Biosurfactants obtained from endophytic bacteria isolated from Chelidonium majus L. production and application

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Abstract

The continuous development of civilization, apart from the undoubted benefits related to the production of useful products, also generates large amounts of waste, the management of which is one of the foundations of the circular economy. The solution to the problem of environmental pollution as well as waste management can be the use of efficient biological tools, such as microorganisms. The analysis of literature data on the search for new microorganisms, that can be a source of innovative biologically active compounds as well as an effective tool in the fight against environmental pollution has become an inspiration to undertake research on a specific group of microorganisms - endophytic microorganisms.

The subject of this research was the development of the biosynthesis of surfactants by endophytic bacteria (isolated from the *Chelidonium majus* L. plant) on waste raw materials and semi-finished products of the agri-food industry; characteristics of the structure and properties of the obtained biosurfactants; their use as plant growth promoting compounds and as factors enhancing microbial degradation of hydrocarbon compounds.

As part of this work, the conditions for surface sterilization of plant material, necessary for the isolation of endophytic microorganisms (sterilization time and concentration of the disinfectant), were developed. Sixteen strains of endophytic microorganisms (10 strains of bacteria and 6 strains of fungi) were isolated. The isolates were screened for the ability to produce surfactants and high degradation activity, as a result of which two bacterial strains (2A and EN18) meeting the above conditions were selected.

It was important to determine the taxonomic affiliation of the selected isolates, which was performed on the basis of the identification of 16S rRNA subunits. Based on the results of the analysis, the species affiliation of the 2A and EN18 strains to the group of *Bacillus pumilus* and *Bacillus cereus* bacteria was determined (due to potential pathogenicity, work on the EN18 strain was abandoned).

One of the most important and, at the same time, the most difficult stages of the biotechnological process is determining the optimal conditions for biosynthesis. Realizing the objectives of this study, using statistical analysis (Taguchi method), the conditions (substrate, temperature, pH, inoculum concentration) of biosurfactant biosynthesis by the endophytic *Bacillus pumilus* 2A strain were determined. Based on the results of the analyzes, the optimal process was determined: carbon source - brewing spent grain, nitrogen source - ammonium nitrate, temperature 30°C, pH 7, inoculum concentration 5%. The developed biosynthesis conditions allowed to carry out the process on a quarter-technical scale in the Infors Techfors fermenter (30 liters), with the yield of 8.68 g/l of biosurfactant.

The composition and properties of the biosurfactant produced by the 2A strain were also determined. The biochemical, spectrometric and chromatographic analyzes performed showed, that it is a glycolipid consisting of hexadecanoic and octadecanoic acids, and also containing the rest of 9,12-octadecadienoic acid. The hydrophilic parts contained in their composition the residues D-glucose, D-xylose and D-arabinose.

As part of the work, the properties of the obtained biosurfactant, undoubtedly influencing its potential application, were analyzed. The obtained glycolipid was characterized by good properties dispersing hydrophobic compounds, high thermostability, and formed stable emulsions. It was also found that the concentration of 1 mg/ml of glycolipid inhibits the adhesion of gram positive and gram negative bacteria to ceramic surfaces in 67 and 78%, respectively, and the concentration of 2.5 mg/ml reduces the adhesion of these bacteria by an average of 50% in the case of a polystyrene surface. These properties may predispose the obtained glycolipid to be used in various branches of the food industry to protect surfaces against pathogens.

A novelty in the conducted research was the demonstration of the effect of endophytic glycolipid on the growth of food plants. It was shown that 0.2% concentration of this compound resulted in a fourfold increase in the growth of beans and radishes, and a twofold increase in the growth of red beet, predisposing the obtained glycolipid to application in agriculture. The results of analyzes carried out on soil contaminated with hydrocarbons showed that the addition of glycolipid also resulted in a significant improvement in the growth of indicator plants, extremely sensitive to contamination, four times on average.

As part of the work, the influence of the obtained surfactant on the microbial degradation of hydrocarbons was also investigated. The biosurfactant was introduced into the soil contaminated with various hydrocarbons (diesel oil, used engine oil, railway grease, creosote oil) at a concentration of 10 mg/ml in order to support their biodegradation using the 2A strain, thanks to which the process efficiency increased by 56% in the case of diesel oil, three times in the case of used engine oil, twice in the case of railwa grease, three times in the case of creosote oil.

Also, the loss of aromatic hydrocarbons in the samples fed with the obtained surfactant was higher by an average of 60% for diesel oil, twice as high for used engine oil, 35% for railway grease and 60% for creosote oil.

The metabolic activity of microorganisms in a polluted environment is extremely important in the processes of microbiological environmental purification. On the basis of analyzes of changes in biochemical parameters, a significant improvement in the metabolic activity of microorganisms was demonstrated in the samples supported by endophytic glycolipid and a reduction in the toxicity of the soil environment (catalase activity on average at the level of 350 μmol H_2O_2/g·min for diesel oil and

used engine oil, 278 µmol H_2O_2/g·min for railway grease and 82 µmol H_2O_2/g·min for creosote oil). In the samples not supported by the addition of glycolipid, the activity of catalase was 1.5 times higher.

The *Bacillus pumilus* 2A strain itself, thanks to its high degradation activity, was used as a biopreparation during the *in situ* treatment of soil contaminated with creosote oil at the Nasycalnia Podładów S.A. in Koźmin Wielkopolski within the project POIR.04.01.02-00-0057/17-00 - "Modern technology for bioremediation of soil contaminated with creosote oil on the premises of Sleeper Treating Plant Spółka Akcyjna in Koźmin Wielkopolski ".

The study also compared the effect of the synthetic surfactant (SDS) and the biosurfactant produced by the 2A strain on the metabolic activity of microorganisms, degradation efficiency and reduction of toxicity (manifested in the intensification of the growth of indicator plants). In the case of the addition of a synthetic surfactant, no changes in the tested parameters were observed, while the positive effect of the addition of a glycolipid to the samples was confirmed, resulting in higher metabolic activity, reduction in the concentration of pollutants (including PAH) and a decrease in toxicity.

The endophytic biosurfactant obtained as part of the work can be widely used both in agriculture to support plant growth, while reducing the use of chemical fertilizers, in the food industry to protect the surface from bacteria, and in environmental protection for the degradation of hydrocarbons and, at the same time, soil reclamation for the purpose of its re-development. . In addition, the possibility of producing glycolipid from the waste of the brewing industry allows for the management of the waste after beer production, which places the technology in the framework of a circular economy.