shown that the *repC* gene was essential and sufficient for plasmid replication, while *repAB* genes together with *parS* centromere-like sites conferred segregational stability of plasmids. At least one *parS* element was required for proper active segregation. In course of this research it was proved that RepB proteins can interact only with parental *parS* elements and no cross-reactivity between individual RepB and *parS* from non-parental *repABC* cassettes occurs. Furthermore the individual RtTA1 RepB were able to form dimers and multimeric fractions in the solution. Using a combined *in vitro* and *in vivo* approach, it was also demonstrated that the N-terminus of RepB is required for interaction with RepA, the domain responsible for *parS* binding with HTH motif is located in the middle of the protein, while the C-terminal part of RepB is responsible for dimerization, and that protein dimerization was prerequisite for DNA binding.

An overall conclusion from the obtained data is that coexistence of several replicons with similar *repABC* cassettes in the multipartite bacterial genome is fine-tuned and largely achieved by the very specific interaction between DNA binding proteins RepB with their target sequences together with the substantial diversity of the RepB proteins and *parS* elements originating from different plasmids.