Investigation of genetic and functional diversity of microsymbionts of red clover plants (*Trifolium pratense*) from two climatic zones, subpolar and temperate, to identify strains with agricultural application potential

Nitrogen is one of the most limited nutrients in soil, which means that artificial nitrogen fertilizers have to be applied to increase plant productivity. However, these compounds are expensive and have a negative impact on the environment and a quality of crops. For these reasons, biological nitrogen fixation (BNF), which yields ~200 million tons of this element annually, represents a good alternative to the use of artificial fertilizers. Among various biological systems, symbiotic interactions of legumes (*Fabaceae*) with soil bacteria from the family *Rhizobiaceae* are characterized by the highest nitrogen fixation efficiency.

Legumes are a large and economically important group of plants encompassing about 18,000 species, many of which are an essential source of plant protein in human diet (beans, peas, soybeans, lentils, peanuts), a rich source of compounds used in medicine, feed (clover, alfalfa, vetch, lupine), and have several other industrial applications (e.g. acacia). Among these plants, the interest in clover cultivation has been recently increasing, since this plant is a rich source of natural isoflavones (genistein, daidzein, formononetin, and biochanin), which are components of several supplements.

Legumes possess the unique ability to establish symbiotic interactions with soil bacteria, which are collectively referred to as rhizobia, under nitrogen starvation in soil. Then, rhizobia induce the formation of nodules, specialized organs on host plant roots, and after their occupation they convert atmospheric dinitrogen to ammonia. However, in the absence of legumes, rhizobia exist as free-living bacteria in soil, when they are exposed to action of various environmental conditions, including nutrient starvation and low temperature stress. The existence of rhizobia in different geographical regions and ecological niches (soil, the rhizosphere and legume root nodules), suggests that these bacteria have developed effective adaptation mechanisms, the elucidation of which might provide a better understanding of the functioning of the legume-rhizobium symbiosis. However, in spite of the great economic and ecological importance of BNF, application of rhizobia in sustainable agriculture is not widespread. One of the reasons is insufficient understanding of adaptation mechanisms of these bacteria to various stress factors and a lack of selected strains of a high plant growth-promoting activity.

The objective of this project is to study the genetic and functional diversity of microsymbionts of red clover (*Trifolium pratense*) plants from two climatic zones, subpolar and temperate, to establish adaptability of these bacteria to low temperature stress and to identify strains with high agricultural potential. These strains should be characterized by a high efficiency of the plant growth and productivity promotion and a high tolerance to low temperatures.

In this project, the analyses will be performed using strains from two collections derived from northern Norway and south-eastern Poland (60 strains in each collection). We plan to establish the genetic diversity and phenotypic and symbiotic properties of these bacteria, and their adaptability to various environmental conditions, including cold stress. Implementation of this project will provide comprehensive data concerning the functioning of the rhizobial strains under both soil conditions and during symbiosis with clover, as well as their adaptation ability to temperature stress. An additional outcome of this project may be identification of strains characterized by a high competitiveness and symbiotic effectiveness.

The genetic diversity of the isolates will be determined based on plasmid profiles, PCR-RFLP analyses of the 16S-23S rDNA intergenic region, and sequence analyses of house-keeping and some symbiotic genes. Metabolic profiles of the strains from both collections will be determined using Biolog plates, whereas their survival and growth rate at various temperatures, adhesion and biofilm formation abilities will be studied using bacteria tagged with genetic markers *gfp* and *dsRed* and fluorescence microscopy and spectrophotometric measurements. Symbiotic properties of the strains will be determined in plant tests with the use of red clover as the host plant. In addition, comparative transcriptomic and proteomic analyses of the chosen strains from each collection cultured at both optimal and low temperatures will be performed, and the results obtained will enable us to identify genes and proteins involved in the rhizobial adaptation to this abiotic factor and to establish differences in the stress response between the strains from the different climatic zones.

In conclusion, the data obtained from the implementation of this project will contribute to a better understanding of symbiotic interactions of rhizobia with legumes and identification of highly effective strains, that may enable more effective use of these bacteria in the future in sustainable agriculture and contribute to increasing of a plant productivity and quality of resulting crops.