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Short Communication

The Ability of *Pseudomonas* sp. SP0113 to Solubilize Tricalcium Phosphate and its Influence on the Development of Spring Wheat

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Abstract

Phosphorus is present in soil in various forms, including as insoluble organic compounds. Many species of soil-dwelling microorganisms release phosphorus from compounds that are sparingly soluble and make it partially available to crop plants. This group of microorganisms includes phosphate-solubilizing bacteria (PSB) that release phosphorus from relatively insoluble forms by producing organic acids, mineral acids, siderophores, CO_2 and H_2S. The ability of *Pseudomonas* sp. SP0113 to solubilize tricalcium phosphate and its influence on the development of spring wheat was determined in this study. Solubilization of tricalcium phosphate (TCP) was evaluated based on changes in the pH of the NBRIP (National Botanical Research Institute's) phosphate growth medium. pH and redox potential were measured immediately after the addition of TCP and every 24 hours. *Pseudomonas* sp. SP0113 proliferated in culture media with pH lower than 7, which indicates that the evaluated strain can be used as plant-growth promoting bacteria (PGPB) in acidic soils. Seed dressing improved the biometric parameters of spring wheat. The applied bacterial strain was capable of solubilizing phosphates. Spring wheat treated with *Pseudomonas* sp. SP0113 was characterized by higher thousand grain weight, kernel yield higher by 7.5%, longer spikes and stems, and a lower dry matter content in comparison with control.

Keywords: *Pseudomonas* sp.; PSB; phosphate solubilizing bacteria; spring wheat

Introduction

Phosphorus, an essential element for the growth and development of all plants, determines the yield and quality of crops [1]. In plants, phosphorus is present as membrane phospholipids, nucleic acids and nucleotides [2]. The phosphorus content of plants ranges 0.1-1.0% DM, and symptoms of phosphorus deficiency are observed below 0.1% DM. Phosphorus concentrations are highest in young plants, and they decrease in vegetative organs with age. The discussed element plays

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a very important role in plants, and it determines the growth rate of the root system [3]. Some plant attributes are associated with phosphorus nutrition, for example seed and flower formation, enzyme activity, crop quality, root development and resistance to plant pathogens. Phosphorus is involved in key plant functions, including photosynthesis, energy transfer and transformation of sugars and starches [1, 4].

In arable soils, phosphorus is present in the form of: organic and mineral compounds in the soil solution, precipitated and sparingly soluble mineral compounds, non-specifically adsorbed compounds that are weakly associated with the solid soil fraction, compounds strongly adsorbed by iron, calcium and aluminum oxide-hydroxides, compounds adsorbed by clay minerals, and insoluble organic compounds [2, 5]. The phosphorus content in soils depends on soil type and ranges 0,002-0,12 DM [1, 7]. The availability of phosphorus for plants is determined mainly by soil pH. In soils with low pH, phosphorus is more readily adsorbed by hydrated iron and aluminum oxides. Soils with a pH of 6-7 are characterized by relatively high concentrations of phosphate anions due to reduced phosphorus binding. Phosphorus fertilization produces the best results in soils with a controlled pH [6].

Microorganisms assimilate soluble phosphorus, preventing it from fixation or adsorption [8]. Fungi are less effective at phosphorous solubilization than bacteria [9]. Microorganisms with the ability to solubilize phosphates in soil increase the amount of available phosphorus for plants and affect positively on plant growth. Inoculation of phosphate-solubilizing bacteria-containing biofertilizers is beneficial to the yield of many plants [4, 10, 11].

Various species of soil-dwelling microorganisms release phosphorus from sparingly soluble compounds and make it available to crop plants [12, 13]. Those microorganisms, known as phosphate-solubilizing bacteria (PSB), represent the following genera: *Pseudomonas, Azotobacter, Bacillus, Rhizobium, Burkholderia* and *Enterobacter* [14-16]. The aim of this study was to evaluate the phosphate solubilzation potential of non-agricultural *Pseudomonas* sp. SP-0113 and confirm its plant growth promotion properties on spring wheat.

Material and Methods

Pseudomonas sp. SP0113, the bacterial strain used in this study, was described in detail by Przemieniecki et al. [17] in 2015. Belonging to the species has been defined using API 20 NE (Biomerieux, France). The strain is capable of solubilizing mineral phosphorus and releasing phosphorus from organic compounds.

The solubilization of tricalcium phosphate (TCP, $Ca_3(PO_4)_2$) was determined based on changes in the pH of the NBRIP (National Botanical Research Institute's) phosphate growth medium, developed according to the method described by Nautiyal et al. [18] in 1999. The experiment was conducted in 250 ml Schott bottles containing 99.9 ml of the medium inoculated with 0.1 ml of the overnight bacterial culture at a concentration of $1 \cdot 10^9$ CFU·ml^{-1}. The pH and redox potential of the medium were measured immediately after the addition of the bacterial strain and every 24 h for 5 days. The clarity (optical density measured at a wavelength of 600nm-OD_{600}) of the solution was determined after sedimentation of the bacterial biomass and the coarse TCP fraction. Sedimentation time was determined based on the results of spectrophotometric tests.

Table 1. Parameters of the pot experiment.

Soil properties	
Textural class	Loamy sand
pH (KCl)	5.8
Total nitrogen [%]	0.013
Phosphorus as P_2O_5 (mg P · 100g^{-1} soil)	24.5
Potassium as K_2O (mg K · 100g^{-1} soil)	20.0
Magnesium (mg Mg · 100g^{-1} soil)	4.5
Pre-sowing nitrogen fertilization (mg N · kg^{-1} soil)	125
Pre-sowing potassium fertilization (mg K · kg^{-1} soil)	125
Addition of $Ca_3(PO_4)_2$ (mg P · kg^{-1} soil)	25

The seed dressing solution was prepared by culturing bacteria on tryptic soy agar (TSA, Merck, Germany) at 27ºC for 48 h. After incubation, the culture was centrifuged (6000 rpm, 10 min), suspended in sterile deionized water and concentrated to $5 \cdot 10^8$ CFU·ml^{-1}. The suspension was combined with sterile carboxymethyl cellulose (CMC, 1% w/v) and silica. The kernel of spring wheat cv. Bombona was sterilized by rinsing in 50% ethanol for 1 min, followed by 1% sodium hypochlorite for 10 min. After sterilization, the kernel was rinsed three times in sterile distilled water and left to dry. Kernel was rinsed in a dressing solution, air dried and placed in soil at a depth of 2 cm. The parameters of the pot experiment are presented in Table 1.

All analyses were performed in three replications. The results were analyzed statistically in the Statistica 10 program with the use of t-student's test. After harvest, wheat plants were measured to determine the length of stems and spikes, spike weight, dry matter content, and kernel weight.

Results and Discussion

Based on the identification test we determined that the strain used in this study was most similar to *Pseudomonas luteola*. A preliminary analysis of the bacterial strain's growth potential on culture media

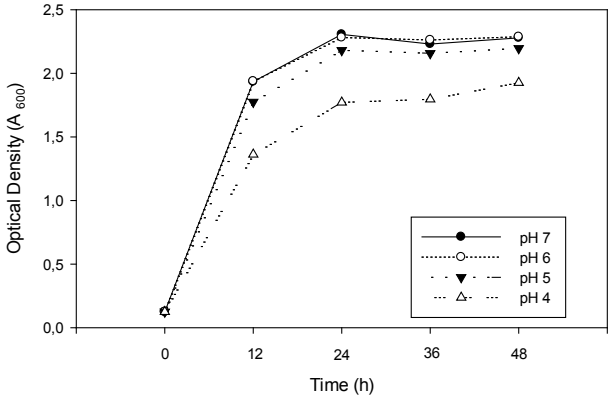

Fig. 1. Growth rate of *Pseudomonas* sp. SP0113 on culture media with different pH.

with various pH revealed that *Pseudomonas* sp. SP0113 proliferated on all examined media. Bacterial concentrations were highest at pH 5-7, whereas at pH 4 bacterial growth was reduced by approximately 20% in the final log phase (after 24 h, Fig. 1).

The pH of the growth medium inoculated with *Pseudomonas* sp. SP0113 decreased relative to control, which indicates that bacterial metabolism led to the production of organic acids. During the 5-day incubation period, pH decreased by 2.4, and the highest decrease of approximately 1 was noted between days 3 and 4. The clarity of the solution containing TCP increased with a decrease in pH, indicating that $Ca_3(PO_4)_2$ was transformed into a soluble compound (Figs 2-3). The highest loss of TCP was observed after 24 h, and the solubilization process continued until day 5, when OD_{600} reached the background value of 0.1 (Fig. 3).

In this study, the solubility of TCP indicates that *Pseudomonas* sp. SP0113 is capable of solubilizing

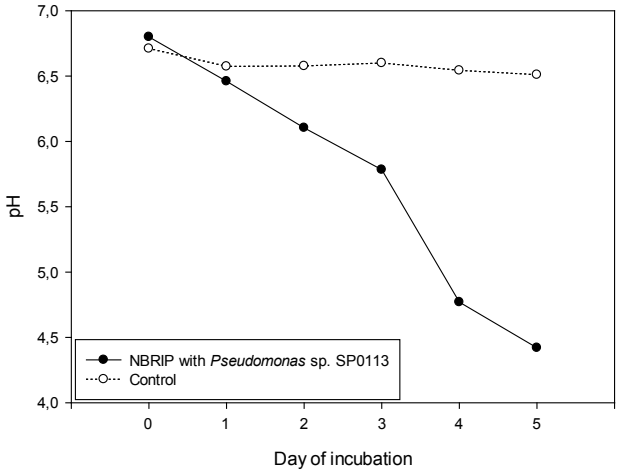

Fig. 2. Changes in the pH of the culture medium under the influence of the metabolites produced by *Pseudomonas* sp. SP0113.

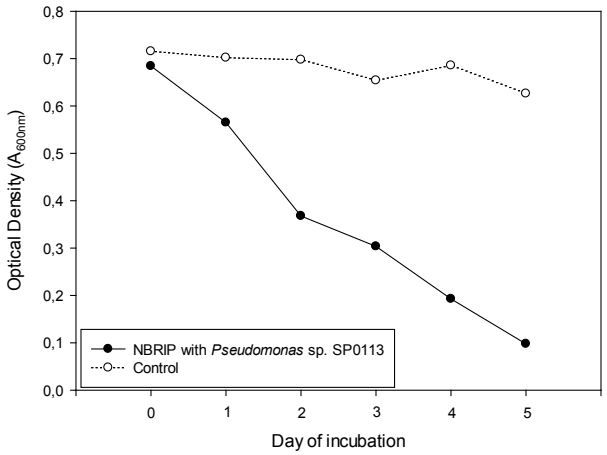

Fig. 3. Decrease in the turbidity of the NBRIP medium resulting from the solubilization of TCP ($Ca_3(PO_4)_2$); medium clarity increased with a decrease in A_{600nm} values.

phosphates. In the preliminary study, the P-solubilization index was high after 14 days of incubation on NBRIP agar [17]. In the liquid NBRIP medium, an absence of sparingly soluble phosphorus compounds was observed already after 5 days of incubation, which indicates that the analyzed bacteria can enhance plant growth in soils rich in phosphates (Fig. 3). Concentration of the organic acids in the NBRIP medium containing TCP was estimated at 20 μg ml^{-1}.

Winter wheat kernel treated with the *Pseudomonas* sp. SP0113 strain was characterized by longer spikes than control; however, these results were not significantly different (Table 2).

The dry matter content of spikes was insignificantly higher in spring wheat whose kernel was not treated with the bacterial strain, which points to a higher percentage of chaff in the spike. Spring wheat whose kernel was dressed with *Pseudomonas* sp. SP0113 produced longer stems than control plants (Table 2). The yield of spring wheat inoculated with *Pseudomonas* sp. SP0113 was 7.5% higher and thousand kernel weight was 5.6% higher in comparison with control (Table 2). The thousand kernel weight of spring wheat inoculated with *Pseudomonas* sp. SP0113 was significantly higher in comparison with control (Table 2).

In the present study *Pseudomonas* sp. SP0113 was capable of solubilizing phosphates, which was confirmed by increasing the pH of medium and clarifying solutions with the insoluble fraction. Nevertheless, the first sign that the tested bacterial strain had the ability to dissolve inorganic phosporates was a selective medium test [17]. Similar results were reported by Park et al. [19], in whose study the *Pseudomonas fluorescens* RAF15 strain isolated from the ginseng rhizosphere led to a decrease in the pH of basal salt solution, similar to that induced by *Pseudomonas* sp. SP0113. In the above experiment, insoluble phosphorus was solubilized in the first 5 days, which is consistent with our findings. Thakker et al. [20] analyzed the growth-enhancing

Table 2. Selected biometric parameters of spring wheat treated with the *Pseudomonas* sp. SP0113 strain as a dressing.

Treatment	Spike length (cm)	Spike weight per pot (g)	Spike dry mass content (%)	Stem length (cm)	1000 kernel weight (g)	Increase in kernel mass relative to control (%)	Increase in kernel yield relative to control (%)
Dressed kernel	7.17±0.53 A*	14.72±0.95	88.62±0.24	41.47±3.84	36.35±0.52 A	5.6	7.5
Control	6.94±0.60 B	14.20±1.23	89.83±0.76	40.76±6.36	34.43±0.48 B	-	-

* The A and B letters accompanying the average mean the results differing from each other. Student's t-test was used in the statistical calculations.

potential of the *Pseudomonas* sp. OG strain isolated from seawater. The examined strain was also able to solubilize phosphates on Pikovskaya's broth. A medium inoculated with *Pseudomonas* sp. OG was characterized by slightly higher concentrations of solubilized phosphorus than the medium containing *Pseudomonas* RAF15. The growth-promoting effect of the analyzed strain was also observed in a greenhouse experiment.

Organic acids are the key factor responsible for P-solubilization by microorganisms. Their concentration in the NBRIP medium containing TCP was estimated at 20 μg ml^{-1}. This value is typical of most bacteria of the genus *Pseudomonas,* which can produce more than 10 organic acids, where gluconic acid accounts for approximately 90% of total organic acids secreted into the medium [21, 22]. However, in some studies solvent effect had been assigned a different organic acid [16, 23].

In the present study the dry matter content of spikes was insignificantly higher in spring wheat whose kernel was not inoculated with the bacterial strain, which points to a higher percentage of chaff in the spike. In a study by Naiman et al. [24], the dry matter content of the aboveground parts of wheat plants inoculated with *Azospirillum brasilense* and *Pseudomonas fluorescens* strains was 20% higher. Rosas et al. [25] inoculated kernel with *Pseudomonas aurantiaca* SR1 and reported a higher dry matter content of stems in comparison with control. Positive influence on the yield of spring wheat after the inoculation of the kernel with the P. putida ART-9 was observed by Przemieniecki et al. [26].

Spring wheat kernel inoculated with *Pseudomonas* sp. SP0113 produced longer stems than control plants. This parameter varied considerably in control plants, which could adversely influence harvest. Zabihi et al. [27] and Kumar et al. [28] demonstrated that PGPB increases the height of wheat plants.

Kernel yield per unit area is influenced by different yield components, including the number of spikes, number of kernel per spike and thousand kernel weight. In the present study spring wheat dressed with *Pseudomonas* sp. SP0113 produced 7.5% higher yield and thousand kernel weight was 5.6% higher in comparison with control (Table 2). Similar results were reported by Naiman et al. [24], Mäder et al. [29] and Kumar et al. [28]. In a study by Rosas et al. [25], inoculation with beneficial bacteria increased kernel yield. The cited authors demonstrated that the application of PGPB can produce kernel yields more than 30% higher than that noted in this study.

In this experiment, thousand kernel weight was the main yield-forming factor. Spring wheat inoculated with *Pseudomonas* sp. SP0113 had significantly higher thousand kernel weight in comparison with control (Table 2). In addition, this strain has a positive effect even under the pressure of herbicides (glyphosate) [30]. In a pot experiment by Shaharoona et al. [31], the thousand kernel weight of wheat treated with PGPB was 6-43% higher than in control.

Conclusions

The evaluated bacterial strain lowers pH in environments containing TCP ($Ca_3(PO_4)_2$) and solubilizes phosphorus compounds unavailable to plant roots. Inoculation with the *Pseudomonas* sp. SP0113 bacterial strain improved the biometric parameters of spring wheat. The applied treatment did not exert a statistically significant effect on some parameters, but it increased kernel yield by 7.5% and thousand kernel weight by more than 5%, indicating that the analyzed strain can be used as a dressing for spring wheat seeds. The test strain efficiently soluble phosphate under laboratory conditions and improves the yield of spring wheat. After optimizing technical properties, the potential use of a bacterial formulation as a bio-fertilizer is justified.

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Conflict of Interest

The authors declare no conflict of interest.

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