Journal of Plant Protection Research eISSN 1899-007X

ORIGINAL ARTICLE

Plant growth promotion of crops using phosphate solubilizing bacterial strains derived from insects

Weronika Zenelt[1], Agata Pruciak-Nowak[2], Krzysztof Krawczyk[3]*

[1] Plant Disease Clinic and Bank of Plant Pathogen, Institute of Plant Protection – National Research Institute, Poznań, Poland

[2] Research Centre of Quarantine, Invasive and Genetically Modified Organisms, Institute of Plant Protection – National Research Institute, Poznań, Poland

[3] Virology and Bacteriology Department, Institute of Plant Protection – National Research Institute, Poznań, Poland

Vol. 64, No. 3: 321–335, 2024

DOI: 10.24425/jppr.2024.151259

Received: March 13, 2024
Accepted: June 03, 2024
Online publication: September 03, 2024

*Corresponding address:
k.krawczyk@iorpib.poznan.pl

Responsible Editor:
Agnieszka Synowiec

Abstract

The aim of this study was to investigate insect derived bacteria for the ability to dissolve insoluble soil phosphate to release soluble phosphorus compounds, available to plants. Bacterial isolates were obtained from *Diabrotica virgifera*, *Hermetia illucens*, *Oulema melanopus*, and *Ostrinia nubilalis*. An *in vitro* evaluation of phosphate solubilization ability on Pikovskaya's medium was done and the phosphate solubilizing index (PSI) was calculated for each isolate. Bacteria were tested in a greenhouse experiment on seeds of oats, wheat, triticale, barley and soybeans. After incubation, the weight and length of their aerial plant parts were measured. The highest increase in the weight of aerial parts was recorded for oats after using strain Om046 for inoculation (88.98%), then, wheat (Dv097, 31.43%), soybean (strain 96, 53.79%), and triticale (bacterial consortium, 36.9%). Bacteria used were identified as *Lactococcus lactis* (strains Om030 and Om046), *Acinetobacter* sp. (Dv123), *Lactococcus garvieae* (Dv097) and *Rothia kristinae* (strains 90 and 96). We showed that a successful application of insect derived bacteria for phosphate solubilization in soil, to promote plant growth, is possible. Innovative agriculture requires constant improvements in increasing crop growth. Thus, new sources of bacterial strains effectively promoting plant growth, are needed. We described a new source of plant growth-promoting bacteria that can be used in agriculture.

Keywords: beneficial bacteria, insect derived bacteria, phosphorus solubilization

Introduction

Phosphorus, along with nitrogen, is the most important nutrient for plants and is essential for plant growth (Rodriguez *et al.* 1999; Alam *et al.* 2002; Chen *et al.* 2006; Hameeda *et al.* 2008; Lambers 2022). It influences a variety of key metabolic processes in plants, including cell division and development, energy transport, signal transmission, and macromolecular biosynthesis. It is also essential for photosynthesis, respiration (Khan *et al.* 2014), formation of flowers, seeds, stalks, and stem strength (Khan *et al.* 2009). Phosphorus mitigates cold stress and strengthens the disease resistance of some plants. A phosphorus deficiency significantly decreases a plant's viability, making it more susceptible to unfavorable conditions (Karpagam and Nagalakshmi 2014). Phosphorus is crucial for plant growth and productivity, as it is involved in various physiological, biochemical, and metabolomic processes such as photosynthesis, respiration, energy generation, and nucleic acid synthesis (Chen *et al.* 2023). It plays a crucial role in plant growth by facilitating energy transfer, photosynthesis, nutrient movement, and genetic transfer (Baroowa *et al.* 2022). As a macronutrient, phosphorus is essential for plant growth and plays a crucial role in improving the productive performance

of crops (Silva *et al.* 2023). Its crucial role exhibits itself in promoting the growth of plant roots, by aiding in the utilization of sugar and starch, supporting photosynthesis, and facilitating nucleus formation and cell division (Gurmu 2023). Understanding phosphorus uptake and utilization by plants is crucial for determining final crop yield. A holistic understanding of phosphorus dynamics from soil to plant is necessary for optimizing phosphorus management and improving phosphorus-use efficiency. This includes reducing the consumption of chemical phosphorus fertilizer, maximizing the exploitation of the biological potential of root/rhizosphere processes for efficient mobilization and acquisition of soil phosphorus by plants, as well as recycling phosphorus from manure and waste (Shen *et al.* 2011)

Despite its crucial role, phosphorus availability is a major limiting factor in agriculture. To provide plants with an optimal amount of phosphorus, mineral fertilizers are commonly used. However, it is estimated that only about 20% of the phosphorus fertilizer used for plant cultivation is accessible to plants (Gupta *et al.* 2014; Roberts and Johnston 2015; Bautista-Cruz *et al.* 2019). This inefficiency necessitates finding environmentally friendly and economically beneficial alternatives.

The problem of phosphate solubility is complex. Both inorganic and organic forms of phosphorus are assimilable by plants however, the organic form is much less assimilable. Furthermore, if linked to humic acids (humo-phosphates), phosphorus is much more assimilable, while in the soil it tends to bind with Ca, Fe, Mg and other elements, becoming insoluble.

To provide plants with phosphorus, in an amount optimal for their growth, mineral fertilizers are commonly used. However, it is estimated that only about 20% of the phosphorus fertilizer used for plant cultivation is accessible to plants (Gupta *et al.* 2014; Roberts and Johnston 2015; Bautista-Cruz *et al.* 2019). Moreover, phosphorus occurs in soil in both organic and inorganic forms, of which the organic form is much less assimilable by plants and constitutes the majority of phosphorus reservoirs (Zaidi *et al.* 2009). As a result, phosphorus deficiency in plants is a frequent phenomenon (Karpagam and Nagalakshmi 2014). Therefore environmentally friendly and economically beneficial alternatives for synthetic phosphorus are desired.

Organic phosphorus is derived from the breakdown of plant and animal residues. It is then converted into inorganic forms of phosphorus, which are easily assimilable by plants. A significant portion of organic phosphorus is mineralized in soil due to rhizosphere-associated bacteria that use a group of enzymes responsible for phosphorus hydrolysis – phosphatases (Rodríguez and Fraga 1999; Gupta *et al.* 2014). They catalyze the process of transforming organic phosphorus into inorganic forms (HPO_{4-} and H_2PO_{4-}), which are absorbed through the roots (He *et al.* 2004; Hussain *et al.* 2013).

Different phosphatase activities related to phosphate solubilizing bacteria (PSB) have been observed in various types of soil (Tarafdar and Jungk 1987; Ponmurugan and Gopi 2006; Jorquera *et al.* 2008; Teng *et al.* 2019; Bautista-Cruz *et al.* 2019). Phosphatase activity is higher in the rhizosphere, and the main source of this activity is of microbial origin (Rodríguez and Fraga 1999; Gupta *et al.* 2014). The highest abundance of all soil bacteria, including PSB, is found in the rhizosphere, the sphere adherent to plant roots, where biochemical and physicochemical conditions are determined by the plant. Chemical substances secreted by plant roots, mainly sugars and organic acids, determine the selection of bacterial species utilizing the root exudates as their carbon source (Oteino *et al.* 2015). The concentration of bacteria in the rhizosphere is up to a thousand times higher than in the soil not affected by plant root exudations (Lugtenberg and Kamilova 2009). Also, the activity of rhizosphere bacteria is higher than in other parts of the soil (Jorquera *et al.* 2008). Plant growth promoting bacteria, including PSB, colonize plant organs. Moreover, roots, stems, leaves, flowers, fruits or seeds are characterized by distinct bacterial communities, which may be the result of chemical preferences or the ability of bacterial strains to colonize different parts of plants (Compant *et al.* 2010). To facilitate plant growth, bacteria must effectively inhabit various plant-related environments such as the rhizosphere, epidermis, and internal tissues, engaging with the host plant through biochemical and physiological traits like motility, attachment, degradation of plant polymers, and evasion of plant defenses (Afzal *et al.* 2019). Key activities like motility and polysaccharide production are vital for the successful colonization of the rhizosphere by endophytic bacteria such as *Alcaligenes faecalis* and *Azospirillum brasilense* (Santoyo *et al.* 2016). Additionally, functions like dissolving inorganic phosphates and synthesizing protease, lipase, and chitinase enzymes support rhizosphere colonization (Krawczyk *et al.* 2016). Phosphate dissolution, for example, is a trait that enhances tomato plant growth, as demonstrated by the utilization of phosphate-dissolving bacteria like *Paenibacillus polymyxa* and *Bacillus megaterium* var. phosphaticum, which yielded results comparable to those of costly fertilizers(Abou El-Yazeid and Abou-Aly 2011).

Plant growth-promoting bacteria (PGPB) are primarily isolated from the rhizosphere, yet they also exist in other environments indirectly linked to plants, such as the rhizosphere or plant surface, where they can be transmitted between plants by insects (Przemieniecki *et al.* 2020) or in the insect's body (Krawczyk *et al.* 2022). Relationships between bacteria and insects

encompass both commensalism and mutualism (Araujo 2004), with the insect gut providing an optimal setting for bacterial conjugation and gene transfer (Dillon and Dillon 2004). Various insect gut bacteria are closely associated with the rhizosphere, phyllosphere, and soil bacteria (Indiragandhi *et al.* 2007), and certain insect gut bacteria, like those isolated from *Plutella xylostella* larvae, exhibit plant growth-promoting characteristics such as nitrogen fixation and the production of salicylic and indole-3-acetic acids (Indiragandhi *et al.* 2008). The intestines of insects could serve as a potential habitat for the discovery of plant growth-promoting (PGP) bacteria, which have the potential to enhance plant growth and mineral uptake. For instance, bacteria were extracted from the intestines of *Plutella xylostella* larvae, yielding three strains: *Acinetobacter* sp., *Pseudomonas* sp., and *Serratia* sp. These bacteria exhibited responses to phosphorus and zinc depletion and were found to influence various solubilization reactions, such as chelation or the conversion of insoluble to soluble phosphorus, which could impact soybean growth in phosphorus-deficient soil (Indiragandhi *et al.* 2008).

Understanding the interactions between insect and plant microbiota is emerging, with implications for modifying plant defenses against foliar insects, shaping plant-associated microbial communities, and influencing multi-trophic interactions (Gadhave and Holloway 2015). The role of rhizobacteria in controlling pest insects in agriculture has also been delineated (Erica 2015). The use of certain strains of PSB may contribute to the reduction of the amount of mineral phosphorous fertilizers used in crops. By facilitating plants with phosphorus uptake, the yield of crops such as oats, barley, soybeans, mustard, or corn can increase up to 20%. Studies on the influence of PSB on cereals and other economically important plants have shown that these microorganisms are capable of improving plant condition (Egamberdiyeva *et al.* 1996; Afzal and Bano 2008; Hameeda *et al.* 2008; Hussain *et al.* 2013; Schoebitz *et al.* 2013; Bakhshandeh *et al.* 2015; Suleman *et al.* 2018; You *et al.* 2020; Mei *et al.* 2021; Song *et al.* 2022a, b; Ait-Ouakrim *et al.* 2023a, b). PSB are able to solubilize phosphorus from its insoluble forms (Chen *et al.* 2006; Zaidi *et al.* 2009; Sharma *et al.* 2011; Kirui *et al.* 2022). The most frequently described bacterial taxa exhibiting the phosphate solubilization ability are representatives of the following genera: *Achromobacter, Agrobacterium, Arthrobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Micrococcus*, *Pantoea*, *Pseudomonas*, *Ralstonia*, *Rhizobium*, *Rhodococcus* and *Serratia* (Rodríguez and Fraga 1999; Chen *et al.* 2006; Afzal and Bano 2008; Hameeda *et al.* 2008; Karpagam and Nagalakshmi 2014; Oteino *et al.* 2015; Bautista-Cruz *et al.* 2019). Bacteria solubilize phosphorus by releasing low molecular weight organic acids, where the hydroxyl and carboxyl groups chelate the cations attached to the phosphate, transforming it into soluble forms (Bhattacharya 2019; Vazquez *et al.* 2000; Alam *et al.* 2002).

Considering the arguments above, plant growth-promoting bacteria, including PSB, can serve as an alternative to mineral fertilizers, which are generally harmful to the environment and are overused (Savci 2012). PSB are considered promising bio-fertilizers that can increase the availability of phosphorus to plants (Afzal and Bano 2008; Zaidi *et al.* 2009; Tang *et al.* 2018). Therefore, new sources of PSB are desired. We turned our attention to insects, an integral part of plant-bacteria-insect relationships, which are extensively studied (Maheshwari 2013; Krawczyk *et al.* 2021; Wielkopolan *et al.* 2021). Insects constitute the most diverse and numerous group of animals inhabiting a wide variety of ecological niches. Their vast diversity enables them, e.g., to metabolize nutrients from various sources. It is known that insect intestines harbor large numbers of bacteria, which contribute to the remarkable adaptability of insects supported by their associated microorganisms (Feldhaar 2011). PGP bacteria have been successfully isolated from cornworm (*Diabrotica virgifera*) (Prischmann *et al.* 2008), moth (*Spodoptera litura*) (Rajashekhar and Kalia 2017), or termite (*Microcerotermes diversus*) (Pourramezan *et al.* 2012). Keeping in mind the above arguments we concluded that it is highly probable that multi-host insect pests of plants like *D. virgifera*, *O. melanopus* and *O. nubilalis* harbor PSB bacteria, especially since the diet of insects often does not provide them with enough nutrients. However, the presence of symbiotic microorganisms can replenish these deficiencies (Feldhaar 2011). Similarly, *H. illucens*, as an insect which breeds mainly on plant debris might also contain phosphate solubilizing bacteria, like other tested insects whose diet is based mainly on plants which do not always meet nutrient needs. Thus, they must have their own mechanism to metabolize the organic phosphorous compounds, which might be of bacterial origin. To assess the potential of bacterial strains for phosphate metabolism we used Pikovskaya medium to determine the ability of bacteria to solubilize phosphates (Sanchez-Gonzalez *et al.* 2023) and we calculated the PSI to assess the potency of phosphate-solubilizing bacteria (Ait-Ouakrim *et al.* 2023a).

Given the limitations of synthetic fertilizers and the potential of PSB, new sources of these beneficial bacteria are desired. Insects, with their diverse microbiota, present a promising yet underexplored source of PSB. Insect gut bacteria have shown plant growth-promoting traits, such as nitrogen fixation and phosphate solubilization (Indiragandhi *et al.* 2008). This

study aimed to explore the potential of insect-derived bacteria as a new source of PSB and their implications for agriculture.

Considering the above, insect derived bacteria may potentially be new sources of PSB. The objectives of this study were:

(1) **To test a wide range of bacteria isolated from the following insects**: the western corn rootworm (*Diabrotica virgifera* family Chrysomelidae), cereal leaf beetle (*Oulema melanopus* family Chrysomelidae), European corn borer (*Ostrinia nubilalis* family Pyralidae) and black soldier fly (*Hermetia illucens* family Stratiomyidae), for the ability to solubilize phosphorus on Pikovskaya medium and to determine their PSI;

(2) **To demonstrate the plant growth-promoting effect of insect-derived PSB bacteria on economically important plants** – including oat (*Avena sativa* L.), wheat (*Triticum aestivum* L.), triticale (×*Triticosecale*), soybean (*Glycine max* L.) and barley (*Hordeum vulgare* L.) under greenhouse conditions;

(3) **To assess the phosphatase activity of rhizosphere soil inoculated with the tested PSB**, to verify if the plant growth promoting effect is a consequence of the PSB activity.

By addressing these objectives, this study aimed to highlight the potential of insect-derived PSB as a sustainable alternative to chemical fertilizers, contributing to enhanced agricultural productivity and environmental sustainability. To our knowledge this is the first report of the application of insect-derived PSB to enhance growth of crops.

Materials and Methods

Insect specimens

The insect samples used in the experiment were specimens of the western corn rootworm (*Diabrotica virgifera* family Chrysomelidae), cereal leaf beetle (*Oulema melanopus* family Chrysomelidae), European corn borer (*Ostrinia nubilalis* family Pyralidae) and black solider fly (*Hermetia illucens* family Stratiomyidae). Imago specimens of *D. virgifera*, *O. melanopus*, and *O. nubilalis* were captured in corn fields in south-eastern Poland. Larval specimens of *H. illucens* were sourced from in-house cultivation and fed on corn debris. All insect specimens were supplied by the Department of Entomology and Animal Agrophages of the Institute of Plant Protection – National Research Institute, Poznań, Poland. Each of the insect samples consisted of five individuals of a given species. In the case of *D. virgifera*, *O. melanopus* and *O. nubilalis* the imago were used, while for *H. illucens*, the larval stage was used, as this stage is foraging.

Bacterial strains

Bacterial isolates were obtained from the imago or the larvae of tested insects. Insect individuals, regardless of the developmental stage, were euthanized and subjected to surface sterilization by placing them in 70% ethanol (3 min), followed by triple washing with sterile distilled water (SDW) to remove the ethanol residues. To confirm the effectiveness of the sterilization process, a 100 µl aliquot from the final wash was plated on tryptic soy agar medium (TSA, Sigma Aldrich Co. Ltd.) and incubated to assess bacterial growth. Next, 20 insect individuals per sample were placed in a mortar and ground with the addition of 5 ml of sterile physiological saline (0.9% NaCl). The resulting homogenous suspension was diluted in a decimal series and the aliquots of 100 µl were taken from the dilutions of 10^{-4} to 10^{-7} cfu ml^{-1}, plated on tryptic soy agar medium (TSA, Sigma Aldrich Co. ltd.) spread with a glass rod, and incubated for 72 hours on TSA medium at 27°C. After incubation, the grown colonies were submitted to a series of purification streaks until a pure culture of each grown morphotype was obtained. The purity of each tested colony was verified on the basis of colony morphology using Gram staining under a light microscope.

Assessing bacterial ability to solubilize phosphates and solubilizing index

The ability of bacteria to solubilize phosphorus from its insoluble forms was tested using Pikovskaya medium (glucose 10 g, yeast extract 0.5 g, ammonium sulphate $(NH_4)_2SO_4$ 0.5 g, magnesium sulphate $MgSO_4 \times 7H_2O$ 0.1 g, calcium phosphate $Ca_3(PO_4)_2$ 5 g, sodium chloride NaCl 0.2 g, potassium chloride KCl 0.2 g, manganese sulphate $MnSO_4 \times H_2O$ 0.002 g, iron sulphate $FeSO_4 \times 7H_2O$ 0.002 g, 15 g agar; pH 7.0) (Sharma *et al.* 2011). A 24-hour, pure culture of each insect derived isolate was spot inoculated on Pikovskaya medium in eight replicates. After 7 days of incubation at 27°C, the appearance of a halo zone around the grown bacterial colony was recorded as a positive result. The size of the halo zones was measured, along with the diameter of bacterial colony. Based on the obtained results, for each isolate, the value of the PSI was calculated using the formula presented below, where z is a diameter of the halo, c is a diameter of the bacterial colony (Alam *et al.* 2002). The average of eight repetitions was calculated:

$$PSI = \frac{c + z}{c}.$$

Twenty isolates with the highest PSI value were selected for further tests including growth rate and the ability to maintain phosphate solubilization after glycerol preservation at –80°C, as indicators of potential bacterial usefulness in practice. The growth rate of 15 chosen isolates was measured after 24 h of incubation on TSA medium at 25°C, using visual assessment with a three grade scale (good, average, poor) in comparison to the fast-growing *Pantoea agglomerans* strain, commonly occurring in soil, water and plants. The ability of phosphate solubilization after glycerol preservation at –80°C was measured the same way with PVK medium, as described previously. Based on these results the isolates were chosen for the greenhouse experiment.

Assessment of the influence of phosphate solubilizing bacteria on plant growth

Plant inoculation

The greenhouse experiment was designed to assess the influence of the six selected bacterial isolates on the growth of the following plants: wheat (*Triticum aestivum* L.), triticale (×*Triticosecale*), oats (*Avena sativa* L.), barley (*Hordeum vulgare* L.) and soy (*Glycine max* L.). The soil used in the research came from agricultural wasteland, belonging to a fawn soil, constituting 80% of the soil in Poland (Ochric humus level. Typical sequence of genetic levels in the profile: O-A-Et-Bt-C) (Kabała *et al.* 2019).

Wheat, triticale, oats, barley and soy seeds were surface sterilized by soaking in 10% sodium hypochlorite solution for 10 minutes. Then the seeds were rinsed three times in sterile distilled water. Surface sterilized seeds were placed in suspensions of 24-hour bacterial cultures, for each isolate individually and for the consortium comprising the six selected isolates. The concentration of each suspension was adjusted to 10^7 cfu ml^{-1}. After soaking in bacterial suspension, the seeds were allowed to dry. Five dried seeds were sown per pot containing agricultural wasteland soil.

Greenhouse experimental design

Isolates Om030, Om046 and Dv097 were tested on oats, wheat and triticale plants, isolates 90, 96 and Dv123 were tested on soybean and barley plants. Three treatments were analyzed: each isolate separately, consortium of all tested isolates and control (seeds treated with sterile distilled water). For measurements, 10 plants were taken randomly from each combination. Pots were placed in a greenhouse cabin (conditions: temp. 20–23°C day, 16–18°C night, humidity approx. 60–65%, lighting 16/8). No fertilizers were applied during plant development. Three replications (3 pots with 5 plants each; pot diameter – 25.5 cm, height – 20 cm, base width – 19.8 cm, capacity 7.5 l) were used for each variable tested: single bacterial isolate, consortium and control (seeds placed in sterile distilled water, without the addition of bacteria). After the plants had grown (oats BBCH-scale 22, wheat BBCH-scale 22, triticale BBCH-scale 61, barley BBCH-scale 71, soy BBCH-scale 70), growth (leaf length) was measured and the above-ground parts were weighed. The root length was not measured since in cereals it has no economic value.

Bacterial phosphatase activity in the rhizosphere soil

One gram of rhizosphere soil was taken from each pot and placed in falcon tubes, 5 cm^3 of Modified Universal Buffer (MUB) together with p-nitrophenylphosphate were added, and then incubated for 1 hour at 37°C. After incubation, the reaction was stopped by adding 1 cm^3 of 0.5 M calcium chloride ($CaCl_2$) and 4 cm^3 of 0.5 M NaOH (Bielinska 2005; Tabatabai and Bremner 1969). Two milliliters of the supernatant of each sample were centrifuged (10 000 g × 2 min.). Next, 100 µl of supernatant was measured with a spectrophotometer at 400 nm. Measurements were made in triplicate, and results were given as an arithmetic mean of three repetitions (Table S1).

Determination of phosphatase activity

Phosphatase activity, expressed as mg of released p-nitrophenol per 1 kg of soil for 1 hour, was calculated based on the standard curve, expressing the relationship between a measurement reading and the corresponding measured quantity value. The standard curve was made by preparing dilutions of the solution containing, respectively: 0, 1, 2, 3, 4 and 5 mg of p-nitrophenol in 5 cm^3 of water, and then proceeded as with soil samples after incubation (Table S2, Fig. S1).

Statistical analysis

The variables in each group were described by the arithmetic mean ± standard deviation. The normality of the distribution was tested with the Shapiro-Wilk test. The groups were compared using ANOVA for independent variables and Dunnett's multiple comparison test. Data distribution significantly different from the normal distribution were compared using the Kruskal-Wallis test and Dunn's multiple comparison test. The significance level was 0.05. The calculations were carried out in the Statistica 12.0 program.

Bacterial identification

The six isolates with the highest values of the PSI were subjected to biochemical and molecular identification.

The biochemical identification involved the use of the BIOLOG® Gen III System (database v. 2.8) (BIOLOG®, Hayward, USA) according to the manufacturer's instructions. Molecular identification was based on the analysis of partial sequences of the 16S rRNA gene. The bacterial isolates were grown on tryptic soy agar (TSA) medium (Sigma Aldrich, Saint Louis, Missouri, USA) for 24 hours. After incubation single colonies were picked and resuspended in 100 µl of sterile distilled water. The bacterial solution was incubated at 96°C for 10 minutes, then cooled and centrifuged (10 000 g × 10 min.). One microliter of the supernatant was used as the template in PCR reaction preformed using GoTaq® Green Master Mix (Sigma Aldrich, Saint Louis, Missouri, USA) and a pair of primers 16SA1 (5′-AGAGTTTGATCMTGGCTCAG-3′) and 16SB1 (5′-TACGGYTACCTTGTTACGACTT-3′). The expected product size was ~1500bp. (Kikuchi *et al.* 2002; Matsuura *et al.* 2014) (Table 2). The PCR reaction profile was as follows: 95°C – 2 min., 32× (94°C – 45 sec., 62°C – 45 sec., 72°C – 1 min. 30 sec.), 72°C – 5 min. Obtained nucleotide sequences were aligned using BioEdit software (v7.2) to obtain consensus sequences. Each consensus sequence was made from two sequencing reads. Next, the consensus sequences were identified using BLAST (https://blast.ncbi.nlm.nih.gov), with the flagged option "Sequences from type material", to retrieve the match with type strains only, for a more rigorous taxonomic assignment. In addition, the sequences were checked using RDP Classifier (http://rdp.cme.msu.edu/classifier/classifier.jsp), which assigns the taxonomy through the naive Bayesian approach (Wang *et al.* 2007). The analyzed sequences were deposited in the GenBank database (https://www.ncbi.nlm.nih.gov).

Results

Bacterial strains identification

Results of identification are given in Table 1. In the case of discrepancies of the molecular and biochemical identifications, the final identification was based on the molecular data, due to a significantly larger database of NCBI than the BIOLOG Gen III system. The identification of strains obtained with using RDP Classifier (http://rdp.cme.msu.edu/classifier/classifier.jsp), which assigns the taxonomy through the naive Bayesian approach, gave the following results with the confidence threshold of 95%: Strain Dv123 – *Acinetobacter* sp., strain Dv097 – *Lactococcus* sp., strain Om030 – *Lactococcus* sp., strain Om046 – *Lactococcus* sp., strain 90 – *Rothia* sp., strain 96 – *Rothia* sp.

The solubilization of phosphorus on Pikovskaya medium

Out of the 171 tested strains, only 20 were not able to solubilize phosphates on Pikovskaya medium (Fig. 1). The values of PSI are presented in a supplement (Table S1).

For the greenhouse experiments the bacterial isolate with the highest PSI value, fast growing and well-enduring the glycerol preservation at –80°C, were selected and used (Table 2).

Table 1. Bacterial strains identification

				NCBI GenBank reference			BIOLOG® results
Isolate	isolate`s biological source	identification based on 16S rRNA sequence (~1.5 kbp)	accession number assigned by GenBank	query covery	Percen-tage of identity	accession	identification
Dv123	*Diabrotica virgifera*	*Acinetobacter guillouiae*	OP891014	100%	99.44%	NR_117626.1	*Acinetobacter* sp.
Dv097	*Diabrotica virgifera*	*Lactococcus garvieae*	OM536191	90%	90.73%	LC145570.1	*Lactococcus garvieae*
Om030	*Oulema melanopus*	*Lactococcus lactis*	OM538405	100%	97.14%	CP065737	*Lactococcus* sp.
Om046	*Oulema melanopus*	*Lactococcus lactis*	OM570559	99%	98.31%	NR_113960.1	*Enterococcus casseliflavus*
90	*Oulema melanopus*	*Rothia kristinae*	OM491326	98%	95.33%	CP065738.1	*Rothia* sp.
96	*Oulema melanopus*	*Rothia kristinae*	OM491517	98%	99.30%	CP065738.1	*Rothia* sp.

Table 2. Isolates' growth rate measured after 24 h of incubation (TSA medium. 25°C) based on visual assessment with 3 grade scale in comparison to fast growing *Pantoea agglomerans* strain; the ability of phosphate solubilization after glycerol preservation in –80°C. Selected isolates used in greenhouse experiments are bolded

No.	Isolate	PSI value	PSI value after glycerol preservation in –80°C	Growth rate
1	Dv123	5	2.77	3*
2	Om001	5	3.29	1
3	Om031	4.75	4.2	2
4	Om030	4.66	4.5	3
5	Om046	4.66	4.3	3
6	Om038	4.44	2.54	2
7	Dv097	4.33	4.04	3
8	Om015	4.33	2.42	2
9	Om029	4.33	3.07	2
10	Dv012	4.12	3.1	2
11	Dv125	4.12	3.6	2
12	Om009	4	2.74	3
13	Om013	4	2.52	3
14	Dv117	3.9	2.5	2
15	Dv135a	3.88	2.42	2
16	Om035	3.88	2.0	2
17	Dv058	3.87	3.1	2
18	90	3.8	3.8	3
19	96	3.8	3.8	3
20	Om045	3.77	2.81	2

*visual assessment with 3 grade scale. where 3 – good. 2 – average. 1 – poor

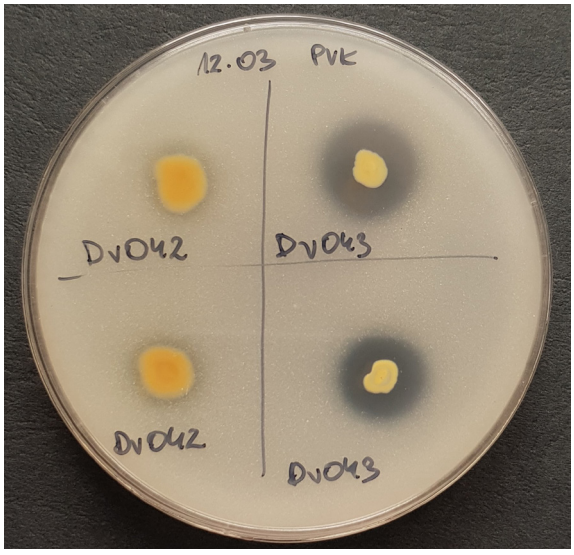

Fig. 1. Example of phosphate solubilization on Pikovskaya`s medium. On the right: halo around bacterial colony of the Dv043 isolate, able to solubilize phosphorus. On the left: isolate Dv042 with no halo and with no ability to solubilize phosphorus

Influence of phosphate solubilizing bacteria (PSB) on plant growth

Plant height

There was no statistically significant difference in plant heights between individual cereals, the significance level was 0.05, *p*-value was less than the predetermined significance level. The height of individual bacteria-treated plants differed from plants not treated with bacteria. Compared to the control, the highest difference in plant growth was observed for soy plants (increase by 28.68%) after inoculation with bacterial consortium (isolates 90, 96 and Dv123) and after inoculation with isolate Dv123 (25.9%). For triticale, the highest difference was noted for the consortium (17.76%) (isolates Om030, Om046, Dv097) and for isolate Dv097 (11.54%). For oats, the highest differences were also recorded for bacterial consortium (Om030, Om046, Dv097) (increase by 7.58%) and for isolate Om030 (7.37%). For wheat, the highest differences were recorded for bacterial consortium (Om030, Om046, Dv097) (5.59%) and for Om046 (3.35%). For barley, the highest differences were recorded for isolate 90 (4.39%) (Table 3).

Weight of the aerial parts

For all tested plants, except for barley and soy, the difference in the weight of the aerial parts was statistically significant when inoculated with selected bacterial strains. Compared to the control group, the highest differences were observed for oats after inoculation with Om046 (increase by 88.98%) and Om030 (73.6%). For soy, the highest differences were recorded for isolate 96 (53.79%) and for bacterial consortium (includes 90, 96 and Dv123) (18.99%). For triticale, the highest differences were recorded for bacterial consortium (Om030, Om046, Dv097) (36.9%) and for isolate Om46 (16.34%). For wheat, the highest differences were recorded for isolate Dv097 (31.43%) and for bacterial consortium (Om030, Om046, Dv097) (27.66%). For barley, none of the isolates included in the bacterial consortium had positive effects on plants (Table 4).

Based on the conducted statistical analyses, there were no statistically significant differences in plant height for all individual cereals. Wheat, triticale and oats inoculated with individual isolates did not show higher growth than the control group. For each cereal, the weight of above-ground parts of plants differed statistically significantly between the analyzed groups. Comparing the weight of the aerial parts of wheat plants between individual isolates and the control group, we obtained significant differences for the Dv097 isolate and the consortium. The above-ground parts of the wheat plants in isolate Dv097 and the consortium were statistically significantly heavier than in

Table 3. Influence of phosphate solubilizing bacteria on plant growth and condition – stalk length. Averages of ten measurement repetitions are presented. The highest values are bolded

	Variable	Om030	Dv097	Om046	Consortium	Control	p-value* for all individual variants
Wheat	stalk length [cm]	49.8 ± 3.8	48.2 ± 2.9	50.8 ± 1.9	**51.9 ± 2.5**	49.2 ± 3.5	0.0746
	% increase compared with control	1.32%	–1.93%	3.35%	**5.59%**	–	–
Triticale	stalk length [cm]	41.6 ± 3.5	43 ± 4.4	41.1 ± 4.9	**45.4 ± 4.1**	38.7 ± 8.4	0.0783
	% increase compared with control	7.91%	11.54%	6.61%	**17.76%**	–	–
Oats	stalk length [cm]	52.4 ± 4.9	50.8 ± 5.9	50.8 ± 4.8	**52.5 ± 4.8**	48.8 ± 5.5	0.506
	% increase compared with control	7.37%	4.09%	4.09%	**7.58%**	–	–
	Variable	90	96	Dv123	Consortium	Control	*p*–value
Soy	stalk length [cm]	29.9 ± 1.98	34.55 ± 5.728	38.4 ± 5.515	**39.25 ± 8.839**	30.5 ± 0.707	0.1131
	% increase compared with control	–1.96%	13.27%	25.9%	**28.68%**	–	–
Barley	stalk length [cm]	**73.6 ± 0.566**	70.3 ± 1.697	68.05 ± 0.7	68.308 ± 14.869	70.05 ± 9.829	0.5035
	% increase compared with control	**4.39%**	0.35%	–2.85%	–2.49%	–	–

**p*-value – the significance level was 0.05. If the *p*-value is less than the predetermined significance level, the null hypothesis is rejected and it is concluded there are statistically significant differences between the groups. There is no statistically significant difference in plant heights among individual cereals

Table 4. Influence of phosphate solubilizing bacteria on over-ground plant parts weight. Averages of ten measurement repetitions are presented. The highest values are bolded

	Variable	Om030	Dv097	Om046	Consortium	Control	*p*-value* for all individual variants
Wheat	overground plant parts weight [g]	2 ± 0.2	**2.6 ± 0.4**	2.1 ± 0.4	**2.5 ± 0.4**	2 ± 0.4	0.001
	% increase compared with control	3.97%	**31.43%**	7.94%	**27.66%**	–	–
	p-value; comparison between isolate and control	0.9727	**0.0024**	0.7629	**0.0086**	–	–
Triticale	overground plant parts weight [g]	2.1 ± 0.3	1.9 ± 0.3	2.2 ± 0.3	**2.5 ± 0.4**	1.8 ± 0.2	0.0002
	% increase compared with control	11.09%	4.38%	16.34%	**36.9%**	–	–
	p-value; comparison between isolate and control	0.4072	0.9426	0.1189	**0.0001**	–	–

Table 4. Influence of phosphate solubilizing bacteria on over-ground plant parts weight. Averages of ten measurement repetitions are presented. The highest values are bolded – continuation

	Variable	Om030	Dv097	Om046	Consortium	Control	*p*-value* for all individual variants
Oats	overground plant parts weight [g]	2.3 ± 0.6	2.3 ± 0.4	**2.5 ± 0.6**	1.6 ± 0.4	1.3 ± 0.6	< 0.0001
	% increase compared with control	73.6%	70.43%	**88.98%**	19.75%	–	–
	p-value; comparison between isolate and control	0.0005	0.0009	**<0.0001**	0.6294	–	–
	Variable	90	96	Dv123	Consortium	Control	*p*–value
Soy	overground plant parts weight [g]	6.61 ± 0.57	**10.12 ± 0.74**	5.54 ± 0.8	8.56 ± 0.52	6.58 ± 0.77	0.8395
	% increase compared with control	0.45%	**53.79%**	–15.8%	18.99%	–	–
Barley	overground plant parts weight [g]	9.69 ± 0.23	8.52 ± 4.67	9.53 ± 2.24	9.73 ± 2.55	12.07 ± 0.31	0.3317
	% increase compared with control	–19.71%	–29.41%	–21.04%	–19.38%	–	–

**p*-value – the significance level was 0.05. If the *p*-value is less than the predetermined significance level, the null hypothesis is rejected and it is concluded there are statistically significant differences between the groups

the control group ($p = 0.0024$; $p = 0.0086$ respectively). Comparing the weight of the aerial parts of triticale plants between individual isolates and the control group, we obtained significant differences in the consortium. The above-ground parts of the triticale plants in the consortium were statistically significantly heavier than in the control group ($p = 0.0001$). Comparing the weight of the aerial parts of oat plants between individual isolates and the control group, we obtained significant differences in isolate Om030, Dv097 and Om046. The above-ground parts of oats in isolates Om030, Dv097 and Om046 were statistically significantly heavier than in the control group ($p = 0.0005$; $p = 0.0009$; $p < 0.0001$, respectively).

Phosphatase activity in rhizosphere soil

The phosphatase activity expressed in mg of released p-nitrophenol per 1 kg of soil for 1 hour and determined on the basis of the standard curve is presented in Table 5. The standard curve is available in the Supplement (Fig. S1).

Regarding the phosphatase activity in rhizosphere soil, in oats, the consortium treatment resulted in a maximum increase in phosphatase activity, which was 165% higher than in the uninoculated control. In wheat, inoculation with the Dv097 strain resulted in a maximum increase of phosphatase activity of 103% higher than the control. The effect of inoculation with the Om046 isolate in triticale plants caused the highest increase in the activity of rhizosphere phosphatase by 114% compared to the control. In soybean, the maximum increase in activity was obtained when inoculated with the bacterial consortium compared to the control by 36%, while the maximum increase of over 275% was observed in barley after inoculation with strain 90 (Table 5).

Discussion

Plant growth promoting bacteria (PGPB) have been shown to serve as biofertilizers in sustainable agriculture (Magnusson *et al.* 2003; Pandey *et al.* 2006; Kim and Indiragandhi 2008; Hussain *et al.* 2013;). Their use has become a tangible alternative for chemical fertilizers. The vast majority of PGPB have been isolated from phyllosphere, rhizosphere, cattle manure, and soil (Kim and Indiragandhi 2008). Amongst the PGPB, phosphate solubilizing bacteria are one of the

Table 5. Spectrophotometric reading (400 nm) – measurement of phosphatase activity in rhizosphere soil inoculated with phosphate solubilizing bacteria (PSB) isolates and approximate value of released p-nitrophenol. The highest values are bolded

Isolate	Oats			Wheat			Triticale		
	average of measurements (DTX reading)	released p-nitrophenol approximate value [µg]	% increase compared with control	average of measurements (DTX reading)	released p-nitrophenol approximate value [µg]	% increase compared with control	average of measurements (DTX reading)	released p-nitrophenol approximate value [µg]	% increase compared with control
Dv097	0.53222	12.5	119%	**0.63404**	**15.05**	**103%**	0.56038	13.05	70.5%
Om030	0.55972	13.2	131.5%	0.39534	9.1	23%	0.5393	12.25	60%
Om046	0.43584	10.1	77%	0.40282	9.25	25%	**0.67486**	**16.35**	**114%**
Consortium	**0.637**	**15.1**	**165%**	0.43002	10.04	35.5%	0.4949	11.45	50%
Control	0.24728	5.7	–	0.33072	7.4	–	0.339	7.65	–

Isolate	Soy			Barley		
	average of measurements (DTX reading)	released p-nitrophenol approximate value [µg]	% increase compared with control	average of measurements (DTX reading)	released p-nitrophenol approximate value [µg]	% increase compared with control
90	1.416	35.5	-12.3%	**overflow**	**> 90**	**> 275%**
96	2.0517	52.5	29.5%	1.447	37.5	56%
Dv123	1.3	32.5	19.5%	1.103	27	12.5%
Consortium	**2.2126**	**55**	**36%**	1.582	41	71%
Control	1.546	40.5	–	1.001	24	–

most important groups due to the significant role of phosphorus in plant growth and development (Alam *et al.* 2002). Although phosphate solubilizing bacteria have been reported in many types of soil, various phyllospheres, and sometimes even in environmentally hostile ecological niches like sub-alpine locations in the Himalayas (Pandey *et al.* 2006), the need for new sources of plant growth promoting bacteria remains significant. That is why we directed our attention to microbial communities isolated from insects because of the well-documented associations between insects and plants. It is known that bacteria inhabiting insects' guts affect the fitness of their hosts. The insect gut provides suitable conditions for bacterial development and gene transfer, as demonstrated through genome analysis (Dillon and Dillon 2004). Bacteria-insect relationships are complex and involve both commensalism and mutualism (Araujo 2004). Therefore, we investigated insects' guts to determine if they contain phosphate solubilizing bacteria, especially since plant-feeding insects consume large amounts of plant tissue with already assimilated phosphates that needs to be dissimilated, potentially with the aid of bacteria.

In our study, out of the 171 strains we tested, only 20 were not able to solubilize phosphate at all, confirming the common occurrence of this feature among bacteria. We used bacterial strains isolated from representatives of four distinct insects: two representatives of the *Coleoptera* order, belonging to the same Chrysomelidae family – the Western corn rootworm *D. virgifera* and the cereal leaf beetle *O. melanopus*; a butterfly (*Lepidoptera*) belonging to the Pyralidae family – the European corn borer *O. nubilalis*; and black fly (*Diptera*) from Stratiomyidae family – *H. illucens*. We observed the phosphate-solubilizing effects of several bacterial genera isolated from insects. Our results are consistent with another study in which the phosphate-solubilizing effects of insect derived *Pseudomonas* sp. PRGB06, isolated from *Plutella xylostella* (*Lepidoptera*), were reported (Kim and Indiragandhi 2008). In our study, phosphate-solubilizing effects were observed for *Acinetobacter guillouiae, Lactococcus garvieae*, *L. lactis* and *Rothia kristinae* (Table 1). Tests conducted in the greenhouse demonstrated the effectiveness of bacteria isolated from insects and their ability to produce phosphatase, thereby increasing the availability of desired, plant-assimilable forms of phosphorus in the soil. There are reports of mycorrhizal and saprotrophic fungi and plant roots as sources of phosphatases in the soil (Tarafdar *et al.* 2001; Margalef *et al.* 2021). However, the only difference between the tested samples and the control samples was the presence of the tested bacterial strains. Therefore, the increased amounts of phosphatase in the tested rhizosphere soil are very likely to indicate the activity of the bacteria used in the experiment. Our research

showed that the overall growth and condition of plants were more influenced by a consortium consisting of several strains of bacteria rather than by single isolates of bacteria. The same result, which is the positive effect of inoculation with phosphate-solubilizing bacteria on plant growth, was also reported for maize plants by using soil-derived *Burkholderia* sp., *Bacillus* sp., *Pseudomonas* sp., and *Flavobacterium* sp. (Hussain *et al.* 2013), where a significant increase in maize height, root length, dry weight of shoots, dry weight of roots, and grain yield was observed in response to inoculation with selected rhizobacteria. This is congruent with our study, where using insect-derived bacteria, we were able to elicit the effect of plant growth promotion. Other reports on the positive effects of inoculation with PSB on plant growth were reported for multiple crops, e.g., for wheat (Sarker *et al.* 2014; Munir *et al.* 2019), cotton (Egamberdiyeva *et al.* 1996), strawberries (Güneş *et al.* 2009) or rice (Bakhshandeh *et al.* 2015). The PSB bacteria were also applied with arbuscular mycorrhizal fungi (Yousefi *et al.* 2011). However, those reports involved using bacteria of rhizosphere origin, not insect-derived bacteria, as in our study.

Plants are able to uptake only the inorganic forms of phosphorus. Importantly, a significant portion of organic phosphorus compounds is mineralized by rhizosphere-associated bacteria phosphatase enzymes (Rodríguez and Fraga 1999; Gupta *et al.* 2014). There are reports of different phosphatase activities observed in different types of soil (Ponmurugan and Gopi 2006; Jorquera *et al.* 2008; Tang *et al.* 2018; Bautista-Cruz *et al.* 2019). In all cases, the use of certain strains of PSB contributes to the reduction of the amount of mineral fertilizers used. For example, by facilitating plants with phosphorus uptake, the yield of crops, such as oats, barley, soybeans, mustard, or corn was increased by up to 20% (Gupta *et al.* 2014), which is consistent with our results obtained with insect-derived bacteria isolates.

It is interesting that we observed that all tested strains of *H. illucens* and *O. nubilalis* derived-bacteria showed low phosphate solubilizing activity (PSI < 3) (Table S1). None of them qualified to be used in the greenhouse test. In *H. illucens*, a possible explanation could be that the larvae of this insect breed primarily on various organic debris of both plant and animal origin. Such debris are often initially decomposed, which makes the presence of bacteria-donated phosphatase enzyme not essential for the insect to obtain an optimal nutrient level. However, this explanation fails in the case of *O. nubilalis* which feeds on crops, including maize, just like *D. virgifera* and *O. melanopus*, in which we reported the presence of phosphate-solubilizing bacteria. It is possible that *O. nubilalis* has its own endogenous phosphatase production and does not require bacteria-donated phosphatase.

We also recorded differences in the influence of phosphate solubilizing bacterial strains on above-ground plant parts weight (Table 3 and 4). The main factor that might have contributed to the varying efficacy of different bacterial strains across plant species is most probably the influence of plant root exudates. It is known that plant root exudates, such as long-chain fatty acids and amino acids, recruit beneficial bacteria like *Pseudomonas*, enhancing plant defense against foliar pathogens by promoting their growth and resistance capabilities (Wen *et al.* 2021). The exudates select beneficial soil microbes, aiding in plant-microbial communication. This phenomenon was documented under drought conditions which alters exudation, impacting microbial recruitment and ecosystem functioning, particularly in fast-growing plants (Williams and de Vries 2020). Furthermore, the plant root exudates contain specific organic acids like citric and fumaric acid, which attract and influence chemotaxis and biofilm formation of beneficial bacteria, aiding in their preferential colonization (Zhang *et al.* 2014). Various plant root exudates select beneficial bacteria by attracting them through specific metabolites, aiding in stress alleviation and nutrient uptake (Vives-Peris *et al.* 2020). Each plant individually selects and recruits bacteria and the performance of the tested bacteria itself might be preferential towards e.g., wheat, oat, barley or soy. Further studies are required on this subject to better understand insect-plant-bacteria relationships. We demonstrated that inoculation with phosphate-solubilizing bacteria had a positive effect on plant growth parameters such as shoots length and the weight of plants. This effect is universal, although its level for each plant is different, as it was observed for wheat, barley, triticale, soy and oats. Furthermore, the plants selected for inoculation – barley, oats, wheat, triticale and soybeans – are among the most economically important crops cultivated in Poland. For years, they have been of utmost importance in the sowing structure. Moreover, soybeans are gaining more and more popularity, and their cultivation in Poland is constantly expanding. This demonstrates that the plant growth-promoting features of bacteria, in this case phosphate-solubilization, are not species-specific or soil-type dependent. This makes the insect-derived bacteria a promising new source of plant growth-promoting bacteria.

In our study we confirmed the presence of the following bacteria: *Acinetobacter* sp., *Lactococcus lactis*, *L. garvieae*, and *Rothia kristinae* in the body of the tested insects. Moreover, we confirmed the ability of those bacteria to solubilize phosphate in the soil, making it more accessible to plants. Identifying those bacterial strains has a new significance and will be discussed more comprehensively.

Acinetobacter sp. has been identified as a beneficial plant growth-promoting bacterium in various studies.

Research has shown that *Acinetobacter* strain SuKIC24 exhibited significant phosphate solubilization and indole-3-acetic acid production, leading to enhanced growth in *Basilicum polystachyon* plants (Minuț *et al.* 2023). Additionally, the application of *Acinetobacter lwoffii* A07, a rhizosphere bacterium, resulted in increased plant biomass, root structure, nutrient content, and soil enzyme activity, promoting the growth of *Pinus sylvestris* var. *mongolica* seedlings and enhancing soil microbial communities (Song *et al.* 2022b). These findings highlight the potential of *Acinetobacter* species in promoting plant growth through various mechanisms, such as nutrient solubilization and enhancing soil health, making them valuable candidates for sustainable agricultural practices (Minuț *et al.* 2023).

Lactobacillus lactis, a type of lactic acid bacteria, has shown potential as a plant growth-promoting bacteria. Studies have highlighted the abilities of lactic acid bacteria, including *Lactobacillus* species, to promote plant growth through various mechanisms (Abhyankar *et al.* 2022; Panetto *et al.* 2023). These bacteria can produce plant growth hormones, enzymes, and exhibit antifungal activity, making them suitable for enhancing plant growth. Additionally, lactobacilli have been recognized for their role in soil nutrient regulation and promoting plant growth in various plant hosts. The use of non-N-fixing lactobacilli inoculants have shown positive effects on plant attributes, such as increased plant biomass and total plant nitrogen, especially in soils treated with compost (Al-Tammar and Khalifa 2022). Therefore, *L. lactis* holds promise as a plant growth-promoting bacteria, contributing to sustainable agriculture practices.

Lactococcus garvieae, traditionally known for its role in animal infections, has recently garnered attention for its potential as a plant-promoting bacterium. While primarily studied in the context of animal diseases like bovine mastitis (Abhyankar *et al.* 2022), *L. garvieae* has also been identified as an emerging zoonotic pathogen (Jaffar *et al.* 2023). Research on *L. garvieae*'s plant-promoting capabilities is limited, with most studies focusing on its pathogenicity in animals. However, given the rise in interest in using lactic acid bacteria (LAB) for plant growth promotion (Lima *et al.* 2022; Lin *et al.* 2023), exploring the potential of *L. garvieae* in this area could be beneficial. Further investigations are needed to understand the mechanisms through which *L. garvieae* could promote plant growth and its potential applications in agriculture and horticulture.

Plant growth-promoting bacteria (PGPB) enhance soil fertility, plant growth, and productivity through various mechanisms (Cropotova *et al.* 2020; Gond *et al.* 2021; Al-Tammar and Khalifa 2022). PGPB play a crucial role in nutrient availability, phytohormone production, and stress mitigation, contributing to sustainable agriculture (de Souza *et al.* 2015). While knowledge about *Rothia kristinae* as PGPB is scant, the broader concept of PGPB aligns with the potential benefits associated with such bacteria, emphasizing their role in sustainable agriculture and food security.

To summarize, insect-derived bacterial strains have significant implications as plant growth-promoting bacteria (PGPB) in agriculture (Huazhong *et al.* 2023; Wang *et al.* 2023). These strains, particularly those capable of phosphate solubilization, offer sustainable solutions by enhancing phosphorus availability to plants, thus improving crop productivity and reducing reliance on costly phosphatic fertilizers (Hyder *et al.* 2023; Li *et al.* 2023). Challenges like high fertilizer costs and low efficiency can be addressed by utilizing these bacteria for efficient phosphorus uptake by plants (Yadav *et al.* 2023). Future research can focus on optimizing the use of insect-derived bacterial strains for enhanced phosphate solubilization, exploring their interactions with plants, and developing tailored microbial inoculants for specific agro-ecosystems. This approach holds promise for sustainable agricultural practices, environmental protection, and improved crop yields in the future.

Acknowledgements

This research was done within the WIR-18 status project of the Plant Protection Institute – National Research Institute, Poznań, Poland.

References

Abhyankar P.S., Gunjal A.B., Kapadnis B.P., Ambade S.V. 2022. Potential of lactic acid bacteria in plant growth promotion. Bhartiya Krishi Anusandhan Patrika 36: 326–329. DOI: https://doi.org/10.18805/bkap374

Abou El-Yazeid A., Abou-Aly H.E. 2011. Enhancing growth, productivity and quality of tomato plants using phosphate solubilizing microorganisms. Australian Journal of Basic and Applied Sciences 5 (7): 371–379; ISSN 1991-8178

Afzal A., Bano A. 2008. Rhizobium and phosphate solubilizing bacteria improve the yield and phosphorus uptake in wheat (*Triticum aestivum*). International Journal of Agriculture and Biology 10 (1): 85–88.

Afzal I., Shinwari Z.K., Sikandar S., Shahzad S. 2019. Plant beneficial endophytic bacteria: Mechanisms, diversity, host range and genetic determinants. Microbiological Research 221 (2): 36–49 DOI: https://doi.org/10.1016/j.micres.2019.02.001

Ait-Ouakrim E.H., Abdelghani C., Cherkaoui E.M., Allal D., Soumia A., Saad I.-K., Bouchra B., Abdelkarim F.-M. 2023. Assessment of potent phosphate-solubilizing bacteria isolated from the olive tree rhizosphere grown on phosphate sludge and their effect on common bean growth. Geomicrobiology Journal 40 (6): 605–617. DOI: https://doi.org/10.1080/01490451.2023.2218839

Ait-Ouakrim E.H., Chakhchar A., El Modafar C., Douira A., Amir S., Ibnsouda-Koraichi S., Belkadi B., Filali-Maltouf A. 2023b. Valorization of Moroccan phosphate sludge through isolation and characterization of phosphate solubilizing

bacteria and assessment of their growth promotion effect on phaseolus vulgaris. Waste and Biomass Valorization 14: 2673–2690. DOI: https://doi.org/10.1007/s12649-023-02054-2

Al-Tammar F.K., Khalifa A.Y.Z. 2022. Plant growth promoting bacteria drive food security. Brazilian Journal of Biology 82: 1–11. DOI: https://doi.org/10.1590/1519-6984.267257

Alam S., Khalil S., Ayub N., Rashid M. 2002. *In vitro* solubilization of inorganic phosphate by phosphate solubilizing microorganisms (PSM) from maize rhizosphere. International Journal of Agriculture & Biology 4 (4): 454–458. DOI: 1560–8530/2002/04–4–454–458

Araujo L. 2004. Symbiosis of evolutionary techniques and statistical natural language processing. IEEE Transactions on Evolutionary Computation 8 (1): 14–27. DOI: https://doi.org/10.1109/TEVC.2003.818189

Bakhshandeh E., Rahimian H., Pirdashti H., Nematzadeh G.A. 2015. Evaluation of phosphate-solubilizing bacteria on the growth and grain yield of rice (*Oryza sativa* L.) cropped in northern Iran. Journal of Applied Microbiology 119 (5): 1371–1382. DOI: https://doi.org/10.1111/jam.12938

Baroowa B., Paul S., Gogoi N. 2022. Role of phosphorus in imparting abiotic stress tolerance to plants. p. 239–262. In: "Climate Change and Agriculture: Perspectives, Sustainability and Resilience" (N. Benkeblia, ed.). Wiley. DOI: https://doi.org/10.1002/9781119789789.ch11

Bautista-Cruz A., Antonio-Revuelta B., del Carmen Martínez Gallegos V., Báez-Pérez A., 2019. Phosphate-solubilizing bacteria improve *Agave angustifolia* Haw. growth under field conditions. Journal of the Science of Food and Agriculture 99 (14): 6601–6607. DOI: https://doi.org/10.1002/jsfa.9946

Bhattacharya A. 2019. Changing environmental condition and phosphorus-use efficiency in plants. p. 241–305. In: „Changing Climate and Resource use Efficiency in Plants". Elsevier, USA.

Bielinska E.J. 2005. Oznaczenie Aktywnosci Fosfataz. Acta Agrophysica. Rozprawy i Monografie 2005 (3): 63–74.

Chen Y.P., Rekha P.D., Arun A.B., Shen F.T., Lai W.A., Young C.C. 2006. Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. Applied Soil Ecology 34 (1): 33–41. DOI: https://doi.org/10.1016/j.apsoil.2005.12.002

Chen Z., Wang L., Cardoso J.A., Zhu S., Liu G., Rao I.M., Lin Y. 2023. Improving phosphorus acquisition efficiency through modification of root growth responses to phosphate starvation in legumes. Frontiers in Plant Science 14 (2): 1–15. DOI: https://doi.org/10.3389/fpls.2023.1094157

Compant S., Clément C., Sessitsch A. 2010. Plant growth-promoting bacteria in the rhizo- and endosphere of plants: Their role, colonization, mechanisms involved and prospects for utilization. Soil Biology and Biochemistry 42 (5): 669–678. DOI: https://doi.org/10.1016/j.soilbio.2009.11.024

Cropotova J., Mozuraityte R., Standal I.B., Ojha S., Rustad T., Tiwari B. 2020. Influence of high-pressure processing on quality attributes of haddock and mackerel minces during frozen storage, and fishcakes prepared thereof. Innovative Food Science and Emerging Technologies 59 (9): 102236. DOI: https://doi.org/10.1016/j.ifset.2019.102236

Dillon R.J., Dillon V.M. 2004. The gut bacteria of insects: nonpathogenic interactions. Annual Review of Entomology 49 (5): 71–92. DOI: https://doi.org/10.1146/annurev.ento.49.061802.123416

Egamberdiyeva D., Juraeva D., Poberejskaya S., Myachina O., Teryuhova P., Seydalieva L. Aliev A. 1996. Improvement of wheat and cotton growth and nutrient uptake by phosphate solubilizing bacteria. Plant and Soil: 58–66.

Erica de O.A. 2015. Rizobacteria in the control of pest insects in agriculture. African Journal of Plant Science 9 (9): 368–373. DOI: https://doi.org/10.5897/ajps2015.1318

Feldhaar H. 2011. Bacterial symbionts as mediators of ecologically important traits of insect hosts. Ecological Entomology 36 (5): 533–543. DOI: https://doi.org/10.1111/j.1365-2311.2011.01318.x

Gadhave K.R., Holloway R. 2015. Interactions between plant growth promoting rhizobacteria, foliar-feeding insects and higher trophic levels. School of Biological Sciences Royal Holloway, University of London Egham, Surrey, TW20 0EX United Kingdom. A thesis [Online] [Available from: https://core.ac.uk/reader/77297480] [Acessed 23 July 2024]

Gond D.P., Jha S.S., Kumar A., Singh S.K. 2021. Plant growth promoting bacteria and its role in green remediation. Sustainable Environmental Clean-up: Green Remediation 149–163. DOI: https://doi.org/10.1016/B978-0-12-823828-8.00007-4

Güneş A., Ataoğlu N., Turan M., Eşitken A., Ketterings Q.M. 2009. Effects of phosphate-solubilizing microorganisms on strawberry yield and nutrient concentrations. Journal of Plant Nutrition and Soil Science 172 (3): 385–392. DOI: https://doi.org/10.1002/jpln.200800121

Gupta G.N., Srivastava S., Khare S.K., Prakash V. 2014. Role of phosphate solubilizing bacteria in crop growth and disease management. Journal of Pure and Applied Microbiology 8 (1): 461–474.

Gurmu S. 2023. Review on effect of phosphorous fertilizer and its availability on growth and development of maize (*Zea mays* L.). Journal of Environment and Earth Science 13 (4): 35–43. DOI: https://doi.org/10.7176/jees/13-4-03

Hameeda B., Harini G., Rupela O.P. Wani S.P., Reddy G. 2008. Growth promotion of maize by phosphate-solubilizing bacteria isolated from composts and macrofauna. Microbiological Research 163 (2): 234–242. DOI: https://doi.org/10.1016/j.micres.2006.05.009

He Z., Griffin T.S., Honeycutt C.W. 2004. Enzymatic hydrolysis of organic phosphorus in swine manure and soil. Journal of Environmental Quality 33 (1): 367–372. DOI: https://doi.org/https://doi.org/10.2134/jeq2004.3670

Huazhong S., Limin L., Coatrieux J.-L. 2023. A look at plant--growth-promoting bacteria plants 27 (5): 116–118. DOI: https://doi.org/10.1109/memb.2008.927990

Hussain M.I., Asghar H.N., Akhtar M.J., Arshad M. 2013. Impact of phosphate solubilizing bacteria on growth and yield of maize. Soil and Environment 32 (1): 71–78.

Hyder S., Rizvi Z.F., los Santos-Villalobos S. de Santoyo G., Gondal A., Khalid N., Fatima S.N. Nadeem M., Rafique K., Rani A. 2023. Applications of plant growth-promoting rhizobacteria for increasing crop production and resilience. Journal of Plant Nutrition 46 (10): 2551–2580. DOI: 10.1080/01904167.2022.2160742

Indiragandhi P., Anandham R., Madhaiyan M., Poonguzhali S., Kim, G.H., Saravanan V.S., Sa T. 2007. Cultivable bacteria associated with larval gut of prothiofos-resistant, prothiofos-susceptible and field-caught populations of diamondback moth, *Plutella xylostella* and their potential for, antagonism towards entomopathogenic fungi and host insect nutriti. Journal of Applied Microbiology 103 (6): 2664–2675. DOI: https://doi.org/10.1111/j.1365-2672.2007.03506.x

Indiragandhi P., Anandham R., Madhaiyan M., Sa T.M. 2008. Characterization of plant growth-promoting traits of bacteria isolated from larval guts of Diamondback moth *Plutella xylostella* (Lepidoptera: Plutellidae). Current Microbiology 56 (4): 327–333. DOI: https://doi.org/10.1007/s00284-007-9086-4

Jaffar N.S., Jawan R., Chong K.P. 2023. The potential of lactic acid bacteria in mediating the control of plant diseases and plant growth stimulation in crop production – A mini review. Frontiers in Plant Science 13 (January): 1–13. DOI: https://doi.org/10.3389/fpls.2022.1047945

Jorquera M.A., Hernández M.T., Rengel Z., Marschner P., De La Luz Mora M. 2008. Isolation of culturable phosphobacteria with both phytate-mineralization and phosphate-solubilization activity from the rhizosphere of plants grown in a volcanic soil. Biology and Fertility of Soils 44 (8): 1025–1034. DOI: https://doi.org/10.1007/s00374-008-0288-0

Kabała C., Charzyński P., Chodorowski J., Drewnik M., Glina B. 2019. Systematyka gleb Polski. 6th ed. Wrocław University of Life Sciences, 56 pp. (in Polish)

Karpagam T., Nagalakshmi P.K. 2014. Isolation and characterization of phosphate solubilizing microbes from agricultural soil. International Journal Of Current Microbiology and Aapplied Sciences 3 (3): 601–614.

Khan A.A., Jilani G., Akhtar M.S., S. M. S. Naqvi, Rasheed M. 2009. Phosphorus solubilizing bacteria: occurrence, mechanisms and their role in crop production. Journal of Agriculture and Biological Sciences 1 (1): 48–58.

Khan M.S., Zaidi A., Musarrat J. (eds.) 2014. Phosphate solubilizing Microorganisms: Principles and Application of Microphos Technology. Springer International, Switzerland, 297 pp. DOI: https://doi.org/10.1007/978-3-319-08216-5

Kikuchi Y., Sameshima S., Kitade O., Kojima J., Fukatsu T. 2002. Novel clade of *Rickettsia* spp. from leeches. Applied and Environmental Microbiology 68 (2): 999–1004. DOI: https://doi.org/10.1128/AEM.68.2.999-1004.2002

Kim K., Indiragandhi P. 2008. *In vitro* evaluation of the mechanism of antagonism and phosphate solubilization by the insect gut bacteria *Pseudomonas* sp. PRGB06 that exhibits plant growth promotion and bio-fertilizing traits. Korean Journal of Soil Fertilizers 41 (1): 18–25.

Kirui C.K., Njeru E.M., Runo S. 2022. Diversity and phosphate solubilization efficiency of phosphate solubilizing bacteria isolated from semi-arid agroecosystems of eastern Kenya. Microbiology Insights 15. Online DOI: https://doi.org/10.1177/11786361221088991

Krawczyk K., Foryś J., Nakoniecznу M., Tarnawska M., Bereś P.K. 2021. Transmission of *Pantoea ananatis*, the causal agent of leaf spot disease of maize (*Zea mays*), by western corn rootworm (*Diabrotica virgifera* virgifera LeConte). Crop Protection 141: 105431. DOI: https://doi.org/https://doi.org/10.1016/j.cropro.2020.105431

Krawczyk K., Szabelska-Beręsewicz A., Przemieniecki S.W., Szymańczyk M., Obrępalska-Stęplowska A. 2022. Insect gut bacteria promoting the growth of tomato plants (*Solanum lycopersicum* L.). International Journal of Molecular Sciences 23 (21): 13548. DOI: https://doi.org/https://doi.org/10.3390/ijms232113548

Krawczyk K., Zwolińska A., Kamasa J., Maćkowiak-Sochacka A., Przemieniecki S. 2016. Identification and characterization of plant growth promoting endophytic bacteria. Progress in Plant Protection 55 (4): 100–109. DOI: https://doi.org/10.14199/ppp-2016-018

Lambers H. 2022. Phosphorus acquisition and utilization in plants. Annual Review of Plant Biology 73: 17–42. DOI: https://doi.org/10.1146/annurev-arplant-102720-125738

Li Q., Yang X., Li J., Li M., Li C., Yao T. 2023. In-depth characterization of phytase-producing plant growth promotion bacteria isolated in alpine grassland of Qinghai-Tibetan Plateau. Frontiers in Microbiology 13 (1): 1–18. DOI: https://doi.org/10.3389/fmicb.2022.1019383

Lima P.J.M., Silva M.M., dos Santos Castro F.F., de Oliveira Batista P.S.G., 2022. Lactococcus garvieae, endocardite de prótese biológica aórtica: relato de caso e revisão de literatura. The Brazilian Journal of Infectious Diseases 26: 102193.

Lin Y., Han J., Barkema H.W., Wang Y., Gao J., Kastelic J.P., Han B., Qin S., Deng Z. 2023. Comparative genomic analyses of Lactococcus garvieae isolated from bovine mastitis in China. Microbiology Spectrum 11 (3): 1–17. DOI: https://doi.org/10.1128/spectrum.02995-22

Lugtenberg B., Kamilova F. 2009. Plant-growth-promoting rhizobacteria. Annual Review of Microbiology 63: 541–556. DOI: https://doi.org/10.1146/annurev.micro.62.081307.162918

Magnusson J., Ström K., Roos S., Sjögren J., Schnürer J. 2003. Broad and complex antifungal activity among environmental isolates of lactic acid bacteria. FEMS Microbiology Letters 219 (1): 129–135. DOI: https://doi.org/10.1016/S0378-1097(02)01207-7

Maheshwari D.K. 2013. Bacteria in Agrobiology – Plang Growrth Responses. Springer, 369 pp.

Margalef O., Sardans J., Maspons J., Molowny-Horas R., Fernández-Martínez M., Janssens I.A., Richter A., Ciais P., Obersteiner M., Peñuelas J. 2021. The effect of global change on soil phosphatase activity. Global Change Biology 27 (22): 5989–6003. DOI: https://doi.org/10.1111/gcb.15832

Matsuura Y., Hosokawa T., Serracin M., Tulgetske G.M., Miller T.A., Fukatsu T. 2014. Bacterial symbionts of a devastating coffee plant pest, the stinkbug *Antestiopsis thunbergii* (Hemiptera: Pentatomidae). Applied and Environmental Microbiology 80 (12): 3769–3775. DOI: https://doi.org/10.1128/AEM.00554-14

Mei C., Chretien R.L., Amaradasa B.S., He Y., Turner A., Lowman S. 2021. Characterization of phosphate solubilizing bacterial endophytes and plant growth promotion *in vitro* and in greenhouse. Microorganisms 9 (9): 1935. DOI: https://doi.org/10.3390/microorganisms9091935

Minuț M., Diaconu M., Roșca M., Cozma P., Bulgariu L., Gavrilescu M. 2023. Screening of *Azotobacter*, *Bacillus* and *Pseudomonas* species as plant growth-promoting bacteria. Processes 11 (1): 80. DOI: https://doi.org/10.3390/pr11010080

Munir I., Bano A., Faisal M. 2019. Impact of phosphate solubilizing bacteria on wheat (*Triticum aestivum*) in the presence of pesticides. Brazilian Journal of Biology 79 (1): 29–37. DOI: https://doi.org/10.1590/1519-6984.172213

Oteino N., Lally R.D., Kiwanuka S., Lloyd A., Ryan D., Germaine K.J., Dowling D.N. 2015. Plant growth promotion induced by phosphate solubilizing endophytic *Pseudomonas* isolates. Frontiers in Microbiology 6 (7): 1–9. DOI: https://doi.org/10.3389/fmicb.2015.00745

Pandey A., Trivedi P., Kumar B., Palni L.M.S. 2006. Characterization of a phosphate solubilizing and antagonistic strain of *Pseudomonas putida* (B0) isolated from a sub-alpine location in the Indian Central Himalaya. Current Microbiology 53 (2): 102–107. DOI: https://doi.org/10.1007/s00284-006-4590-5

Panetto L.D., Doria J., Santos C.H.B., Frezarin E.T., Sales L.R., de Andrade L.A., Rigobelo E.C. 2023. Lactic bacteria with plant-growth-promoting properties in potato. Microbiology Research 14 (1): 279–288. DOI: https://doi.org/10.3390/microbiolres14010022

Ponmurugan P., Gopi C. 2006. *In vitro* production of growth regulators and phosphatase activity by phosphate solubilizing bacteria. African Journal of Biotechnology 5 (4): 348–350. DOI: https://doi.org/10.4314/ajb.v5i4

Pourramezan Z., Ghezelbash G.R., Romani B., Ziaei S., Hedayatkhah A. 2012. Screening and identification of newly isolated cellulose-degrading bacteria from the gut of xylophagous termite Microcerotermes diversus (Silvestri). Microbiology (Russian Federation) 81 (6): 736–742. DOI: https://doi.org/10.1134/S0026261712060124

Prischmann D.A., Lehman R.M., Christie A.A., Dashiell K.E. 2008. Characterization of bacteria isolated from maize roots: emphasis on serratia and infestation with corn rootworms (Chrysomelidae: Diabrotica). Applied Soil Ecology 40 (3): 417–431. DOI: https://doi.org/10.1016/j.apsoil.2008.06.012

Przemieniecki S.W., Gorczyca A., Matras E., Krawczyk K., Mastalerz J., Zakrzewski A. 2020. Bacteria isolated from the aeration chamber of wastewater treatment plants used in the biocontrol and promotion of wheat growth. Agronomy 10 (11): 1792. DOI: https://doi.org/10.3390/agronomy10111792

Rajashekhar M., Kalia V.K. 2017. Biochemical and molecular characterization of *Bacillus* spp . isolated from insects. Journal of Entomology and Zoology Studies 5 (5): 581–588.

Roberts T.L., Johnston A.E. 2015. Phosphorus use efficiency and management in agriculture. Resources, Conservation and Recycling 105: 275–281. DOI: https://doi.org/10.1016/j.resconrec.2015.09.013

Rodriguez H., Fraga R., Rodríguez H., Fraga R. 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol Advances 17 (4–5): 319–339. DOI: https://doi.org/10.1016/S0734-9750(99)00014-2

Sanchez-Gonzalez M.E., Mora-Herrera M.E., Wong-Villarreal A., De La Portilla-López N., Sanchez-Paz L., Lugo J., Vaca-Paulín R., Del Aguila P., Yañez-Ocampo G. 2023. Effect of pH and carbon source on phosphate solubilization by bacterial strains in pikovskaya medium. Microorganisms 11 (1): 1–11. DOI: https://doi.org/10.3390/microorganisms11010049

Santoyo G., Moreno-Hagelsieb G., del Carmen Orozco-Mosqueda M., Glick B.R. 2016. Plant growth-promoting bacterial endophytes. Microbiological Research 183: 92–99. DOI: https://doi.org/10.1016/j.micres.2015.11.008

Sarker A., Talukder N.M., Islam M.T. 2014. Phosphate solubilizing bacteria promote growth and enhance nutrient uptake by wheat. Plant Science Today 1 (2): 86–93. DOI: https://doi.org/10.14719/pst.2014.1.2.25

Savci S. 2012. Investigation of effect of chemical fertilizers on environment. APCBEE Procedia. 1 (January): 287–292. DOI: https://doi.org/10.1016/j.apcbee.2012.03.047

Schoebitz M., Ceballos C., Ciampi L. 2013. Effect of immobilized phosphate solubilizing bacteria on wheat growth and phosphate uptake. Journal of Soil Science and Plant Nutrition 13 (1): 1–10. DOI: https://doi.org/10.4067/s0718-95162013005000001

Sharma S., Kumar V., Tripathi R.B. 2011. Isolation of phosphate solubilizing microorganism (PSMs) from soil. Journal of Microbiology and Biotechnology Research 1 (2): 90–95.

Shen J., Yuan L., Zhang J., Li H., Bai Z., Chen X., Zhang W., Zhang F. 2011. Phosphorus dynamics: from soil to plant. Plant Physiology 156 (3): 997–1005. DOI: https://doi.org/10.1104/pp.111.175232

Silva L.I. da Pereira M.C., Carvalho A.M.X. de Buttrós V.H., Pasqual M., Dória J. 2023. Phosphorus-solubilizing microorganisms: a key to sustainable agriculture. Agriculture (Switzerland) 13 (2): 462. DOI: https://doi.org/10.3390/agriculture13020462

Song C., Wang W., Gan Y., Wang L., Chang X., Wang Y., Yang W. 2022a. Growth promotion ability of phosphate-solubilizing bacteria from the soybean rhizosphere under maize–soybean intercropping systems. Journal of the Science of Food and Agriculture 102 (4): 1430–1442. DOI: https://doi.org/10.1002/jsfa.11477

Song Q., Deng X., Song R., Song X. 2022b. Plant growth-promoting rhizobacteria promote growth of seedlings, regulate soil microbial community, and alleviate damping-off disease caused by *Rhizoctonia solani* on *Pinus sylvestris* var. *mongolica*. Plant Disease 106 (10): 2730–2740. DOI: https://doi.org/10.1094/PDIS-11-21-2562-RE

de Souza R., Ambrosini A., Passaglia L.M.P. 2015. Plant growth-promoting bacteria as inoculants in agricultural soils. Genetics and Molecular Biology 38 (4): 401–419. DOI: https://doi.org/10.1590/S1415-475738420150053

Suleman M., Yasmin S., Rasul M., Yahya M., Atta B.M., Mirza M.S. 2018. Phosphate solubilizing bacteria with glucose dehydrogenase gene for phosphorus uptake and beneficial effects on wheat. PLoS ONE 13 (9): 1–28. DOI: https://doi.org/10.1371/journal.pone.0204408

Tabatabai M.A., Bremner J.M. 1969. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biology and Biochemistry 1 (4): 301–307. DOI: https://doi.org/10.1016/0038-0717(69)90012-1

Tang A., Haruna A.O., Majid N.M.A. 2018. Potential PGPR properties of cellulolytic, nitrogen-fixing, and phosphate-solubilizing bacteria of a rehabilitated tropical forest soil. bioRxiv: 1–22. DOI: https://doi.org/10.1101/351916

Tarafdar J.C., Jungk A. 1987. Phosphatase activity in the rhizosphere and its relation to the depletion of soil organic phosphorus. Biology and Fertility of Soils 3 (4): 199–204. DOI: https://doi.org/10.1007/BF00640630

Tarafdar J.C., Yadav R.S., Meena S.C. 2001. Comparative efficiency of acid phosphatase originated from plant and fungal sources. Journal of Plant Nutrition and Soil Science 164 (3): 279–282. DOI: https://doi.org/10.1002/1522-2624(200106)164:3<279::AID-JPLN279>3.0.CO;2-L

Teng Z., Shao W., Zhang K., Huo Y., Li M. 2019. Characterization of phosphate solubilizing bacteria isolated from heavy metal contaminated soils and their potential for lead immobilization. Journal of Environmental Management (231): 189–197. DOI: https://doi.org/10.1016/j.jenvman.2018.10.012

Vazquez P., Holguin G., Puente M.E., Lopez-Cortes A., Bashan Y. 2000. Phosphate-solubilizing microorganisms associated with the rhizosphere of mangroves in a semiarid coastal lagoon. Biology and Fertility of Soils 30 (5–6): 460–468. DOI: https://doi.org/10.1007/s003740050024

Vives-Peris V., de Ollas C., Gómez-Cadenas A., Pérez-Clemente R.M. 2020. Root exudates: from plant to rhizosphere and beyond. Plant Cell Reports 39 (1): 3–17. DOI: https://doi.org/10.1007/s00299-019-02447-5

Wang C., Pan G., Lu X., Qi W. 2023. Phosphorus solubilizing microorganisms: potential promoters of agricultural and environmental engineering. Frontiers in Bioengineering and Biotechnology 11 (May): 1–5. DOI: https://doi.org/10.3389/fbioe.2023.1181078

Wang Q., Garrity G.M., Tiedje J.M., Cole J.R. 2007. Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Applied and Environmental Microbiology 73 (16): 5261–5267. DOI: https://doi.org/10.1128/AEM.00062-07

Wen T., Zhao M., Yuan J., Kowalchuk G.A., Shen Q. 2021. Root exudates mediate plant defense against foliar pathogens by recruiting beneficial microbes. Soil Ecology Letters 3 (1): 42–51. DOI: https://doi.org/10.1007/s42832-020-0057-z

Wielkopolan B., Krawczyk K., Szabelska-Beręsewicz A., Obrępalska-Stęplowska A. 2021. The structure of the cereal leaf beetle (*Oulema melanopus*) microbiome depends on the insect's developmental stage, host plant, and origin. Scientific Reports 11 (1): 1–19. DOI: https://doi.org/10.1038/s41598-021-99411-9

Williams A., de Vries F.T. 2020. Plant root exudation under drought: implications for ecosystem functioning. New Phytologist 225 (5): 1899–1905. DOI: https://doi.org/10.1111/nph.16223

Yadav R.S., Kumar M., Santra P., Meena H.M., Meena H.N. 2023. Plant growth-promoting microbes: The potential phosphorus solubilizers in soils of arid agro-ecosystem. p. 71–92. In: "Plant Growth Promoting Microorganisms of Arid Region" Springer.

You M., Fang S., MacDonald J., Xu J., Yuan Z.C. 2020. Isolation and characterization of Burkholderia cenocepacia CR318, a phosphate solubilizing bacterium promoting corn growth. Microbiological Research 233 (November 2019): 126395. DOI: https://doi.org/10.1016/j.micres.2019.126395

Yousefi A.A., Khavazi K., Moezi A.A., Rejali F., Nadian H.A. 2011. Phosphate solubilizing bacteria and arbuscular mycorrhizal fungi impacts on inorganic phosphorus fractions and wheat growth. World Applied Sciences Journal 15 (9): 1310–1318.

Zaidi A., Khan M.S., Ahemad M., Oves M. 2009. Plant growth promotion by phosphate solubilizing bacteria. Acta Microbiologica et Immunologica Hungarica 56 (3): 263–284. DOI: https://doi.org/10.1556/AMicr.56.2009.3.6

Zhang N., Wang D., Liu Y., Li S., Shen Q., Zhang R. 2014. Effects of different plant root exudates and their organic acid components on chemotaxis, biofilm formation and colonization by beneficial rhizosphere-associated bacterial strains. Plant and Soil 374 (1–2): 689–700. DOI: https://doi.org/10.1007/s11104-013-1915-6

Journal of Plant Protection Research eISSN 1899-007X

ORIGINAL ARTICLE

Plant growth promotion of crops using phosphate solubilizing bacterial strains derived from insects

Weronika Zenelt[1], Agata Pruciak-Nowak[2], Krzysztof Krawczyk[3]*

[1] Plant Disease Clinic and Bank of Plant Pathogen, Institute of Plant Protection – National Research Institute, Poznań, Poland

[2] Research Centre of Quarantine, Invasive and Genetically Modified Organisms, Institute of Plant Protection – National Research Institute, Poznań, Poland

[3] Virology and Bacteriology Department, Institute of Plant Protection – National Research Institute, Poznań, Poland

Vol. 64, No. 3: 336–339, 2024

DOI: 10.24425/jppr.2024.151259

Received: March 13, 2024
Accepted: June 03, 2024
Online publication: September 03, 2024

*Corresponding address:
k.krawczyk@iorpib.poznan.pl

Responsible Editor:
Agnieszka Synowiec

SUPPLEMENTARY MATERIAL

The authors are fully responsible for both the content and the formal aspects of the supplementary material. No editorial adjustments were made.

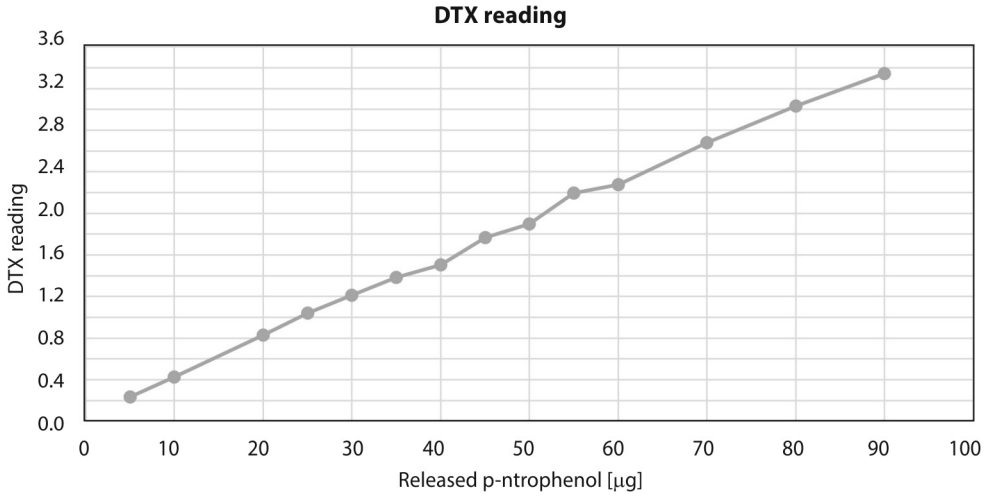

Fig. S1. Standard curve for determination of phosphatase activity

Table S1. The ability of tested bacteria to solubilize phosphorus on Pikovskaya medium before glycerol preservation in –80°C. Calculated PSI based on diameter of the bacterial colony growth and the halo. Given values of diameters are the average of eight replicates

No.	Isolate	ø halo zone [mm]	ø bacterial colony [mm]	Phosphate solubilizing index	Bacteria source
1	Hi001	7.5	4.5	2.66	*H. illucens*
2	Hi002	10	6.5	2.53	*H. illucens*
3	Hi003	7.5	5.5	2.36	*H. illucens*
4	Hi004	0	6.5	1	*H. illucens*
5	Hi007	9.5	6.5	2.46	*H. illucens*
6	Hi005	6.5	5	2.3	*H. illucens*
7	Hi006	6	4.5	2.33	*H. illucens*
8	Hi008	7.5	6	2.25	*H. illucens*
9	Hi009	6	5	2.2	*H. illucens*
10	Hi010	6	4.5	2.33	*H. illucens*
11	Hi011	14.5	14.5	2	*H. illucens*
12	Hi012	6	4	2.5	*H. illucens*
13	Hi013	8	6.5	2.23	*H. illucens*
14	Hi014	11	9	2.22	*H. illucens*
15	Hi015	7.5	5.5	2.36	*H. illucens*
16	Hi016	7.5	6.5	2.15	*H. illucens*
17	Hi017	0	4.5	1	*H. illucens*
18	Hi018	7.5	6	2.25	*H. illucens*
19	Hi019	8	6.5	2.23	*H. illucens*
20	Hi020	0	8.5	1	*H. illucens*
21	Hi021	0	7	1	*H. illucens*
22	Hi022	6	3	3	*H. illucens*
23	Hi023	7	6	2.16	*H. illucens*
24	Dv004	12	10	2.2	*D. virgifera*
25	Dv006b	12	10	2.2	*D. virgifera*
26	Dv006c	15	13	2.15	*D. virgifera*
27	Dv012	12.5	4	4.12	*D. virgifera*
28	Dv016	13	9	2.44	*D. virgifera*
29	Dv021b	12.5	12.5	2	*D. virgifera*
30	Dv021c	0	6.5	1	*D. virgifera*
31	Dv022	14.5	12.5	2.16	*D. virgifera*
32	Dv023	12	10.5	2.14	*D. virgifera*
33	Dv024a	11.5	9.5	2.21	*D. virgifera*
34	Dv024b	11.5	10	2.15	*D. virgifera*
35	Dv025	12.5	12.5	2	*D. virgifera*
36	Dv025a	12.5	11	2.13	*D. virgifera*
37	Dv025b-1	0	9.5	1	*D. virgifera*
38	Dv025b-2	11	8	2.37	*D. virgifera*
39	Dv026A	13.5	13.5	2	*D. virgifera*
40	Dv026B	11	8.5	2.29	*D. virgifera*
41	Dv027	13.5	13.5	2	*D. virgifera*
42	Dv029	13.5	13.5	2	*D. virgifera*
43	Dv030b	13.5	13.5	2	*D. virgifera*
44	Dv032b	12.5	6	3.08	*D. virgifera*
45	Dv033	14	6	3.33	*D. virgifera*
46	Dv034	10.5	10	2.05	*D. virgifera*
47	Dv035	11	11	2	*D. virgifera*
48	Dv036	11	11	2	*D. virgifera*
49	Dv037	18	18	2	*D. virgifera*
50	Dv038	9.5	3.5	3.71	*D. virgifera*
51	Dv039	12	5	3.4	*D. virgifera*
52	Dv040	0	8.5	1	*D. virgifera*
53	Dv041	0	8.5	1	*D. virgifera*
54	Dv042	10.5	10.5	2	*D. virgifera*
55	Dv043	16	6	3.66	*D. virgifera*
56	Dv044	10	10	2	*D. virgifera*
57	Dv045b	16.5	14.5	2.13	*D. virgifera*
58	Dv048	15	11.5	2.3	*D. virgifera*

Table S1. The ability of tested bacteria to solubilize phosphorus on Pikovskaya medium before glycerol preservation in –80°C. Calculated PSI based on diameter of the bacterial colony growth and the halo. Given values of diameters are the average of eight replicates – continuation

No.	Isolate	ø halo zone [mm]	ø bacterial colony [mm]	Phosphate solubilizing index	Bacteria source
59	Dv049	13.5	10	2.35	*D. virgifera*
60	Dv049a	0	13.5	1	*D. virgifera*
61	Dv050	16.5	16.5	2	*D. virgifera*
62	Dv056b	0	8	1	*D. virgifera*
63	Dv058	11.5	4	3.87	*D. virgifera*
64	Dv060	0	8.5	1	*D. virgifera*
65	Dv061	17	7.5	3.26	*D. virgifera*
66	Dv063a	0	19.5	1	*D. virgifera*
67	Dv068	21	20	2.05	*D. virgifera*
68	Dv069	20.5	20.5	2	*D. virgifera*
69	Dv070	14	12.5	2.12	*D. virgifera*
70	Dv071	14	14	2	*D. virgifera*
71	Dv072	14.5	12.5	2.16	*D. virgifera*
72	Dv073	14.5	12.5	2.16	*D. virgifera*
73	Dv092	0	12.5	1	*D. virgifera*
74	Dv096	10	5	3	*D. virgifera*
75	Dv097	11	4.5	4.33	*D. virgifera*
76	Dv101	10	3	3.44	*D. virgifera*
77	Dv102	9	4	3.25	*D. virgifera*
78	Dv107	10	4.5	3.22	*D. virgifera*
79	Dv107a	8	3.5	3.28	*D. virgifera*
80	Dv107b	13.5	11	2.22	*D. virgifera*
81	Dv109	12.5	10	2.25	*D. virgifera*
82	Dv111	14	12.5	2.12	*D. virgifera*
83	Dv111a	12.5	11.5	2.08	*D. virgifera*
84	Dv111b	10.5	9.5	2.1	*D. virgifera*
85	Dv111c	0	12	1	*D. virgifera*
86	Dv112	13.5	6	3.25	*D. virgifera*
87	Dv113	14	13	2.07	*D. virgifera*
88	Dv116	12.5	11.5	2.08	*D. virgifera*
89	Dv117	16	5.5	3.9	*D. virgifera*
90	Dv119	12	11	2.09	*D. virgifera*
91	Dv120	13	5	3.6	*D. virgifera*
92	Dv123	14	3.5	5	*D. virgifera*
93	Dv124	0	10	1	*D. virgifera*
94	Dv125	12.5	4	4.12	*D. virgifera*
95	Dv126a	20.5	8.5	3.41	*D. virgifera*
96	Dv126b	6.5	3	3.16	*D. virgifera*
97	Dv127	0	13	1	*D. virgifera*
98	Dv128	0	7	1	*D. virgifera*
99	Dv129	5	5.5	1.9	*D. virgifera*
100	Dv132	9.5	7.5	2.26	*D. virgifera*
101	Dv133a	6	2.5	3.4	*D. virgifera*
102	Dv135a	24.5	8.5	3.88	*D. virgifera*
103	Dv137	23.5	9.5	3.47	*D. virgifera*
104	Dv138a	18.5	7	3.64	*D. virgifera*
105	Dv139a	17.5	10	2.75	*D. virgifera*
106	Dv140	12.5	10.5	2.19	*D. virgifera*
107	Dv141	13	10.5	2.23	*D. virgifera*
108	OM002	11	9	2.22	*O. melanopus*
109	OM006A	14	12.5	2.12	*O. melanopus*
110	OM006B	14.5	13	2.11	*O. melanopus*
111	OM011	14	11	2.27	*O. melanopus*
112	OM012	12	10.5	2.14	*O. melanopus*
113	OM016	14.5	9.5	2.52	*O. melanopus*
114	OM018	14.5	13	2.11	*O. melanopus*
115	OM019	15	13	2.15	*O. melanopus*
116	OM023	13.5	12.5	2.08	*O. melanopus*
117	On024	12.5	10.5	2.19	*O. nubilalis*
118	On026	13	11	2.18	*O. nubilalis*
119	On027	10	8	2.25	*O. nubilalis*
120	On030	0	3.5	1	*O. nubilalis*
121	On034	9.5	8	2.18	*O. nubilalis*
122	On037	14	12	2.16	*O. nubilalis*
123	Om001	12	3	5	*O. nubilalis*
124	Om002	10	5	3	*O. melanopus*
125	Om003	18	7	3.57	*O. melanopus*
126	Om004	15	8	2.87	*O. melanopus*
127	Om005	11.5	7	2.64	*O. melanopus*
128	Om006	9	7.5	2.2	*O. melanopus*
129	Om007	10	6	2.66	*O. melanopus*
130	Om008	0	20	1	*O. melanopus*
131	Om009	10.5	3.5	4	*O. melanopus*
132	Om011	12	4.5	3.66	*O. melanopus*
133	Om012	10.5	4.5	3.33	*O. melanopus*
134	Om013	10.5	3.5	4	*O. melanopus*
135	Om014	9.5	3.5	3.71	*O. melanopus*
136	Om015	10	3	4.33	*O. melanopus*
137	Om016	17	9	2.88	*O. melanopus*
138	Om017	10	7.5	2.33	*O. melanopus*
139	Om019	10	6.5	2.53	*O. melanopus*
140	Om020	9.5	6	2.58	*O. melanopus*
141	Om021	11.5	6	2.91	*O. melanopus*
142	Om022	11	4.5	3.44	*O. melanopus*
143	Om024	16.5	10.5	2.57	*O. melanopus*
144	Om025	17.5	6.5	3.69	*O. melanopus*
145	Om026	11	4.5	3.44	*O. melanopus*
146	Om027	0	12	1	*O. melanopus*
147	Om028	10.5	8	2.31	*O. melanopus*
148	Om029	10	3	4.33	*O. melanopus*

Table S1. The ability of tested bacteria to solubilize phosphorus on Pikovskaya medium before glycerol preservation in –80°C. Calculated PSI based on diameter of the bacterial colony growth and the halo. Given values of diameters are the average of eight replicates – continuation

No.	Isolate	ø halo zone [mm]	ø bacterial colony [mm]	Phosphate solubilizing index	Bacteria source	No.	Isolate	ø halo zone [mm]	ø bacterial colony [mm]	Phosphate solubilizing index	Bacteria source
149	Om030	11	3	4.66	*O. melanopus*	161	Om042	8.5	5	2.7	*O. melanopus*
150	Om031	15	4	4.75	*O. melanopus*	162	Om043	11	4.5	3.44	*O. melanopus*
151	Om032	11	4.5	3.44	*O. melanopus*	163	Om044	9.5	5.5	2.72	*O. melanopus*
152	Om033	10.5	5.5	2.9	*O. melanopus*	164	Om045	12.5	4.5	3.77	*O. melanopus*
153	Om034	9	4	3.25	*O. melanopus*	165	Om046	11	3	4.66	*O. melanopus*
154	Om035	13	4.5	3.88	*O. melanopus*	166	14	13.5	11.5	1.85	*O. melanopus*
155	Om036	10.5	5.5	2.9	*O. melanopus*	167	27	13.5	6.5	1.48	*O. melanopus*
156	Om037	10	6	2.66	*O. melanopus*	168	67	12	8	1.66	*O. melanopus*
157	Om038	15.5	4.5	4.44	*O. melanopus*	169	90	9.5	4	3.8	*O. melanopus*
158	Om039	11.5	6.5	2.76	*O. melanopus*	170	96	9.5	4	3.8	*O. melanopus*
159	Om040	11	5	3.2	*O. melanopus*	171	106	5.5	4	1.72	*O. melanopus*
160	Om041	15.5	6.5	3.38	*O. melanopus*						

Table S2. Measurement of the color intensity of p-nitrophenol (p-NP) at 400 nm used for the purpose of determination of the standard curve of phosphatase activity

Released Phenol approximate value [µg]	DTX reading
5	0.235
10	0.423
20	0.829
25	1.039
30	1.212
35	1.384
40	1.505
45	1.769
50	1.898
55	2.197
60	2.275
70	2.681
80	3.034
90	3.344
100	overflow
200	overflow
2000	overflow