

**Endophytic bacteria from *Galium aparine* L. – analysis of
biosynthetic gene clusters and their importance in the production of
bioactive compounds**

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Abstract

The progressive degradation of the environment, resulting, among other, from industrial pollution, the emission of harmful substances, and excessive soil exploitation, leads to a decline in soil quality. Consequently, soils lose their fertility as well as their capacity to retain water and essential nutrients, which adversely affects plant growth and development, posing a threat to the stability of agricultural production and food security. Combined with population growth and the increasing demand for food, these factors create a pressing need to develop more efficient and sustainable methods for enhancing agricultural productivity and improving food quality. In this context, increasing attention is being paid to solutions based on the use of microorganisms, including endophytes, which may support plant growth and enhance plant resistance to environmental stresses.

The aim of this dissertation was to characterize and identify taxonomically endophytic bacteria isolated from the tissues of cleavers (*Galium aparine* L.), as well as to select the strain with the greatest potential for metallophore production based on genomic analyses. This doctoral dissertation consists of five thematically coherent scientific publications, including four original research articles presenting the results of the conducted studies.

The scope of the work included sequencing and assembly of genomes of endophytic bacteria isolated from cleavers tissues, functional analysis of genes in the context of strains' ability to produce bioactive compounds beneficial to plants, and laboratory validation of this production. In subsequent stages of the study, the optimization of metallophore production by the selected strain, purification of the metallophore, and a preliminary assessment of its effect on the growth of plants cultivated in soils contaminated with heavy metals were also carried out. The objectives of the study were achieved, and the proposed research hypotheses were verified during the course of the investigation.

Based on the obtained results, all ten examined strains of endophytic bacteria were classified within the family Bacillaceae. Two of them, namely *Priestia* sp. GS2 and *Bacillus* sp. GL1, most likely represent previously undescribed species. Genome analysis of all strains revealed the presence of genes and gene clusters associated with the biosynthesis of numerous bioactive compounds exhibiting plant growth-promoting properties, which was confirmed by laboratory studies. At the same time, the analysis demonstrated the high potential of the investigated bacteria for metallophore production, including unique and less commonly occurring metallophores. All isolated strains exhibited the ability to produce metallophores under laboratory conditions. Among them, *Priestia* sp. GS2 and *Peribacillus frigoritolerans* GR2 were distinguished by high siderophore activity and the profile of chelated metals. *Peribacillus frigoritolerans* GR2 demonstrated the ability to bind toxic heavy metals such as lead, cadmium, and mercury, whereas *Priestia* sp. GS2 showed affinity toward metals essential for proper plant development, including cobalt, zinc, and copper. The optimization and purification of an opine-type metallophore produced by *Priestia* sp. GS2 were successfully performed. Preliminary studies investigating the effect of the purified metallophore on the

growth and development of *Beta vulgaris* L. seeds in heavy metal-contaminated soils demonstrated the potential applicability of both the metallophore and the bacterial strain itself as a biostimulant.

In summary, the obtained results indicate that herbaceous plants constitute a promising source of endophytic bacteria with broad application potential in agriculture and environmental biotechnology. Their ability to produce bioactive compounds, including metallophores that support plant growth and enhance tolerance to stress caused by heavy metals, highlights the possibility of utilizing these strains as natural biostimulants and as components of sustainable strategies aimed at improving soil quality and crop productivity. At the same time, the metallophore biosynthesis pathways present in strains with the highest production potential require further in-depth investigation and more detailed molecular and functional characterization, as the mechanisms underlying their activity remain insufficiently understood. The results of this dissertation provide a foundation for further research on the application of endophytes in the development of modern and environmentally friendly solutions supporting sustainable agricultural production.

Keywords: endophytic bacteria, *Galium aparine* L., biosynthetic gene clusters, bioactive compounds, metallophores