Abstract

Organic production of food is not only a farming strategy that allows to achieve a high-quality product but also decrease the negative effect of farming on the natural environment. It is estimated that about 30% of ecological arable land is located in Europe, and about 6.4% of this land is located in Poland. In addition, the production of organic soft fruit, including raspberries, in Poland between 2012 and 2020 almost doubled from 21 635 tons to 41 870 tons. The intensification of agricultural production that has taken place in recent years has made farmers dependent on chemical methods of plant protection as easy to use and reliable. However, it cannot be ignored that conventional plant protection products can lead to the acquisition of resistance by pathogenic microorganisms and a reduction of the soil microorganisms' biodiversity. Increased interest in the field of organic farming, and in particular the organic production of soft fruits as those, often consumed directly, is another factor that encourages producers to use new solutions for ecological protection and biostimulation of plants, based on inter alia beneficial microorganisms.

Changing the plant protection strategy to a more environmentally friendly one is also in line with the policy of the European Union, which for many years has been trying to promote organic, sustainable and regenerative agriculture among farmers. Council Regulation (EC) No 834/2007 of 28 June 2007 stipulates that the use of conventional, chemical plant protection products must be kept to a minimum, and farmers should use organic plant protection products of natural origin. The European Strategy for Biodiversity 2030, introduced on May 20, 2020, being an important element of the European Green Deal, emphasizes the importance of biodiversity of all-natural environments, as well as the need to increase the biodiversity of agricultural areas. This strategy not only aims to reduce the use of pesticides by 50%, but also to increase the area of organic farming to an amount equal to or greater than 25% of all cultivated land. Farmers using ecological plant cultivation practices are obliged not to use many conventional fertilizers and plant protection products, which may lead to a reduction in yields and an increase in production and final product costs. Mentioned legal acts also define the role and the need to use biological methods of plant protection, such as plant extracts or biopreparations containing microbiological inoculum.

This research concerns the development of an innovative microbiological biopreparation containing a consortium of beneficial bacteria isolated from wild raspberry rhizosphere. Beneficial bacteria used in this research have the ability to inhibit the growth of the chosen, most common fungal and fungal like pathogens that infect raspberry plants. The process of biopreparation development consisted of taking soil samples, isolation of bacterial strains, their identification and selection of beneficial bacteria, assessment of their ability to inhibit the growth of selected phytopathogens, selection of the most effective isolates and functional characteristics of selected bacteria. For selected, 4 strains of bacteria belonging to 3 genera - *Arthorbacter*, *Pseudomonas* and *Rhodococcus*, the composition of the culture medium (carbon and nitrogen sources), the culture conditions (pH value of the medium and culture temperature), the preservation method were optimized and the prebiotic supplementary blend additive was developed. A pot experiment was also carried out to evaluate the effect of the bacterial inoculum on the growth of raspberry plants treated with selected phytopathogens - *Botrytis cinerea*, *Colletotrichum acutatum*, *Phytophthora* sp. and *Verticillium* sp., and on the communities of microorganisms inhabiting the rhizosphere and phyllosphere of raspberries.

The assessment of the ability to inhibit the growth of pathogens by the isolated beneficial bacteria was carried out on the basis of measuring the growth inhibition zones on the culturing plates. Functional characterization and determination of catabolic capacity of individual isolates

were performed using phenotype microarrays by Biolog^{TM}, with the use of GEN III plates. In the pot experiment, 3 methods of application of the microbial inoculum and 5 pathosystems were used, which allowed to study the relationship between the inoculum of beneficial bacteria and phytopathogens, as well as plant growth. The effect of the applied naturalization and the presence of phytopathogens on the microbial communities inhabiting raspberry rhizosphere and phyllosphere were investigated using the Biolog^{TM} system with ECO microplates, and the Next Generation Sequencing (NGS).

The conducted research has shown that the rhizosphere of wild growing raspberries may be a valuable source of beneficial microorganisms, and that properly developed conditions for culturing and drying allow for effective growth and preservation of bacteria. The application of bacterial inoculum to raspberry plants in the pot experiment positively affected the activity of soil dehydrogenases in objects without the presence of pathogens. The application of the microbial inoculum along with watering 4 weeks after planting resulted in the greatest positive effect on the dry mass of the plant. The value of the substrate stress index (SST) for the microbial communities inhabiting the phyllosphere decreased in the experiments where the microbial inoculum was applied. The application of the bacterial inoculum resulted in the reduction of the Shannon's biodiversity index for the fungal communities inhabiting the raspberry phyllosphere. The results obtained on the basis of the conducted research indicate a broad and comprehensive effect of the developed microbiological biopreparation. The results indicate its effectiveness in biostimulating the growth of raspberries, supporting the communities of microorganisms inhabiting the plant's rhizosphere and phyllosphere and inhibiting the growth of phytopathogenic organisms.

Keywords: biopreparations, sustainable agriculture, microbial inoculum, soil microorganisms, regenerative agriculture, phenotypic microarrays, soft fruit phytopathogens, next generation sequencing