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Phosphorus-solubilizing bacteria isolated from the rhizosphere of wild potato *Solanum bulbocastanum* enhance growth of modern potato varieties

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Abstract

Background: Wild potato species harbor a distinctive rhizosphere microbiome relative to their modern counterparts, thus providing a competitive advantage for acquiring phosphorus (P) in their native habitats. Despite this, the effects of transferring phosphorus-solubilizing bacteria (PSB), recruited from wild potatoes rhizosphere, on modern potato varieties' performance has not been investigated. Here, it was hypothesized that PSB isolated from wild potatoes could enhance plant growth and solubilization of various P forms when co-inoculated with commercial potatoes (*Solanum tuberosum*).

Results: To test this hypothesis, three bacteria *Enterobacter cloacae*, *Bacillus thuringiensis*, and *Pseudomonas pseudoalcaligenes* were isolated from the rhizosphere of the wild potato *Solanum bulbocastanum* grown under greenhouse conditions and characterized for their P-solubilizing activities. It was found that both individual bacterial species and the consortium of the three bacteria, dissolved organic (i.e., phytin) and inorganic P (i.e., calcium phosphate) in vitro. The bacterial consortium increased dissolved P by 36-fold for calcium phosphate and sixfold for phytin compared to a sterile control and surpassed the effect of each individual PSB strain. To further evaluate the effect of the PSB consortium on plant growth and P use efficiency, the bacteria were co-inoculated on a commercial potato cultivar and amended separately with phytin, calcium phosphate, commercial P fertilizer, or a combination of the three P sources. The results showed an overall increase in total dry biomass and shoot P content in treatments co-inoculated with PSB.

Conclusions: Our findings indicate that PSB isolated from wild potatoes and inoculated with modern potato varieties have the potential to enhance yield and nutrient uptake.

Keywords: Phosphorus, Wild potato, Phosphorus-solubilizing bacteria, Phosphorus use efficiency, Co-inoculation, Rhizosphere, *Enterobacter cloacae*

Background

Phosphorus (P) is an essential element for all living organisms as well as for the production of food, fiber, and fuel (Bennett and Schipanski 2013). Plant availability of

P in soils is typically constrained due to continuous soil sorption and by the formation of insoluble P complexes by binding with aluminum, iron oxides and hydroxides, and calcium; or through the formation of organic complexes such as orthophosphate esters, phosphonates, and anhydrides (Condron et al. 2005; Shen et al. 2011). The relatively low amount of available P in agricultural soils typically leads to substantially higher P fertilizer rates than required by the crop (Sattari et al. 2012). Accumulated, and often unavailable, P in agricultural soils

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represents a financial loss for farmers, while excess P in runoff and leaching has important environmental implications for aquatic habitats and soil biodiversity (George et al. 2016). It is estimated that residual and unavailable P in some soils would be sufficient to sustain crop yield for the next century and could alleviate expected P shortages in the next 50 years (Zhu et al. 2018). Hence, there is widespread interest in finding ways to utilize accumulated soil P for crop production (Richardson 2001; Withers et al. 2014). Current management strategies to utilize soil residual P tend to focus on the adjustment of the soil pH to the optimal range of P availability (between 5 and 6 pH (Barrow 2017)). However, due to the high amounts of input required to make meaningful, long-term changes to soil pH, this is often not economically feasible for farmers. Other, promising strategies to enhance P mobilization include the addition of phosphate-solubilizing microorganisms, phosphatase enzymes, enzyme activators, low molecular weight organic acids, crop residues, lignin, humic acids, and zeolites (Richardson 2001). Considering the importance of P to agricultural production and anticipated shortages in the future, effective strategies to recover organic and inorganic P sources are urgently needed. Organic P in the soil accounts for 30–65% of the total P and is mainly found as inositol phosphates (commonly called phytate (Condron et al. 2005)). Inorganic P comprises 35–70% of total soil P content in topsoil (Harrison 1987), where calcium phosphate is the primary mineral source under moderately weathered soils with neutral to alkaline pH (Bennett and Schipanski 2013).

Microbes play an important role in altering P availability in soils by increasing plant-available P through the solubilization of mineral P and mineralization of organic P forms, but also by immobilizing and competing with plants for available P (Richardson 2001; Oliverio et al. 2020). Microorganisms accomplish these tasks through the release of mineral dissolving compounds (e.g., organic acids) and the secretion of extracellular enzymes (i.e., phosphatases) (Jones and Oburger 2011). These strategies are dependent on bacterial strain and P source. For instance, Guang-Can et al. (2008) showed that species of the genera *Bacillus* and *Pseudomonas* had phosphate-solubilizing and phosphate-mineralizing abilities coexisting in the same bacterial strain. In addition, a strain of *Enterobacter agglomerans* isolated from the rhizosphere of wheat exhibited significant abilities to solubilize hydroxyapatite and to hydrolyze organic phosphate (Kim et al. 1998). Although certain phosphorus-solubilizing bacteria (PSB) could solubilize organic or inorganic P, it is not clear if this solubilization could affect P use efficiency in commercial crops.

Wild relatives of modern crops typically occupy low-nutrient soil environments in their natural habitats, and unlike managed crops, they are not subjected to frequent additions of synthetic fertilizers. In fact, wild plant species often thrive in nutrient-poor soils and are able to successfully grow, reproduce, and maintain adequate nutrition in the plant tissues (Chapin et al. 1987; Porter and Sachs 2020). Wild and cultivated plants are known to modulate their rhizosphere microbiota, promoting a host-specific community to facilitate plant nutrient acquisition (Berendsen et al. 2012). Increasing evidence suggests that domesticated plants exert a relatively limited selection of their microbiota compared to their wild counterparts (Porter and Sachs 2020). For instance, Schmidt et al. (2020) demonstrated that teosinte appears to have a greater effect than modern corn cultivars on microbial rhizosphere recruitment. Similarly, Pantigoso et al. (2020) showed correlations between enrichment of PSB taxa in the rhizosphere of wild types of potato as compared to commercial varieties. However, it is unclear if specific PSB isolated from wild potatoes (*Solanum bulbocastanum*) could have a direct contribution on P solubilization, and furthermore if these PSBs could be utilized in commercial potato varieties to enhance P use efficiency.

In the present study, we hypothesized that PSB could be isolated from *S. bulbocastanum* and that these PSB could increase P use efficiency and biomass gain when applied to commercial potatoes (*Solanum tuberosum*). Accordingly, bacteria were isolated from the rhizosphere of *S. bulbocastanum*, characterized, and assessed for their potential phosphate-solubilizing ability under in vitro and greenhouse conditions.

Methods
Soil and growing conditions for wild potato type
Botanical seeds of the wild potato *Solanum bulbocastanum* (PI 275184) were obtained from the Potato Gene Bank, Sturgeon Bay, Wisconsin, USA. To improve germination, potato seeds were pre-treated with gibberellic acid at 2000 mg L^{-1} for 2 h after being surface-disinfected with 5% of sodium hypochlorite for 5 min and left soaking for 24 h in distilled water. Seeds were germinated on wet filter paper for approximately 5 days and moved to Murashige and Skoog nutritional media (4.33 g L^{-1}) for 2 weeks.

One seedling of *S. bulbocastanum* was transplanted to pots (15 cm diameter by 14.5 cm deep) containing a mix of two-part sand and one-part soil. The soil was collected in August 2019 from a depth of 15 cm in an organic field at the Agricultural Research, Development, and Educational Center (ARDEC) of Colorado State University, near Fort Collins, CO. The soil had a sandy clay loam texture,

pH of 8.1, and an organic matter content of 2.0%. The P content of the sand–soil mixture was > 11 mg kg^{-1}, which is considered a high level for the AB-DTPA (ammonium bicarbonate-diethylenetriaminepentaacetic acid) extractant. Levels of P in the soil mix used to grow wild potato plants were consistent with those reported by Pantigoso et al. (2020) in an earlier experiment. Wild potato plants were grown in the Greenhouse Facility at Colorado State University for approximately 45 days under standard light and temperature conditions receiving irrigation as needed.

Rhizosphere soil collection and bacteria isolation

Rhizosphere soil was sampled by carefully excavating the roots of *S. bulbocastanum*, removing soil particles attached to the root, and collecting this material in a plastic bag. For isolation of soil bacteria, 1 g of rhizosphere soil was vigorously stirred in 20 mL of sterile water. An aliquot of this soil extract was placed in NBRIP (National Botanical Research Institute Phosphate) liquid medium containing tricalcium phosphate $(Ca_3(PO_4)_2)$ as the phosphate source (Nautiyal 1999). The NBRIP medium is comprised of glucose (10.0 g), $Ca_3(PO_4)_2$ (5.0 g), NaCl (0.2 g), $MgSO_4 \cdot 7H_2O$ (0.5 g), $(NH_4)_2SO_4$ (0.5 g), KCl (0.2 g), $MnSO_4$ (0.03 g), $FeSO_4 \cdot 7H_2O$ (0.003 g) with a pH of 7.0–8.0. Five mL of NBRIP media was inoculated with 10 µL of soil extract and incubated in a rotary shaker at 170 rev min^{-1} at room temperature for 48 h. The culture media was then diluted 10^{-6}, and a 1 mL aliquot was plated on NBRIP solid media (1.2% Bacto agar) and incubated at 30 °C overnight for 12 h. PSB candidates that differed in size, shape, and color were separated into different petri dishes containing NBRIP several times to purify single colonies. Eight selected bacteria candidates were subsequently grown in Luria–Bertani liquid media (Bertani 1951) before its use and/or mixed with glycerol and frozen for storage.

Bacterial isolate identification

Eight bacterial isolates were identified to the genus level using a 64-well VITEK 2 GN card containing biochemical tests measuring C source utilization, inhibition, and resistance; enzymatic activities were processed in the Veterinary Diagnostic Lab at Colorado State University, Fort Collins, Colorado. For species-level identification, 526 base pairs of the 16S rRNA amplicon sequencing were amplified using the universal primers: 0005F (5′-TGGAGAGTTTGATCCTGGCTCAG-3′) and 0531R (5′-TACCGCGGCTGCTGGCAC-3′). The 16S identification analysis was performed in the MIDI labs, Inc., Newark, DE. Three isolates out of the eight were different species.

Qualitative and quantitative determination of phosphorus-solubilizing ability

Using a 2.5-mm platinum wire loop, a streak of bacteria culture obtained from pure cultures of each of the three selected isolates (*Enterobacter cloacae*, *Pseudomonas pseudoalcaligenes*, and *Bacillus thuringiensis*) was dipped into liquid Luria–Bertani medium (Bertani 1951) and incubated separately in a rotary shaker at 170 rev min^{-1} at room temperature overnight until reaching the mid-exponential growth phase. An aliquot of diluted (OD$_{600}$ = 1; 1×10^8) cultured bacteria was placed on NBRIP solid media containing calcium phosphate or phytin as the P source to quantitatively evaluate their phosphate-solubilizing ability. Then, the bacterial isolates were qualitatively evaluated such that a clear halo observed around the bacteria colonies was interpreted as evidence of phosphate solubilization ability.

For the quantitative experiment, a 50 µL diluted (OD$_{600}$ = 1; 1×10^8) aliquot from each pure culture of *E. cloacae*, *B. thuringiensis*, and/or *P. pseudoalcaligenes* was added to a 5 mL liquid NBRIP medium separately and incubated in a rotary shaker for 72 h. For the inoculation of bacterial consortium, a proportion of each strain was prepared and mixed at the same final concentration (OD$_{600}$ = 1; 1×10^8) and incubated, also for 72 h. After incubation and as preparation for downstream analysis, the solution was centrifuged at 6000 rpm for 20 min to remove the suspended bacterial cells and the remaining calcium phosphate/phytate. Liquid NBRIP media without the addition of bacteria was used as a control. The concentration of phosphate in the supernatant was analyzed according to the protocol of Soltanpour et al. (1983) and measured with an inductive coupled plasma-optical emission spectrometer (ICP-OES; Perkin Elmer 7300DV) at the Soil, Water and Plant Testing Laboratory of Colorado State University.

Potato growth response to P amendments and bacterial consortia

Certified tubers of the commercial potato (*Solanum tuberosum*) cultivar "Defender" (Russet-type) were pre-sprouted for a week, and whole tubers of similar size were planted in plastic pots (15 cm diameter by 14.5 cm deep) containing three parts of sand and one-part peat moss for a total weight of 555 g. To reduce the population of microbes and to assess the sole effect of P-solubilizing activity of the co-inoculated bacteria, sand and peat moss were heat-sterilized on an autoclave for three 30-min cycles at 121 °C and thoroughly mixed prior to P amendment and tuber planting. A sample of this substrate mixture was analyzed for Olsen P resulting in 16.4 mg kg^{-1} of available P. The soil amendments used in this experiment were composed of four P sources: (1) fertilizer triple

superphosphate (P), (2) calcium phosphate ($Ca_3O_8P_2$), (3) calcium phytate ($C_6H_6Ca_6O_{24}P_6$), and (4) a combination of the three previous phosphate sources. All four treatments received approximately 1 g of elemental P per kg of soil, both at planting and after 30 days. A fifth P un-amended treatment was included as a control. Each of the five P fertilization treatments was grown with and without the inoculation of PSB, in a full-factorial and fully randomized design, with five replicates per treatment. The PSB applied comprised a consortium of the same bacteria *E. cloacae*, *B. thuringiensis*, and *P. pseudoalcaligenes* mentioned above. A proportion of each strain at the same final concentration ($OD_{600} = 1$) was mixed, and 1 mL aliquot of the consortium was applied to the soil near the main stem of potato plants at 2, 3, and 4 weeks after planting. A representative soil sample of each treatment was collected for pH and nutrient analysis at harvest time and sent to the Soil, Water and Plant Testing Laboratory of Colorado State University for analysis (Additional file1: Table S1).

This study was performed under greenhouse conditions from August to October 2020 in the Plant Growth Facility (PGF) at Colorado State University, Fort Collins, Colorado. Plants were harvested 2 weeks after the final inoculation and 60 days after tuber planting. Fresh biomass of shoot, roots, and tubers was recorded separately, and then each plant component was oven-dried for 4 days at 80 °C and weighed. Total P in the aboveground plant shoot tissue was analyzed by digesting the material in a block digester with HCl and HNO_3 and cleared with H_2O_2. Then, the sample was brought to a volume of 50 mL and a total P was read on an ICP-OES.

Calculations

Phosphorus utilization efficiency (PuTE) for potato plants in each pot was calculated as

$$P \text{ utilization efficiency } = \text{ tuber biomass}/P \text{ applied.}$$

Phosphorus uptake efficiency (PuPE) by potato plants in each pot was calculated as

$$P \text{ uptake efficiency } = P \text{ content in shoots}/P \text{ applied.}$$

Equation:

$$\text{PuTE} \left(g \text{ tuber } g^{-1} P \text{ applied} \right)$$
$$= \text{TuDM} \left(\text{tuber dry matter} \right)/P \text{ supply}$$

$$\text{PuPE} \left(\text{mg } P \, g^{-1} P \text{ applied} \right)$$
$$= \text{SPC} \left(P \text{ content in shoots} \right)/P \text{ supply}$$

Data analysis

One-way ANOVA was used to analyze differences in dissolved P between individual bacteria isolates and consortium incubated in NBRIP phytin and calcium phosphate solutions. Two-way ANOVA was used to examine the effect of P amendment type and PSB inoculation and their interaction on plant biomass and shoot P content. Homogeneity of variance and normality were assessed, and log transformations were applied as needed to meet these assumptions. A probability level of $p = 0.05$ was considered statistically significant (Girden 1992).

Results

Differences in relative abundance from wild versus cultivated potatoes

16S rRNA sequencing provided species-level resolution for the three bacterial isolates. Phosphorus-solubilizing bacteria were identified in GenBank with 100% similarity with *Enterobacter cloacae* GU191924, 99% similarity to *Pseudomonas pseudoalcaligenes*, and 99% similarity to *Bacillus thuringiensis*. Phylogenetic trees for the three isolates at genus level are provided in Additional file1: Fig. S1.
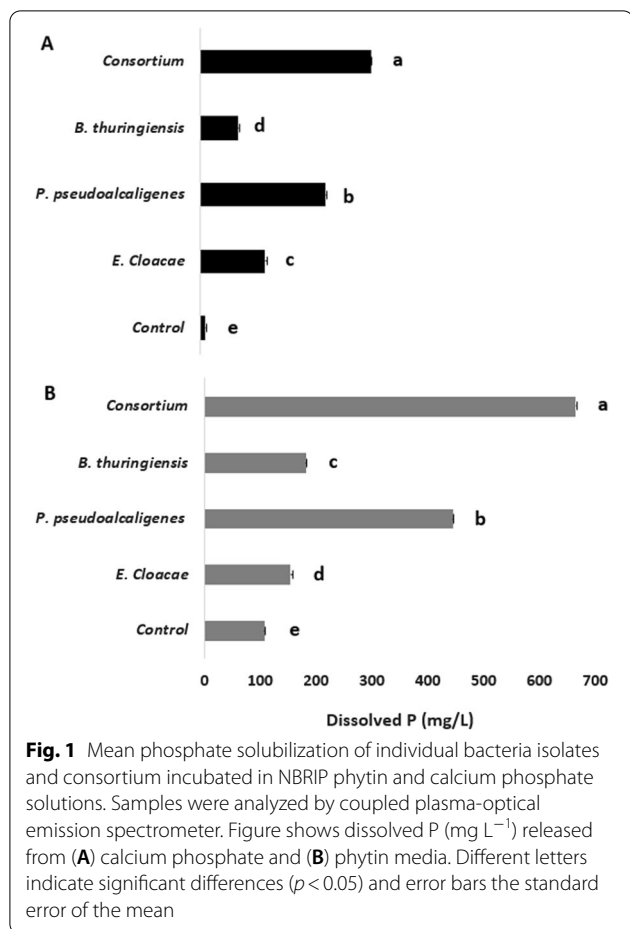
To further investigate differences in the relative abundance of the three bacteria genera isolated from *S. bulbocastanum*, and to compare their abundance with cultivated relatives, the 16S rRNA sequenced data from Pantigoso et al. (2020) were analyzed. The analysis showed a greater relative abundance for Enterobacteriaceae and Pseudomonadaceae in wild potato species when compared to cultivated potato cultivars (Additional file1: Fig. S2).

Bacteria isolates solubilized plant-unavailable phosphate

The phosphate-solubilizing effect of the bacterial isolates was qualitatively tested using phytin and calcium phosphate NBRIP solid media. Individual bacteria and a consortium of the three isolates formed a clear halo around the colony indicating their ability to release free phosphate from calcium phosphate and phytin complexes (Additional file1: Fig. S3).

The phosphate-solubilizing activity of the bacteria isolates was analyzed by ICP-OES. This method allowed us to evaluate the amount of dissolved P (plant-available phosphate) after incubation of bacterial strains in calcium phosphate and phytin solutions. The results showed that individual isolates and the bacterial consortium significantly increased the content of dissolved P under both P solutions relative to the control ($p < 0.001$; Fig. 1).
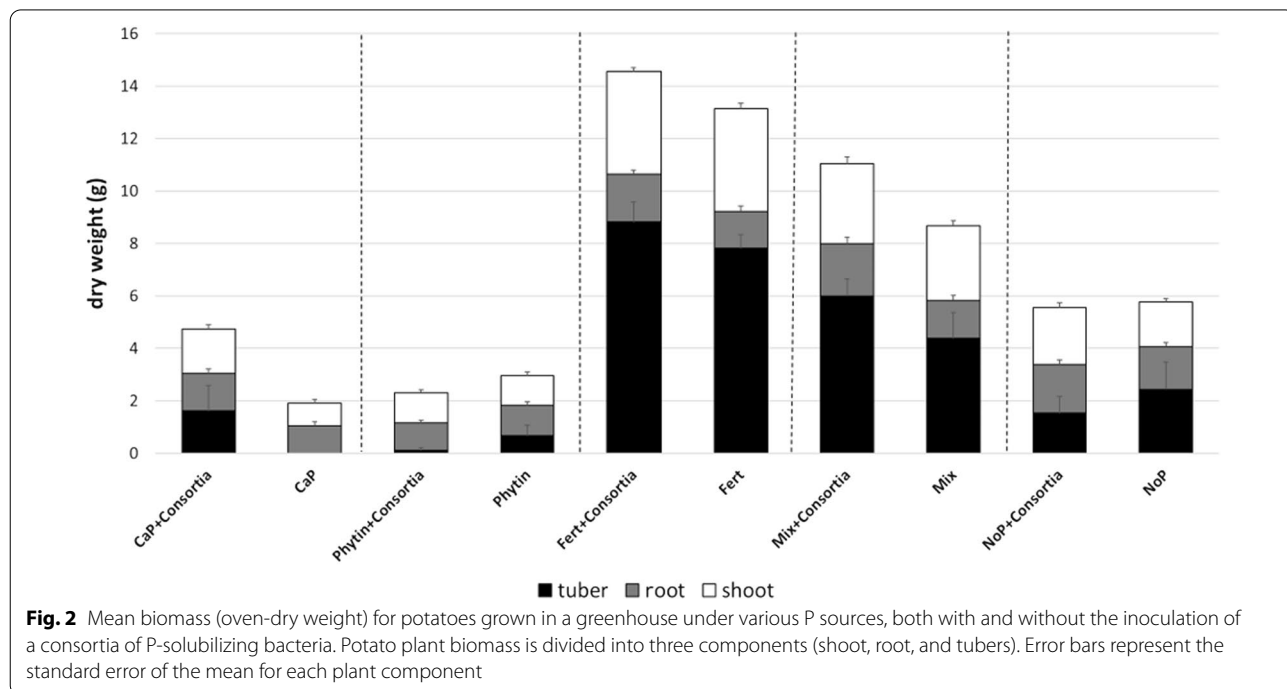
Absolute values for dissolved P were lower under calcium phosphate (Fig. 1A) than under phytin solutions (Fig. 1B), but the relative difference between the bacteria-inoculated treatments and the control for calcium phosphate

Fig. 1 Mean phosphate solubilization of individual bacteria isolates and consortium incubated in NBRIP phytin and calcium phosphate solutions. Samples were analyzed by coupled plasma-optical emission spectrometer. Figure shows dissolved P (mg L^{-1}) released from (**A**) calcium phosphate and (**B**) phytin media. Different letters indicate significant differences ($p < 0.05$) and error bars the standard error of the mean

was greater than differences for phytin when compared to their respective controls. Under calcium phosphate media, the bacterial consortium resulted in a 36-fold increase in dissolved P relative to the control, while increases for individual strains were: *P. pseudoalcaligenes* 26-fold, *B. thuringiensis* eightfold, and *E. cloacae* 13-fold. In the phytin solution, the consortium dissolved approximately sixfold more P than the control, *P. pseudoalcaligenes* four times, *B. thuringiensis* twofold, and *E. cloacae* 1.44-fold.

Effects of phosphorus source and bacterial consortia on plant growth

The effect of different P sources and PSB consortia inoculation on total potato biomass revealed clear treatment differences due to P source ($p < 0.001$), such that the treatments containing TSP generally produced the most biomass, while those amended with CaP and phytin were the lowest. We also observed the PSB consortia to have a significant increase in total biomass relative to the uninoculated control ($p = 0.043$), but there was no significant interaction between P source and PSB inoculation (Fig. 2). Similarly, P source had a significant effect on each of the plant components (shoots, roots, and tubers; $p < 0.002$). Inoculation with the PSB consortia significantly increased shoot ($p = 0.031$) and root ($p = 0.015$) biomass, on average, across treatments. However, no PSB effect was observed for tubers, as tuber biomass was highly variable. There were no significant interactions between P source and PSB consortia for any of the plant components (Table 1). When analyzing P concentration

Fig. 2 Mean biomass (oven-dry weight) for potatoes grown in a greenhouse under various P sources, both with and without the inoculation of a consortia of P-solubilizing bacteria. Potato plant biomass is divided into three components (shoot, root, and tubers). Error bars represent the standard error of the mean for each plant component

of the aboveground biomass (shoot tissue), statistical differences between treatments were observed for P source ($p < 0.001$), but not for PSB consortia inoculation (Table 1).

Table 1 Two-way ANOVA for total dry biomass P concentration as well as biomass of the individual plant components (roots, shoots, and tubers)

Total biomass	*p* value
P_source	<.0001
PSB	0.0431
P_source*PSB	0.2065
Shoot biomass	**p value**
P_source	<.0001
PSB	0.0314
P_source*PSB	0.3043
Root biomass	***p* value**
P_source	0.0012
PSB	0.0148
P_source*PSB	0.4865
Tuber biomass	***p* value**
P_source	<.0001
PSB	0.2135
P_source*PSB	0.2177
P concentration	***p* value**
P_source	<.0001
PSB	0.8593
P_source*PSB	0.0554

Plant P content, P uptake efficiency, and P use efficiency responses

Aboveground P content, P uptake efficiency, and P utilization efficiency revealed clear treatment differences due to P source ($p < 0.001$; Table 2). Treatments containing TSP represented the highest value, while those with CaP and phytin were the lowest. PSB inoculation significantly increased P content ($p = 0.039$) and had a marginally significant effect on P uptake efficiency ($p = 0.085$) and P utilization efficiency ($p = 0.052$). No significant interaction between P source and PSB inoculation was observed.

Effects of phosphorus source and bacterial consortia on soil pH

Soil pH at the end of the greenhouse experiment tended to vary with P source but not bacterial inoculation. The average soil pH, from inoculated and non-inoculated treatments, was relatively uniform for treatments that received calcium phosphate (6.75), phytin (6.85), and the control (6.65), while in the TSP treatment pH was roughly a unit lower (5.5) and the treatment with a combination of P sources was intermediate (6.1; Additional file1: Table S1).

Discussion

Wild potato (*S. bulbocastanum)* recruits a distinct rhizosphere microbiome compared to its modern relative when grown under the same soil conditions. Recent studies suggest that differences in rhizosphere microbiome structure between wild types and cultivated crops significantly correlate with nutrient uptake favoring the wild type (Schmidt et al. 2020; Brisson et al. 2019, 2021).

Three P-solubilizing bacteria strains were identified as *E. cloacae, P. pseudoalcaligenes*, and *B. thuringiensis.*

Table 2 Phosphorus use efficiencies of potato plants amended with four P sources and a control in greenhouse conditions with and without inoculation of a consortium of P-solubilizing bacteria

Treatments	P content		P uptake efficiency		P utilization efficiency	
	(mg P plant^{-1})		(mg P shoot g^{-1} P applied)		(g tuber g^{-1} P applied)	
	With PSB	**Without PSB**	**With PSB**	**Without PSB**	**With PSB**	**Without PSB**
Calcium phosphate	0.956	0.666	2.078	1.448	3.509	0.000
Phytin	0.856	0.726	1.861	1.578	0.223	1.448
Fertilizer	4.268	3.484	9.278	7.574	19.162	16.967
Mix	1.749	1.852	3.803	4.026	12.886	9.520
No P	1.023	0.836				
Statistical significance	***p* value**					
P_source	<.0001		<.0001		<.0001	
PSB	0.0395		0.0854		0.0518	
P_source*PSB	0.7695		0.6263		0.2981	

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Based on 16S sequencing reads, we showed a higher relative abundance of Enterobacteriaceae and Pseudomonadaceae and a marginally lower abundance of Bacillaceae families in the rhizosphere of *S. bulbocastanum* when compared to the same families present in the rhizosphere of modern relatives. These observations agree with mounting evidence suggesting that domesticated plants either exert a relatively limited selection or select for different microbial communities with less obvious functionality, in terms of P acquisition, compared to their wild counterparts (Porter and Sachs 2020; Martín-Robles et al. 2018; Escudero-Martinez and Bulgarelli 2019; Jaiswal et al. 2020). Several studies between wild accessions, ancestral and modern varieties have shown evidence of significant shifts in composition of their rhizosphere microbiota in different crops such as maize, rice, and bean (Peiffer et al. 2013; Edwards et al. 2015; Perez et al. 2007; Walters et al. 2018). We have recently shown effects of P fertilization on the rhizosphere microbiome in cultivated and non-cultivated potatoes. These effects revealed differences in bacterial community structure and P absorption favoring non-cultivated potatoes (Pantigoso et al. 2020). However, it is unknown if the differential plant recruitment of microbes observed in microbiomes of modern and wild types constitutes a functionally important component for plant nutrition (Cordovez et al. 2019). In addition, the selection relaxation hypothesis predicts that germplasm bred under more intensive agricultural conditions will exhibit greater functional trait disruption than germplasm bred under less intensively managed conditions (Porter and Sachs 2020). Consistent with this hypothesis, numerous crop taxa have evolved a reduced ability to associate with mycorrhizae under high levels of P fertilization (Brisson et al. 2021; Hetrick et al. 1993; Xing et al. 2012). Similarly, De la Torre-Hernandez et al. (2020) investigated bacterial community composition and plant growth-promoting rhizobacteria traits associated with wild and cultivated cactus plants. Interestingly, the bacteria isolated from the wild types showed a higher number of such bacteria with functional traits including a higher number of P-solubilizing strains. Genera *Bacillus* and *Pseudomonas* were found in both samples, but *Pseudomonas* was particularly abundant in the wild cactus type. These findings are further supported by Coleman-Derr et al. (2016) and Hilton et al. (2013) who reported a marked reduction in microbial diversity in rhizosphere soils of cultivated agave compared to native agave plants.

Effects of *E. cloacae, P. pseudoalcaligenes,* and *B. thuringiensis* on availability of phosphorus

In a previous study, we showed a correlation between P rate supplied to soils, P content in shoot tissues, and

differential abundance of bacterial taxa for *S. bulbocastanum* when compared to modern potato relatives grown under the same conditions (Pantigoso et al. 2020). In this study, we postulate that effective PSB recruitment of wild potato enhances P solubilization improving plant growth. Our findings are supported by recent studies including Schmidt et al. (2020) who demonstrated that teosinte appears to have a greater effect than modern corn cultivars on rhizosphere recruitment and individual plant–microbe interactions related to nitrogen uptake. Brisson et al. (2019) showed that root exudate metabolite profile and rhizosphere microbial communities were distinct between teosinte and modern maize and shifted in response to P availability. In addition to the known plant genotype effect on differential bacterial recruitment, the soil environment from which microbial inoculants were collected significantly contributes to the effect on plant growth promotion in terms of plant biomass and assimilation of key nutrients (Gu et al. 2020). Most PSB species identified in the literature are ubiquitous in soils, and their occurrence has been reported in numerous geographic areas, in a wide range of soil conditions, and under variable nutrient environments, highlighting the importance of wild-type plants and their specific root exudation signature for specialized microbial recruitment across soil types (Johri et al. 1999; Rodríguez and Fraga 1999; Chen et al. 2006; Oliveira et al. 2009; Pérez-Jaramillo et al. 2018; Aranda et al. 2011).

The most common PSB strains isolated so far belong to the genera *Pseudomonas, Bacillus, Enterobacter, Rhizobium, Arthrobacter, Flavobacterium,* and *Azospirillum* (Johri et al. 1999). Numerous reports from in vitro, greenhouse and field studies have shown that soil microbial inoculants comprised of *Bacillus, Enterobacter,* and *Pseudomonas* enhance productivity in horticultural crops when applied alone or in combination with commercial fertilizer treatments (Richardson 2001; Baas et al. 2016). In addition, *Bacillus, Pseudomonas,* and *Enterobacter* strains have also shown abilities for solubilizing and mineralizing organic and inorganic P forms (Kim et al. 1998; Guang-Can et al. 2008; Afkairin et al. 2021). In agreement with this, we showed that a single PSB strain can solubilize more than one plant-unavailable form of P (both calcium and phytin) in vitro and that soils inoculated with a consortium of PSB species increase potato plant biomass and P content.

Effects of individual and co-inoculated PSB on organic and inorganic P sources

Under in vitro conditions, we observed that the increase in dissolution of P by the bacterial consortium was significant for both forms of P tested, but more effective for calcium phosphate than for the organic form (phytin)

suggesting a higher efficiency of the consortia to solubilize calcium phosphate (Adnan et al. 2017). This is consistent with Guang-Can et al. (2008) who showed greater abilities of P solubilization under inorganic P sources, and with Baas et al. (2016) who demonstrated increased P uptake and productivity in various plant crops by P-mobilizing consortia. The affinity of bacteria isolates to utilize a given P form can also be attributed to their specific metabolism or temporal requirements of P. In addition, under our study conditions, calcium phosphate was the primary form of plant-unavailable P used for isolation of bacteria from soil slurries of wild potato rhizosphere. This supports the idea that PSB may be more effective depending on the type of plant-unavailable P used for its isolation as well as the type of P source that PSB encountered after its inoculation to soils.

Effects of PSB co-inoculation on plant growth and soil P under greenhouse conditions

In our greenhouse experiment, potato plants were grown under heat-sterilized soil to lower the population of microbes and enhance the influence of the newly introduced bacterial strains and their P solubilization activity. A global assessment of biofertilizer performance showed significant yield responses and improved P uptake for various crops inoculated with PSB under field conditions, thus evidencing that PSB have the potential to be effective with or without a competition from a native soil microbial community (Schütz et al. 2018). In agreement with this, we observed an overall significant effect of PSB inoculation in total plant biomass. However, the increase in plant biomass was absent for phytin treatment and the control, despite bacterial inoculation. This contradicts our *in vitro* studies where it was shown that PSB significantly dissolved P under both calcium phosphate and phytin. These discrepancies could be explained by the potentially insufficient bacterial P solubilization from phytin to supply the P demand for plant growth (Baas et al. 2016). In addition, our in vitro study showed that the bacterial consortium was more effective at solubilizing calcium phosphate, producing several folds more dissolved P than under phytin. Furthermore, our initial isolation process used primarily a calcium phosphate source, which further explains why the bacterial isolates preferred the mineral P over the organic source (Kim et al. 1998).

The highest availability of soil P in soils for plants is thought to be within the range of 5–6 pH units (Barrow 2017). P availability likely decreases as it approaches neutrality and beyond. We observed that soil pH from calcium phosphate, phytin and the combination of P source treatments remained stable. However, P fertilizer (TSP) treatment reduced the pH from 6.7 to 5.5. This reduction

in pH could have further increased the availability of soil P for plants, affecting both, PSB inoculated and non-inoculated treatments equally. However, we suspect that the primary reason for TSP showing a substantial increasing in plant growth, compared to all other treatments, is its highly soluble and readily available characteristics of this P source. Conversely, non-inoculated treatments with plant-unavailable P (calcium phosphate and phytin) did not substantially increase or decrease the pH. Despite this, these same plant-unavailable P treatments reduced plant growth when compared to non-inoculated, non-amended control treatment. This may be due to the binding properties of calcium phosphate and phytin on the existing orthophosphate in the substrate or phytin, as organic substrate could have caused microbial growth and immobilization. However, available P values in the phytin treatments were relatively similar to TSP. This observation may indicate that phytin had a toxic effect on plant growth. It has been reported that organic P (i.e., phytin) enhances the phosphatase activity of plants surpassing plant's demand and that high availability of phytin (20 mg P/100 mL) had a toxic effect to plants (Tarafdar and Claassen 1988). Interestingly, upon inoculation, PSB appears to have counteracted the binding effect of P unavailable forms by increasing plant growth under the calcium phosphate treatment but not under phytin. This observation agrees with Adnan et al. (2017) who found that PSB inoculation nullified the antagonistic effect of soil calcification on bioavailable P under both mineral and organic P sources.

Effects of PSB co-inoculation on P uptake and utilization efficiencies

Co-inoculation of *Enterobacter* sp., *Bacillus* sp., and *Pseudomonas* sp. increased plant P content across treatments. This result agrees with previous research indicating a greater P availability and plant crop uptake after soil inoculation of P-solubilizing microorganisms (Alori et al. 2017; Bargaz et al. 2018). Further, the efficiency of the plant to uptake P and to transform this P into biomass in response to bacterial inoculation was evaluated. Impacts of PSB on P uptake and P utilization were only marginally significant; however, our findings suggest that PSB inoculation can improve P use efficiency after only 60 days of plant development. These findings are consistent with several reports documenting the improvement in nutrient use efficiency in potato under controlled and field conditions with PSB addition (Johri et al. 1999; Munda et al. 2015; Adesemoye et al. 2008; Naqqash et al. 2016). Lastly, in our study we demonstrated the efficacy of soil microbial inoculation on the potato plant's ability to acquire and utilize various forms of P. However, it is

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important to consider that PSB can have multiple mechanisms other than P solubilization. These include secretion of phytohormones, antibiotics used as biocontrol, or the substances that chelate specific nutrients, which were not tested under our experimental conditions (Gómez-Muñoz et al. 2018; Etesami and Maheshwari 2018; Ahmed and Holmström 2014). Most importantly, we showed that PSB inoculation is effective under soils containing insoluble P forms such as calcium phosphate, a prevalent form of plant-unavailable P in agricultural soils. Thus, higher P bioavailability of sparingly soluble P can be translated into reduced P fertilizer inefficiencies and potentially greater profit for farmers. In summary, these results demonstrate the ability of PSB isolated from wild plants' rhizosphere soil to enhance crop P nutrition and their potential to increase availability of sparingly soluble forms of P in soils, thereby increasing crop yield and reducing P fertilizer inefficiencies.

Conclusions

In this study, we show that wild potato *S. bulbocastanum* effectively recruit PSB with the ability to assimilate organic and inorganic P forms under in vitro and *in planta* conditions in modern potato cultivars. Furthermore, we found that co-inoculation of *E. cloacae*, *P. pseudoalcaligenes*, and *B. thuringiensis* has the potential to increase plant biomass, yield, and P nutrient uptake in potatoes. Lastly, our findings indicate that PSB can be used as a sustainable alternative to promote phosphorus availability for potatoes; however, its efficacy in the field requires further testing.

Abbreviations

P: Phosphorus; PSB: Phosphorus-solubilizing bacteria; NBRIP: National Botanical Research Institute Phosphate; AB-DTPA: Ammonium bicarbonate-diethylenetriaminepentaacetic acid; C: Carbon; OD: Optical density; ICP-OES: Coupled plasma-optical emission spectrometer; TSP: Triple super phosphate.

Supplementary Information

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Additional file 1. Supplementary File.

Author contributions

HAP and JMV conceived and designed the experiment. HA and YH conducted the experiments. HAP analyzed the data under the guidance of JV, DKM, and SJF. All authors contributed to the interpretation of the data, discussion of results, writing, and revision of the manuscript prior to submission. All authors read and approved the final manuscript.

Availability of data and materials

All data generated during the current study are included in this published article and its supplementary information files.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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