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Study of the plant growth-promoting capacity of *Pseudomonas putida* 1046 in a model plant system

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Abstract

Plant Growth Promoting Rhizobacteria (PGPR) represent a microbial community that exerts growth-promoting capabilities in plants by various mechanisms. Among the PGPR genera, *Pseudomonas* spp. deserves special attention. It is due to its characteristic traits, like the production of phytohormones and siderophores, solubilization of minerals and phosphates, and plant protection from biotic and abiotic stress. These PGPR properties depend on the microorganism and its plant counterpart. The use of microbial strains as bioinoculants must consider the physiological and economic aspects of the process, and the plant growth stimulating effect has to be checked and proved. This study aimed to explore the PGP capacity of *Pseudomonas putida* 1046 strain in a model plant system of the economically important corn culture (*Zea mays*). The effect of the strain's metabolic status on the plant germination capacity was evaluated. Bacterial cultures, grown 16 h and 48 h, were explored for the treatment of the corn seeds at three experimental concentrations: 0.1, 0.2, and 0.4%, and monitoring of their germination capacity through the growth indicators length of the radicle, length of the coleoptile, and the number of lateral roots. The data obtained outline the positive effect of *Pseudomonas putida* 1046 on the germination capacity of corn when applied at 0.2% concentration. The *in vitro* treatment of the model plants with 0.2% suspension resulted in a 22.87%–28.33% increase in the length of the radicle, a 35.96%–49.56% increase in the length of the coleoptile, and a 5.41–16.67% increase in the number of the lateral roots. High values of the vigour index (2125 for 16 h and 2721 for 48 h culture) were also registered. The strain's ability to produce siderophores of hydroximate type and exhibit phosphate solubilizing activity is proved. The optimal treatment parameters of the corn seeds comprise the application of 0.2% suspension of 16 h grown *Pseudomonas putida* 1046 strain for five days.

Keywords
biofertilizer, PGPR, rhizosphere microorganisms, *Zea mays*

Introduction

The soil microbial communities influence plant growth and development. The playground for this effect is the rhizosphere since the plant root system and the soil microorganisms interact there. This interaction is a complex process influenced by various environmental factors, such as temperature, humidity, pH, availability of nutrients, etc. (Mimmo et al. 2018). The plant-associated bacteria can execute beneficial or deleterious effects on plant growth (Dobbelaere et al. 2003). The soil bacteria that positively affect the plant are called Plant Growth Promoting Rhizobacteria (PGPR) (Kloepper and Schroth 1978). PGPR constitute a vital component of the rhizosphere. They support the development of the plant's root and shoot systems. PGPR positively affect plant morphogenesis, flowering, and photosynthesis efficiency (Hossain et al. 2017).

The need for environmentally friendly fertilizers or biofertilizers is constantly increasing nowadays (Meliani et al. 2017) on account of soil, water, and air contamination due to chemicals such as fertilizers and pesticides. A few alternatives to fertilizers that are environmentally friendly have been developed (Gupta et al. 2015). Thus, the impact of PGPR on agriculture steadily increases as they offer an attractive way to replace chemical products. Fertilizers containing microorganisms are an appropriate alternative to chemical ones since they are a natural component of healthy soil that enriches rather than pollutes it (Vessey 2003; Nadeem et al. 2016). Moreover, they produce growth-promoting substances in large quantities that influence the plant's morphology (Bhattacharyya and Jha 2012).

Many species belonging to g. *Pseudomonas* possess plant growth-promoting (PGP) characteristics that allow their application as bacterial fertilizers. They are abundantly present in the rhizosphere. *Pseudomonas* spp. are known for their well-established growth-promoting mechanisms. They encompass better root colonization, the production of enzymes, metabolites, phytohormones and siderophores, mineral and phosphate solubilization, and plant protection from biotic and abiotic stress (Nadeem et al. 2016).

The application of the microbial PGP properties depends on the microorganism and its plant counterpart. Corn (*Zea mays*) is one of the most important cereal crops in the world after wheat and rice. It is used as livestock feed, human food, and raw material for several industries (Kumar and Jhariya 2013). The corn growth, development, and yield are well-focused biotechnological targets, and the plant attributes, such as germination rate, drought tolerance, and yield components, are objects of profound research and technological interest. PGPR could be used for maximizing these parameters and applied as a potent tool in sustainable agriculture. The contribution of g. *Pseudomonas* representatives in the enhancement of some biochemical and agronomic

parameters of corn under normal conditions or exposure to various abiotic stress is well-recognized in the literature (Mubeen et al. 2021). However, the use of pseudomonads and their corn counterpart in biofertilization programs must consider the physiological and economic aspects of the process, and the plant growth stimulating effect has to be checked and proved.

This study aimed to explore the PGP capacity of bacteria belonging to *Pseudomonas putida* species in a model plant system of the economically important corn culture *Zea mays*.

Materials and methods

Bacterial strain and test plants

Pseudomonas putida 1046 strain was used in this study. It possesses the biochemical capacity to catabolize aromatic hydrocarbons and their derivatives. Corn seeds (Dekalb – DKC 5830 HD (Hybrid 101)) were used as a model plant test system. They are characterized by a fast initial development, high quality of the grain, tolerance to high sowing norms, and a response to high fertilizers and sowing norms. In some of the experiments, soybean seeds (*Glycine max* L.) were used.

Culture media and cultivation conditions

The experimental strain was maintained on Nutrient agar (BB-NCIPD Ltd., Bulgaria). Batch cultures in Nutrient broth were obtained after cultivation at 28 °C for 16 h or 48 h on a rotary shaker (220 rpm).

Biochemical analysis

Pseudomonas putida 1046 biochemical profile was established applying ApiZYM rapid systems for the detection of bacterial enzymes and Api 20 NE standardized system for the identification of non-fastidious, non-enteric Gram-negative rods. The tests performance was according to the methods described by Humble et al. (1977) and Donnelly (2006). Siderophore detection was performed following the method of Alexander and Zuberer (1991). The assay for phosphate solubilization was done according to Shahid et at. (2015).

PGP effect determination

The seed germination method was applied to study the PGP effect of *Pseudomonas putida* 1046 strain on corn (*Zea mays*) seeds. The corn seeds were subjected to surface sterilization before the germination test. Sodium hypochlorite (0.02%) solution was applied for 2 min., followed by intense rinsing with sterile distilled water. The seeds'

germination capacity was assessed through the growth parameters of the plant root system (Sharma et al. 2014). Water suspensions with a defined concentration of 0.1%, 0.2%, and 0.4% were prepared from the 16 h and 48 h test microorganisms containing 10^8 CFU/ml. The corn seeds were immersed in these suspensions and cultured for five days at 25 °C. The growth parameters: length of the radicle, length of the coleoptile, and the number of lateral roots were measured. The positive effect of the test strain on the germination capacity of the corn plants was calculated as a percentage of untreated control. The plant vigour index was also calculated using the following formula: Vigour index = (mean radicle length + mean coleoptile length) × % germination (Baki et al. 1973). To prove the relationship (and its strength) between *Pseudomonas putida* 1046, and the growth parameters of the plant root system the seed germination method was applied to another technical crop, soybean (*Glycine max* L.), at the same experimental conditions. Each treatment was performed following a completely randomized design with three replicates and 10 seeds/replicate.

Correlation analysis

Correlation analysis was performed by calculating the correlation coefficients with MS Excel software of two variables data sets – *Pseudomonas putida* 1046 suspension concentration (0% (control), 0.1%, and 0.2%) and the growth parameters of the plant root system (the indices length of the radicle, length of the coleoptile, the number of the lateral roots, and the vigour index).

Data analyses

All presented data are mean values of at least 3 individual experiments. The data were analysed by MS EXCEL build-in function, and the results were presented as means with standard deviations (n=3).

Results

Pseudomonas putida 1046 strain has been selected on the basis of preliminary biochemical analyses that indicate its potential plant growth-promoting capacity. The biochemical profile of the strain was established applying ApiZYM and Api 20 NE detection systems. The biochemical profile data presented in Table 1 showed activity of the enzymes alkaline phosphatase, acid phosphatase, and naphthol-AS-BI-phosphohydrolase, indicative for PGP potential due to their involvement in the phosphate solubilization and mineralization. Additional phytohormones analysis showed the biosynthetic capacity of the test strain for the phytohormones gibberellic acid (GA3), indole acetic acid (IAA), and jasmonic acid (data not shown).

The effect of the strain metabolic status on the test plants' germination was evaluated in order to check the PGP capacity of *Pseudomonas putida* 1046 strain. The PGP

Table 1. Biochemical characterization of the *Pseudomonas putida* 1046 strain.

ApiZYM		Api 20NE	
Alkaline phosphatase	positive	NO_3 Reduction of nitrates to nitrites	negative
Esterase (C 4)	positive	Reduction of nitrates to nitrogen	positive
Esterase Lipase (C 8)	positive	TRP Indole production (tryptophan)	negative
Lipase (C 14)	negative	GLU Fermentation (glucose)	negative
Leucine arylamidase	positive	ADH Arginine dihydrolase	positive
Valine arylamidase	negative	URE Urease	negative
Cystine arylamidase	negative	ESC Hydrolysis (βglucosidase) (esculin)	negative
Trypsin	negative	GEL Hydrolysis (protease) (gelatin)	negative
α-chimotrypsin	negative	PNGP β-Galactosidase (paranitropheny l-ßDgalactopyranosidase)	negative
Acid phosphatase	positive	GLU Assimilation (glucose)	positive
Naphthol-AS-BI-phosphohydrolase	positive	ARA Assimilation (arabinose)	positive
α-galactosidase	negative	MNE Assimilation (mannose)	positive
β- galactosidase	negative	MAN Assimilation (mannitol)	positive
β-glucuronidase	negative	NAG Assimilation (N-acetylglucosamine)	positive
α-glucuronidase	negative	MAL Assimilation (maltose)	negative
β-glucosidase	negative	GNT Assimilation (potassium gluconate)	positive
N-acetyl-β-glucosaminidase	negative	CAP Assimilation (capric acid)	positive
α-mannosidase	negative	ADI Assimilation (adipic acid)	positive
α-fucosidase	negative	MLT Assimilation (malate)	positive
		CIT Assimilation (trisodium citrate)	positive
		PAC Assimilation (phenylacetic acid)	positive
		OX Cytochrome oxidase	positive

capacity of the bacterial strain was assessed in respect to its test plants. The seeds germination of the test plants was used as an assessment indicator through the quantitative measurement of three indices: length of the radicle, length of the coleoptile, and number of lateral roots. The corn seeds at three experimental concentrations (0.1, 0.2, and 0.4%) were treated with bacterial cultures, grown for 16 h and 48 h and monitored for their germination capacity. The length of the radicle, length of the coleoptile, and the number of lateral roots were used as growth indicators.

Length of the radicle

The values for the growth indicator length of the radicle are shown in Figs 1A, 2. They indicate a positive effect of the bacterial culture on the plant physiology, best represented during the treatment with 0.2% suspension. An increase of 22.87% for the 16 h culture and 28.33% for the 48 h one was observed. The values for the remaining two tested concentrations (0.1% and 0.4%) showed variations in the range of 1.7 to 9.9% compared to the untreated control.

Length of the coleoptile

The data for the length of the coleoptile show that all three tested concentrations exhibit a positive effect on its growth (Figs 1B, 2). The values indicated an increase in this

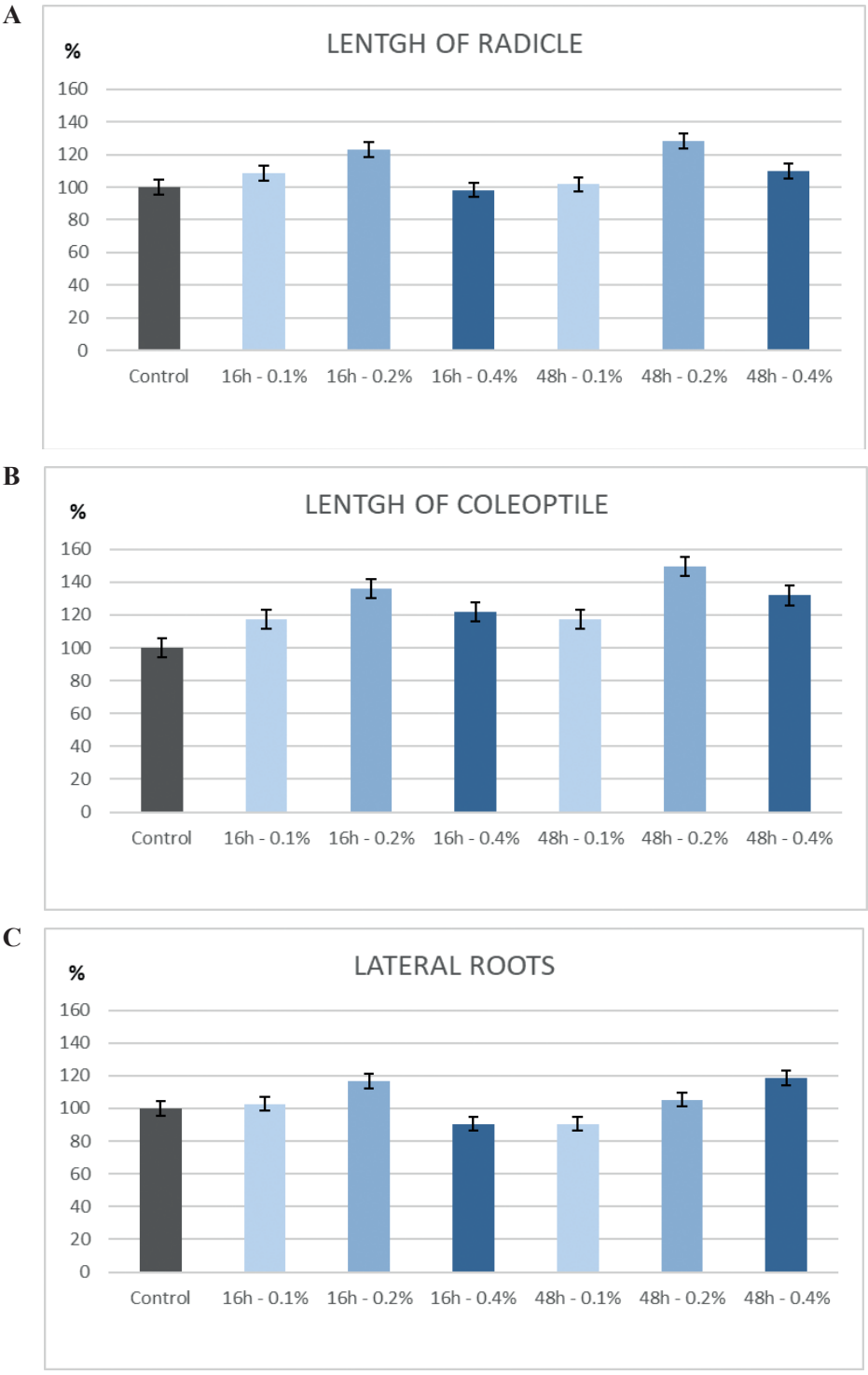

Figure 1. PGP effect of *Pseudomonas putida* 1046 on the germination of corn seeds evaluated through length of the radicle (**A**), length of the coleoptile (**B**), and the number of lateral roots (**C**).

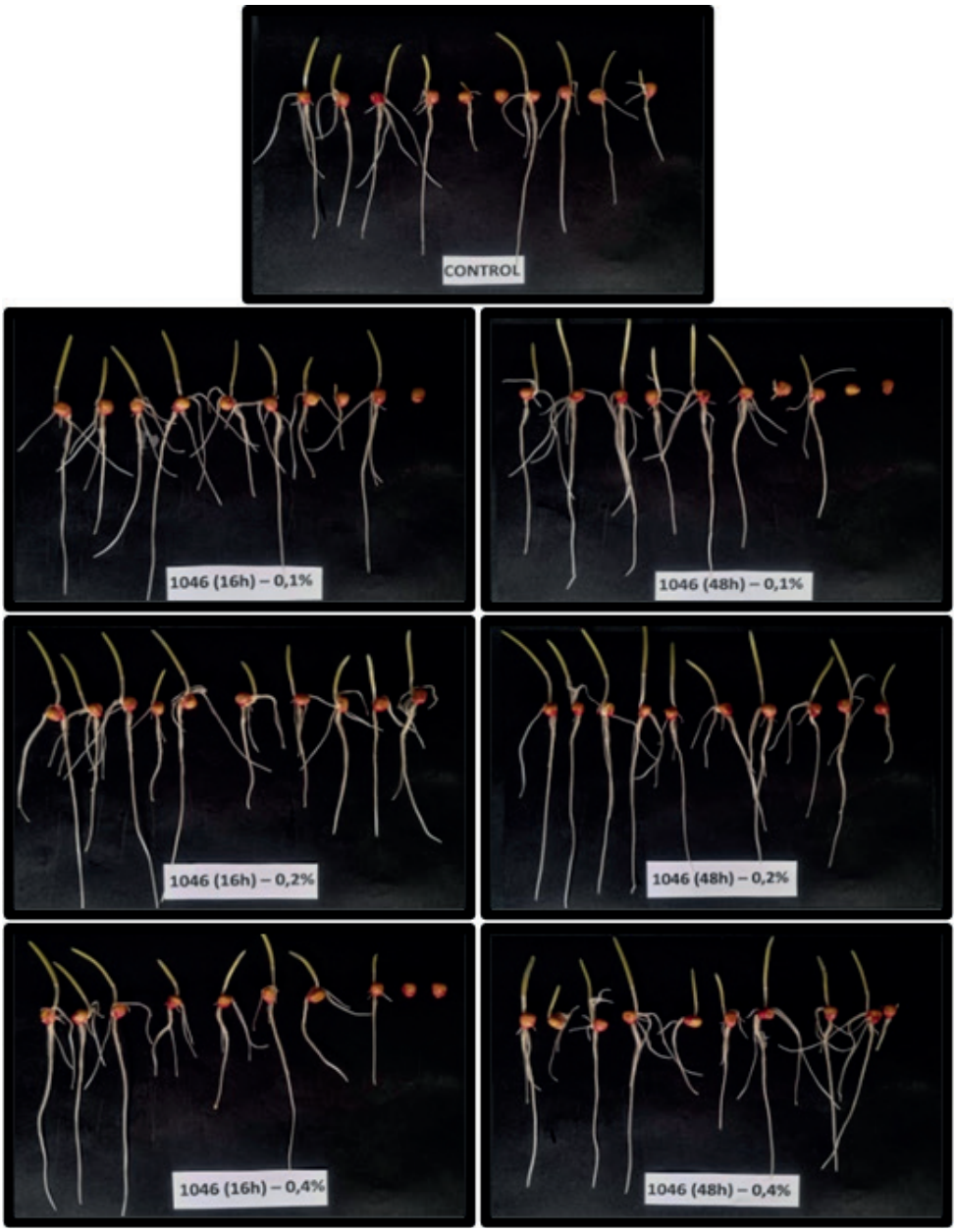

Figure 2. Visual representation of *Pseudomonas putida* 1046 PGP effect on corn seeds germination capacity.

growth parameter between 17.4% and 49.6%. The tendency for the best stimulatory effect of 0.2%, acknowledged for the length of the radicle, was also observed here. An increase of 35.96% for the 0.2% and 49.56% for 0.4% suspensions was registered, respectively. Comparing the physiological status of *P. putida* culture, it is evident that at 0.1%, the stimulatory effect was the same (11.7%) regardless of the culture age. With

the increase of the bacterial strain concentration (0.2% and 0.4%), the 48 h population stimulated the coleoptile development more efficiently (10% to 15%) compared to the 16 h one.

Number of the lateral roots

The third index (number of the lateral roots), used for assessment of the corn germination capacity, showed a relatively lower stimulatory effect (2.78%–18.60%) as compared to the previous two indicators. However, it is evident that the tendency for the highest positive results obtained by the 0.2% suspension of the bacterial strain is kept (Figs 1C, 2). The 16 h bacterial culture showed higher growth promotion of the lateral roots compared to the 48 h (16.67% vs. 5.41%).

Among the three tested bacterial concentrations, the 0.2% suspension was the right choice for the seeds' treatment. The 0.1% expressed no or mild positive effect on the number of lateral roots and the length of radicles, as shown in Figs 1, 2. Compared to the 0.2%, the highest tested concentration of 0.4% did not significantly affect any of the analysed indices.

Vigour index

The values for the vigour index are presented in Fig. 3. The corn plants were treated with 0.1%, 0.2%, and 0.4% suspensions of 16 h and 48 h *P. putida* culture and

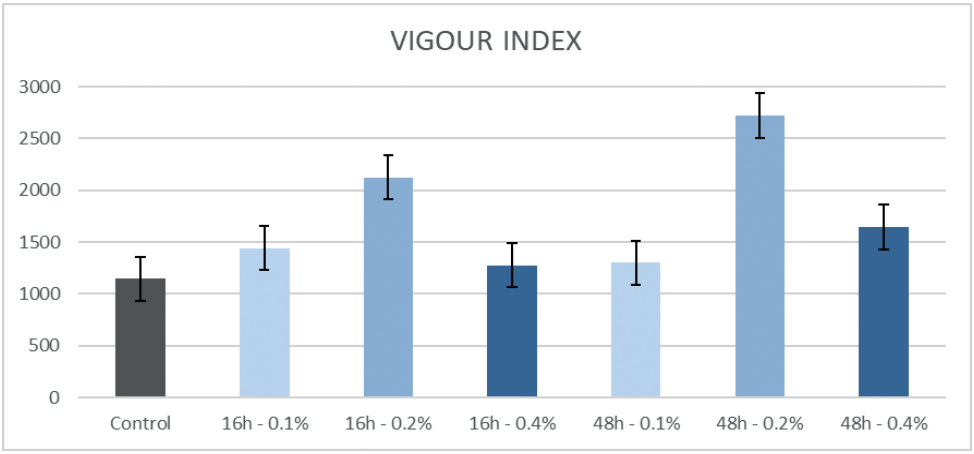

Figure 3. Effect of bacterial inoculation on corn vigour index after 5 days *in vitro* germination.

Table 2. Correlation coefficients for the relationship *Ps. putida* and plant root system growth parameters.

Time (h) / Index	Radicle	Coleoptile	Lateral roots	Vigour index
16	0,98927	0,99982059	0,95572399	0,975104
48	0,891681	0,99194071	0,36147266	0,90716648

evaluated five days after *in vitro* germination. The highest values of the vigour index were registered after treatment with 0.2% suspension of the bacterial strain: 2125 for 16 h and 2721 for 48 h culture.

Correlation analysis

Based on the experimental data for the plant growth indices evaluated, correlation analysis for the bacterial and plant data sets was performed as described in the section Materials and Methods. The results are presented in Table 2. They prove a positive relationship between the *Pseudomonas putida* 1046 and the growth parameters of the plant root system and the vigour index. In quantitative aspect, the correlation coefficient (r) can be evaluated as a very strong one since its values fall into the range 0.9–1.0 for the 16 h grown culture and 0.4–1.0 for the 48 h one.

Pseudomonas putida 1046 PGP effect on soybean

In order to confirm the PGP effect of *Pseudomonas putida* 1046 strain on the corn test plant, two different approaches were used: testing another plant model system and extended research on PGP characteristics of the bacterial strain.

Soybean (*Glycine max* L.) was exploited as a second model system. The crop has a global position as one of the most significant agri-cultures, a source of food, protein, and oil. The PGP effect of *Pseudomonas putida* 1046 was evaluated using two of the indices applied for the corn: length of the radicle and the epicotile (coleoptile). The tendency for enhanced germination due to the increased number of lateral roots and enlarged epicotile length, promoted by *Pseudomonas putida* 1046, is confirmed by the data depicted in Fig. 4A, B.

The ability of the model bacterial strain to produce siderophores and solubilize phosphates was examined by qualitative analyses. These activities were tested due to their promotional effect on plant growth: enhanced metal accumulation in plants and putative application for phytoremediation purposes of siderophores, and the growth-promoting ability attributed to phosphate solubilization. The data about these PGP characteristics are presented in Figs 5, 6. They indicate the production of siderophores of hydroxamate type (hallo coloured in yellow-orange) and the typical colourless hallo of the solubilized phosphates.

Discussion

Nowadays, extensive research is focused on PGPR as a versatile replacement of fertilizers, pesticides, and other agrochemicals for the promotion of plant growth. PGPR influence in a positive way (directly or indirectly) the soil structure and fertility, decomposition of organic matter and organic pollutants, solubilization of nutrients of mineral nature, production of plant growth regulators, stimulation of the root growth, and execution of biocontrol against soil and seed-borne pathogens. (Gupta et al.

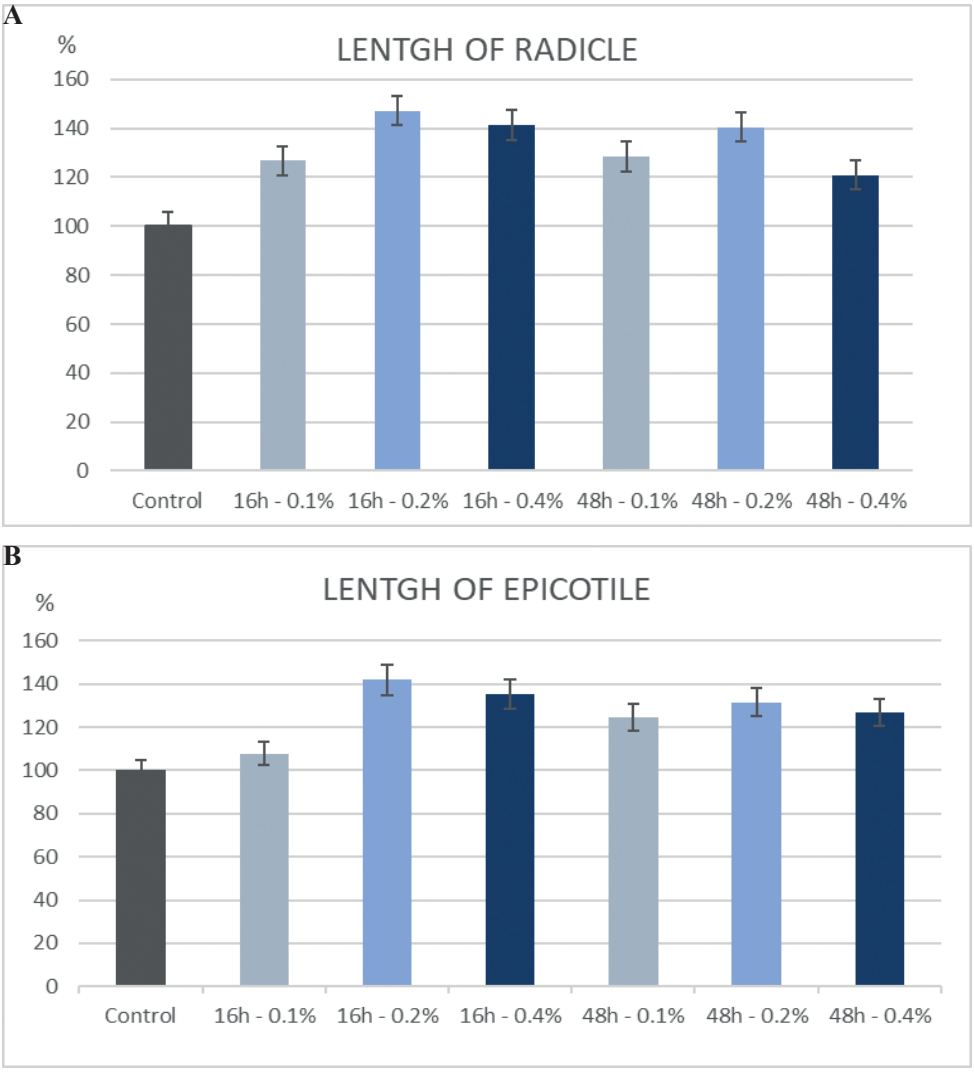

Figure 4. PGP effect of *Pseudomonas putida* 1046 on the germination of soybean seeds presented through the growth parameters length of radicle (**A**) and length of epicotile (**B**).

2015). Assessment of the plant growth-promoting potential of rhizobacteria requires a complex approach that includes, among others, the monitoring of plant germination capacity in test systems.

The data presented in the Results section outline the positive effect of *Pseudomonas putida* 1046 strain on the germination capacity of the technical crop *Zea mays*. Its biochemical profile speculated PGP potential, and the determined PGP traits confirmed it. The *in vitro* treatment of the model plants with bacterial strain improved their germination capacity. Gholami et al. (2009) reported similar results regarding improved seeds germination potential of corn. Other authors observed the same tendency for various crop cultures, such as canola (Glick et al. 1997), wheat (de

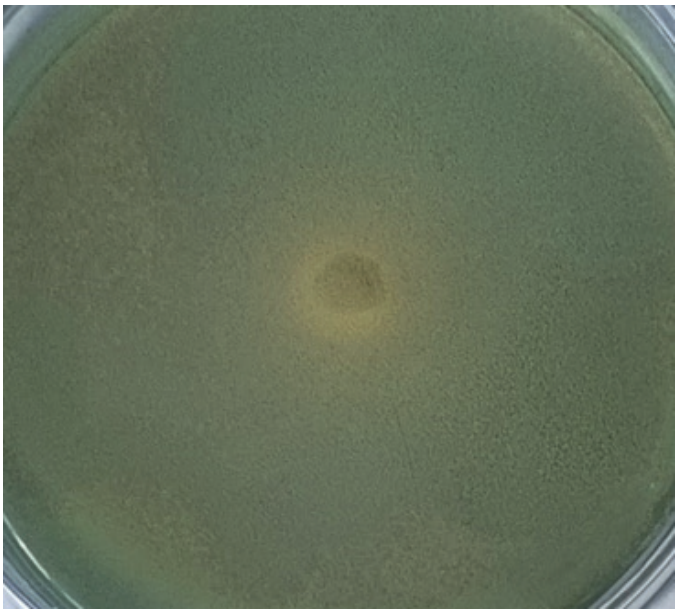

Figure 5. Siderophore production by *Pseudomonas putida* 1046.

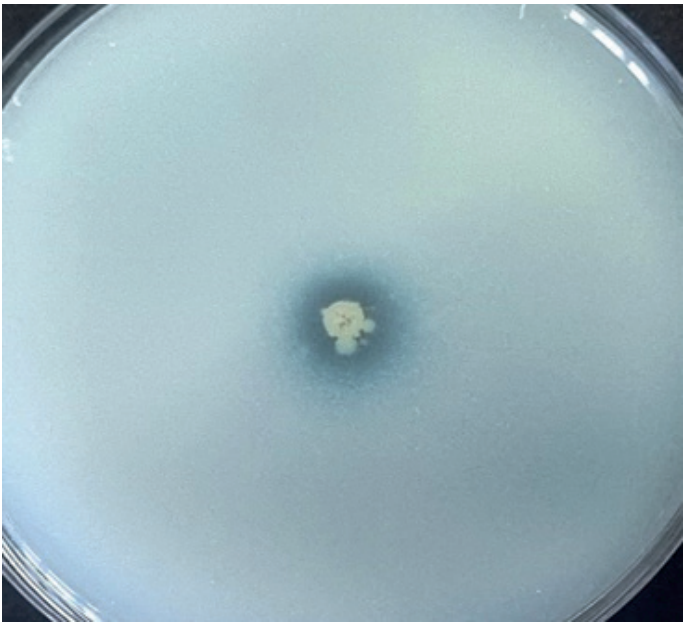

Figure 6. Phosphate solubilization activity of *Pseudomonas putida* 1046.

Freitas and Germida 1992), sunflower (Shaukat et al. 2006), and potato (Frommel et al. 1993), where significant enhancement of the seeds' emergence was registered – up to 100% of untreated control. It can be speculated that these findings are due to

the activity of hydrolytic enzymes that assure intensive substrate assimilation (e.g., α-amylases for the starch) and promote early germination. The enhanced hormones biosynthesis might trigger the activity of these hydrolytic enzymes. In addition, hormone dependency seems to increase the vigour index due to promoted synthesis of indoleacetic acid, the most abundant representative of auxins family of phytohormones. Its role in the root initiation and elongation and in differentiation and proliferation of plant tissues has been well documented (Meliani et al. 2017). Our results with soybean additionally enlarged the spectrum of the crop cultures subjectable to the PGP effect of *Pseudomonas putida*.

The physiological status of the culture is a parameter correlating with its biosynthetic capacity. The metabolically active 16 h culture expressed high values for the three tested indices and strong correlation coefficients of the bacterial and model plant data sets resulting in a better germination capacity. *Pseudomonas putida* strains and plants are in commensal relationships. The root exudates of the plant feed the bacteria, and the bacteria stimulate plant growth through the production of hormone precursors and antibiotics that suppress the pathogens' growth. It also supports nutrients immobilization (Molina et al. 2019). The data comparison for the 16 h and 48 h cultures indicated that the former executed its stimulatory function efficiently enough to result in high values for the all tested indices. The values for the length of the radicle and the coleoptile for the 0.2% suspension of the 48 h culture were slightly higher compared to those for the 16 h one (22.87% *vs.* 28.33%; and 35.96% *vs.* 45.96%). However, the shorter cultivation period (16 *vs.* 48 h) for the bacterial strain can compensate the difference of 5.5% to 10% since this is a defined technological advancement. Furthermore, the third index (the number of the lateral roots) is better presented in the 16 h compared to the 48 h bacterial culture (16.67% *vs.* 5.41). In fact, the formation of lateral roots contributes to the increase of the root surface reflecting the nutrients' assimilation.

Conclusions

The results demonstrate that the *Pseudomonas putida* 1046 strain possesses defined plant growth-promoting capacity. It enhances the process of corn germination due to the increased number of lateral roots and enlarged length of the coleoptile and the root. The optimal treatment parameters of the corn seeds, considering both the physiological and economic aspects of the process, comprise the application of 0.2% suspension of 16 h grown *Pseudomonas putida* 1046 strain for five days.

The positive germination capacity effect exerted by *Pseudomonas putida* 1046 strain contributes to improve our knowledge of the variety of approaches and mechanisms that are implicated in the plant growth promotion by these bacteria. However, greater understanding of the interaction between bacteria and host plants requires further study. From an economic point of view, in light of the possible application of *Pseudomonas putida* 1046 strain as a bioinoculant, the use of less concentrated suspension is more feasible and cost-effective.

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