

9. Abstract

Biological nitrogen fixation is the process in which atmospheric nitrogen is reduced to ammonia by prokaryotic organisms and in this form it is introduced into the biosphere and metabolized in cells of living organisms. The main role in this process play the nodule bacteria collectively called rhizobia, which in symbiotic interaction with legumes fix from 30 to 50% of the total N₂ assimilated by all diazotrophic organisms. Mutualistic interaction of rhizobia with Fabaceae plants is agriculturally and ecologically important and hence, the great interest in this symbiosis and their partners, i.e. nodule bacteria (microsymbionts) and legumes (macrosymbionts).

The aim of this dissertation was the determination of genomic polymorphism of rhizobia forming symbiosis with wild growing legume plant *Lembotropis nigricans* (black broom), which have not yet been described in scientific literature, their taxonomic position at the genus and species levels, phylogenetic relationship with other bacteria and efficiency of atmospheric nitrogen fixation in symbiosis with Fabaceae plants.

In the first stage, black broom microsymbionts were characterized in terms of their phenotypic properties. Numerical analysis of phenotypic traits showed that root nodule isolates of *L. nigricans* are phenotypically most similar to bacteria of *Bradyrhizobium* genera.

The genomic polymorphism of the studied rhizobia, determined by three PCR-based DNA fingerprinting methods (BOX-PCR, ERIC-PCR and AFLP) together with 16S rDNA and analysis of restriction patterns obtained by RFLP-16S rDNA allowed for selection of strains, representing different genomic groups, for the 16SrRNA, core, and symbiotic gene sequences analysis.

The taxonomic position of black broom symbionts at the genus level and their phylogenetic relationship with other bacteria have been determined using comparative analysis of 16S rRNA gene sequences. The determined taxonomic rank of studied rhizobia has been verified by multilocus sequence analysis (MLSA).

Application of MLSA, i.e. concatenated the sequences of four housekeeping genes enabled affiliation of *L. nigricans* symbionts into *Bradyrhizobium japonicum* species. In the evolutionary studies of nodulation genes of diazotrophic rhizobia specific for black broom, symbiotic genes: *nodC* and *nodZ* (whose products participate

in the synthesis of the basic structure of Nod factors essential for root nodule formation) have been used.

Bearing in mind, that the aim of this work were diazotrophic symbionts of *L. nigricans* (one of two species in the genus *Lembotropis*) the analysis of their host range (laboratory plant inoculation test) and determination of the efficiency of nitrogen fixation by these bacteria in symbiosis with black broom (stem dry weight and acetylene test) were also important tasks of the presented research. The plant test results have shown the essential role of studied rhizobia in the stimulation of *L. nigricans* growth and generally, in the global nitrogen cycle. It should be also emphasized that information about the host range is one of the required criteria for the description of a new nodule bacteria species.

Multidirectional research of black broom symbionts concerning their phenotypic properties, genome diversity and their phylogenetic relatedness to other rhizobia, based on analysis of core and symbiotic gene sequences as well as the determination of DNA–DNA hybridization rate of these bacteria with reference rhizobia allowed us to classify *L. nigricans* microsymbionts to *Bradyrhizobium japonicum* symbiovar *genistarum*.

A handwritten signature in blue ink, appearing to read 'Wojciech Kopylov', is positioned below the text.