ENDOPHYTIC BIOSURFACTANTS-CHITOSAN BIOPREPARATION: AN INNOVATIVE FORMULATION FOR ENHANCING CROP PLANT GROWTH

Piotr Drożdżyński

Institute of Molecular and Industrial Biotechnology, Faculty of Biotechnology and Food Sciences, Lodz University of Technology, Stefanowskiego 2/22 Str., 90-537 Lodz, Poland
ORCID: 0000-0002-7371-5125
corresponding author: piotr.drozdzynski@p.lodz.pl

Abstract

Endophytic microorganisms are crucial in enhancing plant growth and augmenting resilience to environmental stresses. This study evaluated the synergistic effect of a biosurfactant produced by B. pumilus 2A, an endophyte derived from Chelidonium majus L., combined with chitosan dissolved in citric acid. Biopreparations at concentrations of 0.1-0.4% were formulated and tested on five crop species: Zea mays (corn), Secale cereale (rye), Fagopyrum esculentum (buckwheat), Avena sativa (oats), and Hordeum vulgare (barley). The optimal formulation was identified at 0.2%, significantly enhancing root and stem growth, particularly in buckwheat and rye (up to 316% relative to control). While the chitosan solution acidified the soil, potentially affecting some plant responses, the combined formulation proved highly effective. The results highlight the agricultural potential of combining chitosan and biosurfactants for use in agricultural systems, promoting plant growth even under stress conditions.

Keywords: *chitosan, biosurfactant, plant growth promotion, biocomposite, crop plants*

Received: 23.04.2025
Accepted: 29.05.2025

1. Introduction

Endophytes are unique microorganisms that inhabit plant tissues without causing any adverse effects on their host [1–3]. These bacteria and fungi are known to directly stimulate the growth of the host plant and synthesise secondary metabolites that increase the plant's resistance to biotic and abiotic factors [4]. Recently, it has been assumed that endophytes can also biosynthesise compounds that were once thought to be produced exclusively by plants. Based on their functional roles, endophytes with biotechnological potential can be divided into four categories [5–8]:

- endophytes that produce phytostimulants and increase the bioavailability of elements obtained from the environment,
- endophytes that degrade harmful substances, thus improving environmental conditions,
- endophytes that activate the systemic resistance of the plant host,
- endophytes that inhibit the proliferation of plant pathogens by synthesising bioinsecticides, antibiotics, or surface-active compounds known as biosurfactants.

Biosurfactants are characterised by an amphipathic structure that contains both hydrophilic and hydrophobic groups. This unique configuration confers valuable properties such as foaming, emulsification, cleaning, and dispersion. In addition, biosurfactants can modulate bacterial motility and biofilm formation and exhibit antimicrobial and anticancer effects [5, 6]. One particularly promising endophytic strain is *Bacillus pumilus* 2A, which was isolated from plant tissues of *Chelidonium majus* L. and showed a high capacity for biosurfactant production [9]. Emulsifying activity analyses showed that this strain exhibited the highest emulsifying capacity (OD500 1.96) among all isolated endophytes. The biosurfactant synthesised by *B. pumilus* 2A shows potential for environmental applications. Phytotoxicity tests have shown that this biosurfactant promotes seed germination in soils contaminated with diesel fuel (137%) and used engine oil (120%) [9]. Furthermore, the presence of the biosurfactant positively affects the growth of *Sinapis alba,* an indicator plant in soils contaminated with hydrocarbons, which highlights its potential as a plant growth promoter and its usefulness in bioremediation activities [9].

Combining the potential of biosurfactant produced by *B. pumilus* 2A and the chitosan, with its unique properties, can be an innovative approach in agricultural biotechnology. Chitosan is a naturally occurring biopolymer formed by the deacetylation of chitin. In industry, chitosan is produced by treating chitin with a 40 - 50% sodium hydroxide solution, which removes about 80% of the acetyl groups and converts *N*-acetyl-D-glucosamine into D-glucosamine [10, 11].

Chitosan, as a biodegradable and biocompatible biopolymer, offers numerous benefits, including antioxidant, antimicrobial, and bioadhesive properties, making it valuable in biotechnology and agriculture. Its ability to form coatings and gels further expands its application potential [11]. As the second most abundant renewable carbon source after lignocellulosic biomass, chitosan is both economical and sustainable. In synergy with biosurfactants from *B. pumilus* 2A, chitosan can significantly improve plant growth, especially under environmental stress. Combining the protective and growth-promoting properties of chitosan with the emulsifying and biostimulating properties of biosurfactants from *B. pumilus* 2A offers a promising biotechnological solution to increase crop productivity and facilitate bioremediation of contaminated soils.

This study sought to evaluate the synergistic effect of a biosurfactant produced by the endophytic strain *Bacillus pumilus* 2A and chitosan dissolved in citric acid on the enhancement of crop plant growth. The work focused on the development of

biopreparations with different concentrations (0.1 - 0.4%) and their application to five species of crop plants in order to determine the optimal formulation influencing the growth of the root system and above-ground parts.

2. Materials and Methods

2.1. Materials

Chitosan with a degree of deacetylation (DDA) of 95% and a viscosity of 1000 mPas was obtained from Heppe Medical Chitosan GmbH (Germany). The Phytotoxkit® set was purchased from MicroBioTests Inc. (Belgium). All other reagents were of analytical grade.

2.2. Microorganism

The microorganism used for these studies was endophytic *Bacillus pumilus* 2A, which originated from *Chelidonium majus* L. and belongs to the resources of the Institute of Molecular and Industrial Biotechnology [9]. The strain was stored as a glycerin stock in a freezer at –80°C. The strain was activated to produce surface-active compounds by transplanting onto LB medium, and after 24 hours of cultivation, the preinoculum was used to inoculate the culture for biosurfactants production.

2.3. Biosurfactant Production and Purification

Biosurfactant production was performed on the MINIFORS2 (Infors HT) fermenter with *B. pumilus* 2A. The working volume of the mineral medium was 4 L. The 2A strain was inoculated onto a mineral medium with composition as follows: 1 g/L NaH_2PO_4, 1 g/L Na_2HPO_4, 0.2 g/L $MgSO_4$, 1 g/L NH_4NO_3, 50 mg/L $FeCl_3$, 50 g/L of brewer's grains as a carbon source [12].

The fermentation was carried out for 5 days, and after that time, bacterial biomass was separated (10,000 rpm, 4°C), and biosurfactant was isolated. Shortly after, the culture liquid was acidified with 2 M HCl to pH 2 and left overnight to precipitate; the sediment was dissolved with 0.1 M NaOH and extracted with chloroform:methanol (1:1, v/v). The solvents were evaporated on the rotary evaporator (40 mbar, 35°C, 100 rpm), and the extracted biosurfactants were used to prepare biosurfactant-chitosan biopreparations [12].

2.4. Chitosan-Biosurfactant Biopreparation Formulations

The chitosan was dissolved in the citric acid with a citric acid:chitosan 6:1 ratio. Then, the biosurfactant was added and mixed vigorously to obtain a final concentration of 0.1 - 0.4%. Then, biopreparation was used to evaluate its effect on the growth of crop plants.

2.5. pH Measurements

The pH of biopreparations with biosurfactant concentrations ranged from 0.1 to 0.4%, and soil containing these biopreparations was measured at an Accumet AE150 pH-meter (Thermo Scientific).

2.6. Crop Plant Growth Enhancement Evaluation Tests

The enhancing effect of obtained biopreparations at biosurfactant concentrations of 0.1 - 0.4% was evaluated on crop plants: *Zea mays* (corn), *Secale cereale* (rye), *Fagopyrum esculentum* (buckwheat), *Avena* L. (oats), and *Hordeum* L. (barley), using the Phytotoxkit® set (MicroBioTests Inc.). The Phytotoxkit test was carried out according to the standard procedure of this study using three seeds of all the above-mentioned crop

plants [13]. Shortly, the 90 ml of soil (117 g) was moistened with 30 ml of biopreparations at concentrations of biosurfactants of 0.1 - 0.4%, a 0.4% solution of biosurfactant (KBS), and a solution of chitosan in citric acid (KCH) and water. The level of soil saturation with water equal to 30 ml was determined before phytotests [13]. The final concentration of biosurfactant in the soil equals 25.6 ± 1.2, 51.3 ± 2.6, 76.9 ± 3.9, and 102.5 ± 5.3 mg/100 g of soil for 0.1, 0.2, 0.3, and 0.4%, respectively, while the chitosan concentration was 254.6 ± 12.7 mg/100 g of soil. After 6 days, pictures of the plates were taken, and the growth of the plants was evaluated using the FIJI software. The results were presented as percentage growth enhancements against the growth of crop plants on plates containing water only.

3. Results and Discussion

3.1. Biosurfactant Production

The crude biosurfactant produced by *Bacillus pumilus* 2A was obtained with 8.43 ± 0.75 g/L efficacy. After purification, the yield of a partially purified biosurfactant was 5.69 ± 0.22 g/L.

3.2. pH Measurements

The pH of the soil in which plants grow is very important. Soil pH is a metabolic regulator that influences nutrient availability, microbial activity, and the effectiveness of fertilisation and biopreparations [14, 15]. Maintaining pH within the ideal range (5.8 - 6.5 for most plants) is essential for maximising yields, promoting healthy plant growth, and ensuring the efficacy of biotechnological interventions [16].

Table 1. Measurements of pH in biopreparations and treated soil.

Biopreparation	pH of biopreparation	pH of soil treated with biopreparation
KBS	6.61 ± 0.1	6.10 ± 0.1
KCH	2.20 ± 0.1	3.91 ± 0.1
0.1%	2.19 ± 0.1	4.19 ± 0.1
0.2%	2.19 ± 0.1	4.17 ± 0.1
0.3%	2.20 ± 0.1	4.00 ± 0.1
0.4%	2.22 ± 0.1	4.11 ± 0.1

Note. KBS, biosurfactant solution (0.4%); KCH, chitosan in citric acid solution (1:6 ratio).

The biosurfactant *B. pumilus* 2A (KBS) is non-toxic to soil and preserves its pH at an optimal level for plant growth. Chitosan dissolved in citric acid markedly acidifies the soil environment, potentially adversely affecting plant growth, particularly with prolonged application or in soils with inherently low pH levels. To obtain the full bioactive characteristics of both of these compounds, the biopreparations were prepared.

3.3. Growth Enhancement of Crop Plants

The preparations used - both a solution of chitosan in citric acid and a 0.4% solution of biosurfactant produced by the *Bacillus pumilus* 2A strain - supported the development of cultivated plants used during the experiment. However, only the combination of both preparations resulted in additional synergistic enhancement of plant growth.

Figure 1 depicts the percentage variation in the root system length of five plant species (corn, oats, barley, rye, and buckwheat) following the application of different biopreparations: pure biosurfactant (KBS), chitosan solution in citric acid (KCH), and their combination at concentrations of 0.1 - 0.4% of biosurfactant. Water (H_2O) served as the control sample and established the reference value at 100% and was subtracted. A score over 0 signifies a stimulating effect, whilst a value below it indicates an inhibitory effect. The most significant growth-promoting effect was noted in buckwheat, with its length increasing by three to nearly four times (200 - 300%) relative to control, particularly following the administration of 0.2% and 0.1% solutions. Rye exhibited significant sensitivity to stimulation, achieving peak growth with the 0.2% formulation (about 280%). Corn had a modest response, with a stimulating impact (150 - 200%) predominantly observed following the application of KBS and reduced dosages of the biosurfactant-chitosan combination. The impact of biopreparations on barley and oats was minimal. Barley exhibited minimal reactivity across all tested concentrations (< 50%), but oats demonstrated a negative response, with the majority of formulations leading to a reduction in root length below the control value, indicating growth inhibition. Such behaviours may suggest phytotoxicity linked to environmental acidity or an overabundance of active compounds.

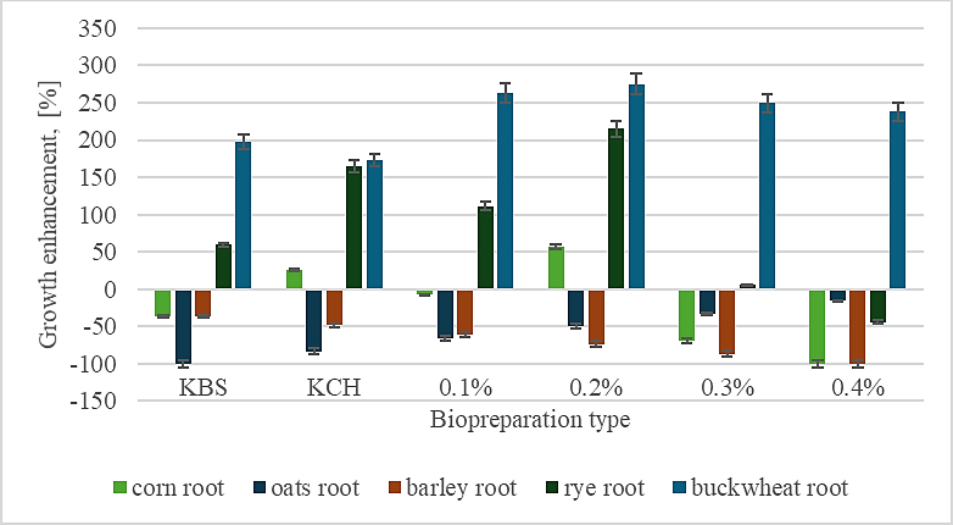

Figure 1. Growth enhancement of crop plants' roots.

The greatest favourable effects on root growth were achieved with the 0.2% formulation, indicating that the synergistic interaction between *Bacillus pumilus* 2A biosurfactant and chitosan at this concentration is the most efficacious option. The observations underscore the necessity of considering species specificity in plant responses to biopreparations, particularly for species that are more sensitive to variations in pH and soil microbiological composition.

Figure 2 shows similarly the growth of stems of tested crop plants. In samples with oats and barley, the growth of stems was completely inhibited. The maximum stimulation of stem growth occurred following the application of a biosurfactant and chitosan mixture at a concentration of 0.2%. The length of buckwheat stems in this variety rose by over fourfold compared to the control (exceeding 300%), whereas rye exhibited an increase of

more than threefold (about 280%). Corn exhibited a moderate growth response of approximately 150%. A comparable, if marginally diminished, impact was observed at a reduced concentration of 0.1%, with the stem elongation values for all three species fluctuating between 150 and 240%.

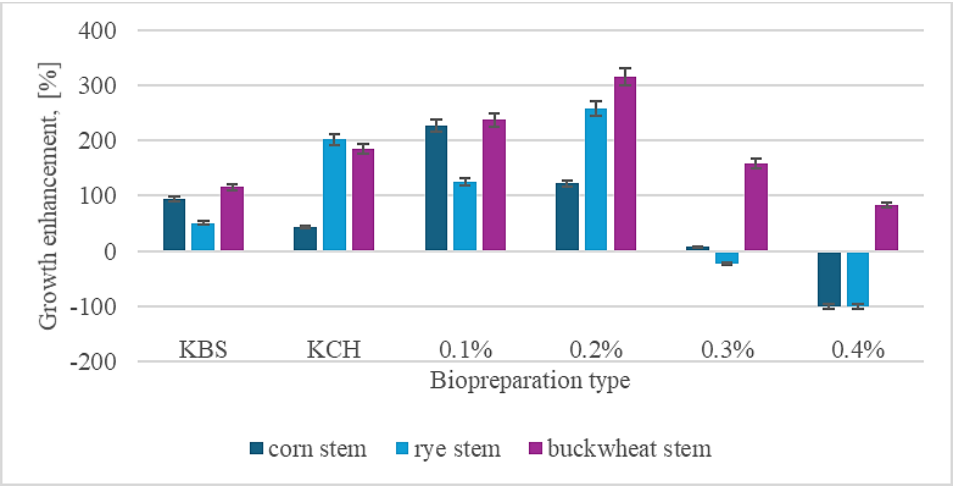

Figure 2. Growth enhancement of crop plants' stalks.

Formulations exclusively including single components (KBS and KCH) had a stimulating effect, but to a lesser degree. Stem growth for all plant species ranged from 20 to 100%. Variants with elevated concentrations (0.3 and 0.4%) exhibited diminished efficacy or a pronounced inhibitory effect, particularly on rye and maize. Thus, it may be caused by phytotoxicity induced by the low pH of the formulations or the overabundance of active compounds.

The experimental results indicate the efficacy of a biopreparation comprising chitosan and biological surfactants derived from the *Bacillus pumilus* 2A strain in agriculture, as it markedly enhances crop plant growth relative to the control sample of water-moistened reference soil. The authors of the study described in patent PL441772A1, titled "Method of obtaining a biological preparation for stimulating plant growth and plant protection" [17], also achieved plant growth induction. The researchers employed indicator plants for the investigation: *Sorghum saccharatum, Lepidium sativum*, and *Sinapis alba*. An application of 25% by weight concentration of chitosan (DDA 80%, 160 kDa) resulted in plant growth enhancements of 43, 39, and 42%. Piekarska *et al.* [18] demonstrated that chitosan and glycolipid biocomposites enhanced the growth of the evaluated indicator plants, including both roots and stems. The most effective formulation of the biopreparation was identified as chitosan lactate supplemented with 5% glycolipids. The *Lepidium sativum* stem exhibited a 100% increase in growth compared to the water-irrigated samples, while the root length was 80% greater than that of the control sample. The modifications of the experiment conducted in this research: the chitosan solvent - citrate, as opposed to lactate, along with reduced concentrations of surface-active compounds (0.1 - 0.4%), resulted in an induction of crop plant growth ranging from 6.3% for rye roots to 274.8% for buckwheat roots, and from 7.8% for corn stems to 316.21% for buckwheat stems.

4. Conclusions

The combination of *Bacillus pumilus* 2A biosurfactant with chitosan in citric acid showed a strong synergistic effect, significantly improving plant growth, especially at a 0.2% concentration, which was found to be optimal for the root and shoot elongation of buckwheat and rye. Although chitosan formulations clearly acidified the soil, the effect of pH was species dependent, with moderate acidification benefiting some crops but causing phytotoxicity in more sensitive species such as oats. These results underline the importance of tailoring bioformulations to specific crop needs. The bioformulation shows great potential for use in sustainable agriculture, especially in stressed or degraded soils, and it offers a promising solution for organic and low-input farming systems. Furthermore, the results support further commercialisation of biosurfactant–chitosan blends as environmentally friendly, multifunctional crop productivity enhancers and soil quality improvers.

5. References

[1] Schulz B, Boyle C; (2006) What are Endophytes? In: Schulz BJE, Boyle CJC, Sieber TN (eds), Microbial root endophytes. Soil Biology, Springer, Berlin, Heidelberg, Vol. 9, 1–13. **DOI:** 10.1007/3-540-33526-9_1

[2] Fontana DC, De Paula S, Torres AG, De Souza VHM, Pascholati SF, Schmidt D, Dourado Neto D; (2021) Endophytic fungi: Biological control and induced resistance to phytopathogens and abiotic stresses. Pathogens 10(5), 570. **DOI:** 10.3390/pathogens10050570

[3] Shahzad R, Khan AL, Bilal S, Asaf S, Lee I-J; (2018) What is there in seeds? Vertically transmitted endophytic resources for sustainable improvement in plant growth. Front Plant Sci 9, 24. **DOI:** 10.3389/fpls.2018.00024

[4] Santos ML, Berlitz DL, Wiest SLF, Schünemann R, Knaak N, Fiuza LM; (2018) Benefits associated with the interaction of endophytic bacteria and plants. Braz Arch Biol Technol 61, e18160431. **DOI:** 10.1590/1678-4324-2018160431

[5] Tidke S, Kl RK, Ramakrishna D, Kiran S, Kosturkova G, Gokare RA; (2017) Current understanding of endophytes: Their relevance, importance, and industrial potentials. IOSR JBB 3(3), 43–59. **DOI:** 10.9790/264X-0303024359

[6] Wang Y, Dai C-C; (2011) Endophytes: A potential resource for biosynthesis, biotransformation, and biodegradation. Ann Microbiol 61(2), 207–215. **DOI:** 10.1007/s13213-010-0120-6

[7] White JF, Kingsley KL, Zhang Q, Verma R, Obi N, Dvinskikh S, Elmore MT, Verma SK, Gond SK, Kowalski KP; (2019) Review: Endophytic microbes and their potential applications in crop management. Pest Manag Sci 75(10), 2558–2565. **DOI:** 10.1002/ps.5527

[8] Ryan RP, Germaine K, Franks A, Ryan DJ, Dowling DN; (2008) Bacterial endophytes: Recent developments and applications. FEMS Microbiol Lett 278(1), 1–9. **DOI:** 10.1111/j.1574-6968.2007.00918.x

[9] Marchut-Mikolajczyk O, Drożdżyński P, Pietrzyk D, Antczak T; (2018) Biosurfactant production and hydrocarbon degradation activity of endophytic bacteria isolated from Chelidonium majus L. Microb Cell Fact 17(1), 171. **DOI:** 10.1186/s12934-018-1017-5

[10] Kaczmarek MB, Struszczyk-Świta K, Li X, Szczęsna-Antczak M, Daroch M; (2019) Enzymatic modifications of chitin, chitosan, and chitooligosaccharides. Front Bioeng Biotechnol 7, 243. **DOI:** 10.3389/fbioe.2019.00243

[11] Struszczyk-Świta K, Kaczmarek MB, Antczak T, Marchut-Mikołajczyk O; (2024) Continuous production of chitooligosaccharides in a column reactor by the PUF-immobilized whole cell enzymes of Mucor circinelloides IBT-83. Microb Cell Fact 23(1), 258. **DOI:** 10.1186/s12934-024-02529-4

[12] Marchut-Mikołajczyk O, Drożdżyński P, Polewczyk A, Smułek W, Antczak T; (2021) Biosurfactant from endophytic Bacillus pumilus 2A: Physicochemical characterization, production and optimization and potential for plant growth promotion. Microb Cell Fact 20(1), 40. **DOI:** 10.1186/s12934-021-01533-2

[13] MicroBioTests Inc.; (2023) Test protocols. URL: http://www.microbiotests.be/toxkit-microbiotests/test-protocols/ (accessed: 14.04.2025).

[14] Goulding KWT; (2016) Soil acidification and the importance of liming agricultural soils with particular reference to the United Kingdom. Soil Use Manage 32(3), 390–399. **DOI:** 10.1111/sum.12270

[15] Adams F; (1984) Soil acidity and liming. In: Adams F (ed), Soil acidity and liming. Agron Monogr 12, 2nd ed. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison, WI, 1–380 pp. **DOI:** 10.2134/agronmonogr12.2ed

[16] Rousk J, Brookes PC, Bååth E; (2010) The microbial PLFA composition as affected by pH in an arable soil. Soil Biol Biochem 42(3), 516–520. **DOI:** 10.1016/j.soilbio.2009.11.026

[17] Piekarska K, Wiśniewska-Wrona M, Machrut-Mikołajczyk O, Struszczyk-Świta K, Drożdżyński P, Olszak-Przybysz H, Korbecka-Glinka G; (2025) Sposób otrzymywania biodegradowalnych kompozytów chitozanowych z dodatkiem biosurfaktantu. Patent PL441772A1.

[18] Piekarska K, Wiśniewska-Wrona M, Machrut-Mikołajczyk O, Struszczyk-Świta K, Drożdżyński P, Olszak-Przybysz H, Korbecka-Glinka G; (2022) Development of a recipe for the production of chitosan/glycolipids biocomposites for plant protection. In: Vassilopoulos A, Michaud V (eds), Proceedings of the 20th European Conference on Composite Materials – Composites Meet Sustainability (ECCM20), Vol 1–6. EPFL Press, Lausanne, p. 435.