Abstract

Legumes (*Fabaceae*) are organisms that play an important role in human life. They are a rich source of protein, serve as natural fertilizers and improve soil fertility. Under nitrogen deficiency, legumes are capable of establishing symbiosis with soil bacteria, which transform free atmospheric nitrogen into ammonium (forms available to plants) in the process of so-called Biological Nitrogen Fixation (BNF). The most important group of microorganisms capable of carrying out BNF are bacteria from the *Rhizobiaceae* family, commonly called rhizobia.

The symbiosis between legumes and rhizobia is affected by many environmental factors, such as temperature, pH, soil salinity, oxidoreductive potential and heavy metal ions. The most dangerous among them, in the context of environmental pollution and human health, are heavy metals. Their presence in soil is mainly caused by constantly progressing industrialization and urbanization. The accumulation of heavy metals in crops leads to inhibition of their growth and symbiosis with nitrogen-fixing bacteria. The element that deserves special attention is zinc. On the one hand, in physiological concentrations it plays the role of a micronutrient and it is necessary for the proper development and functioning of both plants and rhizobia. On the other hand, its concentration exceeding the organism's demand leads to disorders in cellular metabolic processes, which may result in their death. To survive adverse conditions and preserve the ability to establish symbiotic interactions with plants, rhizobia has developed a number of defensive mechanisms. The most important among them is the ability to produce and release a significant amount of polysaccharide (EPS) to the environment. This polymer is a natural barrier that protects free-living bacteria against unfavorable soil conditions. It is also an essential factor enabling symbiosis with the plant host. Moreover, the properties of EPS create the opportunity to use rhizobia in various remediation techniques, such as bioremediation, or as plant growth promoting rhizobacteria (PGPR).

Thus far, no comprehensive research has been conducted into the effects of zinc ions on the phenotypic and symbiotic properties of rhizobia. Not much is known about the contribution of EPS in protecting these bacteria from heavy metal stress. Therefore, the purpose of the experiments described in this dissertation was to determine the role of this polymer in the adaptation of the bacterium *Rhizobium leguminosarum* bv. *trifolii* to environmental stress caused by the presence of zinc ions. A detailed characterization of the effect of Zn^{2+} ions on rhizobia cells and the rhizobia-legumes symbiosis was conducted.

Additionally, the efficiency of biosorption of these ions by EPS molecules was determined. The conducted experiments showed that the presence of Zn^{2+} ions negatively affects many physiological processes and phenotypic properties of rhizobia cells, but also stimulates their protective mechanisms. Zinc ions have been shown to inhibit the growth of the studied microorganisms and negatively affect their survival rate, as well as reduce the mobility and efficiency of rhizobia cell respiration. Among the tested bacteria, the strains that produced an increased amount of EPS [Rt24.2 (pBA1) and Rt24.2 (pBR1)] proved to be the most resistant to the harmful effects of zinc compared to the wild strain (Rt24.2). In addition, it has been observed that the presence of Zn^{2+} ions in the growth environment stimulates bacteria to increase the production of EPS and other surface polysaccharides, form larger amounts of biofilm, and also increase activity of cellular enzymes responsible for the removal of free radicals. The presence of this stress factor causes a variety of changes in the physicochemical properties of the bacterial cell, especially in electrophoretic mobility, dependingly on the amount of EPS synthesized, the phase of bacterial growth, the number of dead cells as well as the concentration of zinc ions and the pH of environment. Additionally, Zn^{2+} ions reduce effectiveness of the symbiosis between rhizobial strains and clover, negatively affecting both microsymbiont and plant host. It was observed that strains overproducing EPS were characterized by much better adhesion to clover roots and the efficiency of symbiosis. The EPS isolated from the Rt24.2 strain showed good ability to sorb Zn^{2+} ions.

Summarizing, EPS plays a key role in the adaptation of the free-living bacteria *R. leguminosarum* bv. *trifolii* to stress conditions caused by the presence of zinc ions, as well as during their symbiosis with clover. Overproduction of EPS by rhizobia increases the effectiveness of their adaptation to stress conditions, and also applicability in the remediation processes. Moreover, good sorption properties of EPS make it potentially useful in biotechnology.

Key words: *Rhizobium*-legumes symbiosis, heavy metals, zinc, exopolysaccharide

Magdalena
Kopycińska