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RESEARCH ARTICLE

Valorization of phosphate sludge and its bacterial biomass as a potential bioformulation for improving tomato growth

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Abstract

Phosphorus (P) is a vital limiting nutrient element for plant growth and yield. In Morocco, the natural phosphate rock extractions generate significant amounts of phosphate wash sludge (PS), which could be reused productively, thus creating another added value for farmers. The present study aimed to demonstrate the combination effect of soil amendment by two different PS concentrations (1% and 5%) associated with three phosphate-solubilizing bacteria (PSB) consortia (C1, C2, and C3), isolated from phosphate mining sludge, on plant growth and nutrient uptake in tomato seedlings (*Solanum lycopersicum*). The results obtained showed that this bioformulation significantly improved P solubilization and plant growth compared to control conditions. Of all the combinations, C3-inoculated soil amended with 5% PS was the most effective in significantly improving plant height and dry and fresh biomass of shoots and roots. P solubilization and its availability for tomato seedlings uptake were maximal with the bioformulation (C3 + 5% PS). This latter enhanced P and potassium (K) uptake by 27.89 and 38.81% in shoots and 38.57% and 74.67% in roots, respectively, compared to non-inoculated soil amended with 5% PS. The highest flowering rate (200 %) was recorded in C3-inoculated soil amended with 5% PS. Supporting these results, the principal component analysis discriminated this bioformulation (C3 + 5% PS) from the other combinations. Our results open up prospects for upgrading phosphate sludge enriched with PSB consortia as a biofertilizer that can be used in ecofriendly agriculture integrated into the circular economy.

Keywords Phosphate sludge · *Solanum lycopersicum* · Phosphate-solubilizing bacteria · Plant growth · Biofertilizer

Introduction

Morocco has the largest phosphate rock reserve, approximately 70% of the estimated global phosphate reserve (El Bamiki et al. 2021). During the treatment of phosphate rock, large quantities of derivatives are produced, including phosphate wash sludge, which contains significant amounts of insoluble phosphorus (Ait-Ouakrim et al. 2023a, 2023b). They are stored in large ponds covering large areas, and their accumulation causes a storage capacity problem (Hakkou et al. 2016). Therefore, recycling phosphate sludge is mandatory to exploit optimally the annual quantities produced and integrate it into a circular economy. Phosphate sludge can be reused as a source of phosphorus for plants (Ait-Ouakrim et al. 2023a, 2023b; Benbrik et al. 2020). However, most phosphorus in sludge exists in various insoluble forms (Hakkou et al. 2016), whereas only the primary and secondary orthophosphate ions (H_3PO_4, $H_2PO_4^{-}$, HPO_4^{2-}, HPO_4^{3-}) are assimilated by plants as nutrients (Bargaz et al.

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2021). Mobilization of insoluble phosphate from the soil in an assimilable soluble form for plants may be ensured by various microorganisms, including the solubilizing bacteria of phosphate (PSB) (Ait-Ouakrim et al. 2023a, 2023b; Aliyat et al. 2022; Benbrik et al. 2020). This group of bacteria is characterized by an important mineral phosphate solubilizing and organic phosphorus mineralization activity.

The solubilization of inorganic phosphorus depends mainly on the ability of PSB to produce organic acids and secrete H^+ protons, releasing phosphorus in the assimilable form into the soil by lowering the pH of the rhizosphere (Wei et al. 2018). Organic acids can directly dissolve mineral phosphate through the exchange of a phosphate anion with an acid anion or through the chelation of the associated Fe, Al, and Ca ions in the soil complexes (Rawat et al. 2021; Sharma et al. 2013). Other studies have reported that PSB could also solubilize potassium, improve nitrogen fixation, and produce phytohormones (Mitra et al. 2020; Rawat et al. 2021). PSB are widely examined for their plant growth-promoting properties and biocontrol abilities such as increased plant growth, plant biomass, availability of nutrients, and mitigation of diverse biotic and abiotic stresses in plants (Aliyat et al. 2022; Bargaz et al. 2021; Benbrik et al. 2020; Pandey et al. 2012). The plant growth promotion (PGP) traits function additively and synergistically, and multiple mechanisms are responsible for promoting plant growth and increasing yield, including increasing available mineral nutrients, moderating phytohormone rates, and acting as biocontrol agents of phytopathogens (Emami et al. 2019; Kong and Liu 2022).

Tomato (*Solanum lycopersicum*) is among the major field and greenhouse vegetable crops grown all over the world. The latest FAO data reveal that tomato production has increased globally over the past 60 years, with 5.03 million ha annually producing over 180.8 million tons of tomato fruits (FAOSTAT 2023). Tomatoes serve important functions in human nutrition and health since they are substantial sources of carotenoids and other elements for the diet that promote health (Meng et al. 2022). Utilizing safe and non-toxic biofertilizers to the environment, based on high-quality PSB formulations, to manage vegetable crop production systems in an integrated, intensive, and sustainable manner can improve fertilization efficiency, lower fertilizer production costs, boost soil productivity, and support the sustainability of agricultural land (Billah et al. 2019). Previous studies above-provided insight regarding the potential of native phosphate solubilizing bacteria (isolated from phosphate sludge) to boost plant development and yields by making phosphorus available to plants (Ait-Ouakrim et al. 2023a, 2023b). A wiser agricultural co-application of P sludge and PSB assumes paramount importance to enhance agronomic profitability, environmental sustainability, and economic viability of phosphorus nutrient utilization.

Our study aimed to evaluate the phosphate sludge and its bacterial biomass as a potential source of biofertilizers for soil enrichment and tomato growth promotion by assessing two different concentrations of P sludge with three different PSB consortia.

Material and methods

Bacterial consortium preparation

Six PSB strains were selected for their ability to dissolve inorganic phosphate and other plant growth promotion activities. Three strains were isolated from phosphate sludge and identified as *Brevibacterium frigoritolerans* HFBP01, *Bacillus vallismortis* HFBP15*, Streptomyces venezuelae* HFBP26 (Ait-Ouakrim et al. 2023a), and three others from rhizosphere soil of olive tree grown in phosphate sludge identified as *Pseudomonas moraviensis* HFBPR01, *Bacillus cereus* HFBPR04, and *Bacillus aryabhattai* HFBPR40 (Ait-Ouakrim et al. 2023b) (Supplementary data 1). The *s*ix strains were prepared separately in 150 mL of tryptic soy broth (10%) liquid medium and incubated at 28 °C in a rotary shaker (120 rpm) for 48 h. After incubation, the optical density was adjusted to 10^8 CFU mL^{-1}. Three consortia (C) were formulated as follows: C1 (HFBPR01, HFBPR04, and HFBPR40), C2 (HFBP01, HFBP15, and HFBP26), and C3 (C1+C2). The biocompatibility of these PSB strains was tested, and the results obtained showed that they have no antagonistic effects between them (Ait-Ouakrim et al. 2023a, 2023b).

Tomato seed inoculation

Tomato seeds (*Solanum Lycopersicum* 'Campbell 33') were disinfected in 10% of sodium hypochlorite and sown directly in commercial potting soil. Two-week-old seedlings were transplanted into plastic pots (15 cm in diameter and 20 cm in depth) previously disinfected and filled with 2 kg of soil (S) mixed with different concentrations of phosphate sludge (PS) in the following proportions: PS 0%, PS 1%, and PS 5%. After that, tomato seedlings were inoculated with 10 mL of the bacterial consortia near the root area after transplantation and 1 month later. The negative controls received 10 mL of physiological saline solution. The experiment included 12 treatments as shown in Table 1. Ten pots of each treatment were established and kept for 80 days under controlled greenhouse conditions (25 ± 2 °C, 60 ± 5% relative humidity, and a photoperiod of 16:8 h) and watered every 48 h. Each pot contained a single tomato seedling.

Table 1 Experimental design of the conducted study describing the different treatments applied

Treatment	Description
S	Soil without inoculation (negative control)
S + C1	Soil inoculated with C1
S + C2	Soil inoculated with C2
S + C3	Soil inoculated with C3
S + PS1%	Soil amended with 1% of PS
S + PS1% + C1	Soil amended with 1% of PS and inoculated with C1
S + PS1% + C2	Soil amended with 1% of PS and inoculated with C2
S + PS1% + C3	Soil amended with 1% of PS and inoculated with C3
S + PS5%	Soil amended with 5% of PS
S + PS5% + C1	Soil amended with 5% of PS and inoculated with C1
S + PS5% + C2	Soil amended with 5% of PS and inoculated with C2
S + PS5% + C3	Soil amended with 5% of PS and inoculated with C3

Soil analysis

pH and conductivity

Both soil pH-H_2O and KCl-pH were measured by mixing 10 g of soil (+3 g of KCl for the KCl-pH) with 20 mL of distilled water; the suspension was agitated for 30 min before the pH measurement. Electrical conductivity was determined using a conductivity meter by dissolving 10 g of soil in 100 mL of distilled water.

Available P and total P in the soil

Available P in soil was measured using the method of Olsen et al. (1954). In order to monitor changes and trends of available P in the soil during the experiment and taking into account the variety of tomato studied, its development cycle, and the substrate used and its volume, the measurements were performed four times, at T0 (time before inoculation) corresponding to control, T40 (40 days after inoculation), T60 (60 days after inoculation), and T80 (80 days after inoculation). Soil (1 g) was dissolved in 20 mL of sodium bicarbonate; the suspension was stirred for 1 h and filtered using filter paper. A 1 mL of suspension was mixed with 5 mL of molybdate-hydrazine sulfate reagent (10 mL of 0.15% hydrazine sulfate was mixed with 20 mL of sodium molybdate solution). The mixture was incubated for 10 min at 70 °C in a water bath. The amount of phosphorus was estimated using a standard curve of KH_2PO_4 by measuring the optical density at 820 nm. Total P was determined by colorimetry method after mineralization of soil samples using the same method described above. A 0.1 g of soil sample mineralized at 550 °C for 6 h was dissolved in 2 mL of HCL 10%, and then, 20 mL of distilled water was added after evaporation of HCl. The obtained suspension was filtered, and the filtrate was used for the determination of total P.

Mineral elements K^+, Na^{2+}, and Ca^{2+}

Soil samples were mineralized for 4 h at 500 °C. Each sample (100 mg) corresponding to each treatment was mixed with 2 mL HCl (10%). After the evaporation of HCl, 20 mL of distilled water was added, and then, the mixture was filtered using filter paper. The resulting solutions were used to determine the concentration of each element by flame atomic absorption spectrometry (Gaines and Mitchell 1979).

Plant material analysis

Plant growth promotion traits

After transplanting the tomato seedlings, their initial shoot length was measured. Thereafter, the monitoring of this parameter was carried out weekly throughout the growing period. The flowering rate was determined as soon as the flowers appeared. In order to assess the biomass allocation within tomato seedlings in response to different treatments, the fresh and dry biomass was measured. The fresh weight (both shoot and root, separately) was measured after plant harvesting and root cleaning, while the dry weight was measured after drying the plant material for 72 h at 65 °C. There were ten replicates per treatment (one plant per replicate) for shoot length and three for biomass weight.

Determination of total phosphorus and potassium

The amount of total phosphorus and potassium in both shoots and roots was determined using 100 mg of dry matter previously ground and mineralized in the same manner as that described above in "Available P and total P in the soil" and "Mineral elements K^+, Na^{2+}, and Ca^{2+}", respectively. There were three replicates per treatment (one plant per replicate).

Statistical analysis

Results were analyzed statistically with the SPSS V25 software for Microsoft Windows. The analysis includes the analysis of variance (ANOVA) followed by a comparison of the means ($p \geq 0.05$) with Tukey's post hoc test after checking the normality and the homoscedasticity of data. A principal component analysis (PCA) was also performed to identify the discriminating variables between different treatments.

Results

Physicochemical characteristics of the soils used

The three substrates used in this study with different concentrations of phosphate sludge (0, 1, and 5 % PS) were characterized physicochemically (Table 2). Results showed that PS-deficient soil has an alkaline pH by referring to both pH-KCl and pH-H_2O measurement and an electrical conductivity of about 52.30 µS cm^{-1}. Regarding pH-H_2O, it was kept relatively stable and alkaline after soil amendment with PS without any significant statistical difference between the three substrates ($p \leq 0.05$). Soil amendment significantly increased the amount of total phosphorus, available phosphorus, Na, and Ca. The highest concentrations of these mineral elements were recorded in the soil amended by 5% PS. The concentration of 5% PS increased the available P content in soil by about 71.6 and 31.9% compared to PS-deficient soil and soil amended by 1%, respectively (Table 2). However, K content has not changed significantly between PS-deficient soil and PS-amended soil.

Dynamics of available phosphorus in soil

The dynamic of available phosphorus in the soil during the tomato-growing period exhibited significant differences with time and between treatments ($p \leq 0.05$) (Table 3). Available P varied in proportion to the PS concentration, such that the concentrations of 1 and 5% significantly increased the available P by about 19.8 and 73.3%, respectively, compared to PS-deficient soil at time 0. The levels of available P also rose significantly in the PS-deficient soil inoculated with PSB consortia in comparison with the non-inoculated ones. Regardless of the time factor, the combination of PS and PSB consortia thus significantly increased available P. Nonetheless, the available

Table 2 Physical and chemical characteristics of different substrates used

Parameter	Deficient soil	Deficient soil + PS (1%)	Deficient soil + PS (5%)
Total P (ppm)	1018.44 ± 20.45c	1743.24 ± 47.38b	2459.31 ± 153.70a
Available P (ppm)	107.18 ± 3.11c	139.47 ± 1.78b	183.97 ± 1.85a
K^+ (ppm)	29.23 ± 2.78a	30.00 ± 1.46a	31.60 ± 3.50a
Na^{2+} (ppm)	31.60 ± 0.74b	36.00 ± 1.58b	46.20 ± 2.55a
Ca^{2+} (ppm)	143.90 ± 1.73c	204.10 ± 4.00b	353.10 ± 24.58a
pH-H_2O	8.35 ± 0.10a	8.37 ± 0.4a	8.5 ± 0.1a
pH-KCl	7.82 ± 0.09	-	-
Electrical conductivity (µS cm^{-1})	52.30 ± 0.30	-	-

Values are the mean of $n = 3$ (mean ± standard deviation). Values with different letters are significantly different at $p \leq 0.05$

Table 3 Dynamics of assimilable phosphorus in the soil under different combinations (phosphate sludge (PS) and PSB consortia (C))

Combination	Assimilable phosphorus (ppm)			
	0 d	40 d	60 d	80 d
S	80.18 ± 3.09Ac	63.17 ± 7.99Bg	51.72 ± 4.68Be	56.11 ± 4.22Be
S + C1	80.18 ± 3.09Bc	94.57 ± 4.24Aef	83.66 ± 4.22ABd	88.04 ± 5.15ABd
S + C2	80.18 ± 3.08Ac	91.44 ± 5.61Aef	80.33 ± 6.83Ad	85.40 ± 9.76Ad
S + C3	80.18 ± 3.09Bc	92.98 ± 5.82Aef	87.02 ± 2.45ABd	91.67 ± 5.37ABd
S + PS1%	96.05 ± 4.56Ab	75.76 ± 8.88Bfg	81.65 ± 10.33ABd	74.33 ± 5.66Bde
S + PS1% + C1	96.05 ± 4.56Cb	144.34 ± 5.46ABcd	132.02 ± 4.68Bbc	152.67 ± 7.86Aab
S + PS1% + C2	96.05 ± 4.56Bb	137.50 ± 8.47Ad	125.71 ± 8.75Ac	136.33 ± 6.24Abc
S + PS1% + C3	96.05 ± 4.56Bb	159.73 ± 9.26Abc	141.39 ± 12.10Aabc	159.33 ± 4.70Aa
S + PS5%	138.96 ± 3.19Aa	110.27 ± 5.09Be	85.04 ± 4.67Cd	89.33 ± 6.12Cd
S + PS5% + C1	138.96 ± 3.19Ca	165.64 ± 7.81Ab	151.30 ± 3.30BCab	160.67 ± 4.69ABa
S + PS5% + C2	138.96 ± 3.19Ba	159.36 ± 8.38Abc	137.17 ± 7.49Babc	130.00 ± 5.01Bc
S + PS5% + C3	138.96 ± 3.19Ca	189.90 ± 4.96Aa	157.67 ± 7.05Ba	166.34 ± 9.18Ba

Values are the mean of $n = 3$ (mean ± standard deviation). Values with different lowercase letters in each column and uppercase letters in each row are significantly different at $p \leq 0.05$

P concentration continuously reduced over time in PS-deficient soil and non-inoculated soil amended with PS. Conversely, the presence of PSB consortia increased the available P over time, reaching maximum values only after 40 days for most treatments (Table 3). The highest available P amounts were recorded in C3-inoculated soil amended with 5% PS, with an increase of approximately 72.21, 85.41, and 86.20 % noted after 40, 60, and 80 days of the tomato-growing period, respectively, in comparison with the corresponding non-inoculated soil amended with 5% PS.

Length of shoot of tomato seedlings

Results of the effect of different concentrations of phosphate sludge and PSB consortia on tomato shoot length are presented in Table 4. Significant statistical differences were recorded in this growth trait according to time and treatment factors ($p \leq 0.05$). Regarding the time effect, change in shoot length usually began to increase significantly from the second week in different treatments, compared to the control (0 W). The maximum shoot length is reached in the last week of the tomato-growing period, especially in C3-inoculated soil amended with 5% PS (59.56 cm) followed by C1-inoculated soil amended with 5% PS (53.40 cm). Compared to soil amended with 5% PS, C3, and C1 increased significantly tomato shoot length by about 33.84 and 20.0 %, respectively.

Dry and fresh biomass of tomato seedlings

Shoot and root dry and fresh biomass varied significantly according to the consortium type and the phosphate sludge concentration ($p \leq 0.05$) (Figs. 1 and 2). Tomato seedlings growing in the PS-deficient soil and inoculated with different consortia showed no significant difference in the shoot and root dry and fresh biomass compared to non-inoculated ones. However, C3 significantly improved these growth traits in both soils amended with 1% and 5% PS compared to other consortia, except for shoot dry biomass, where both C1 and C3 showed the highest values in soil amended with 1% PS. Among treatment combinations between PS and PSB, C3-inoculated soil amended with 5% PS was the favorable and optimal association to significantly increase shoot and root dry and fresh biomass by ensuring good vegetative growth and development. Indeed, C3 improved shoot dry biomass by about 15.76% (Fig. 1a), root dry biomass by 36.25% (Fig. 1b), shoot fresh biomass by 22.32% (Fig. 2a), and root fresh biomass by 46.93% (Fig. 2b), in soil amended with 5% PS compared to that enriched with 1% PS. Nonetheless, the values of fresh and dry biomass of tomato shoots are greater than those recorded in roots (Figs. 1 and 2).

Phosphorus and potassium uptake in tomato seedlings

The addition of PS significantly increased P and K in tomato shoots and roots ($p \leq 0.05$) (Table 5). This accumulation was more pronounced in soil amended with 5% PS than that amended with 1% PS. P concentration increased by about 52.77 and 93.09% in shoots and roots, respectively, while K concentration was 6.72% in shoots and 103.32% in roots, compared to the control (PS-deficient soil). Furthermore, inoculation by PSB consortia significantly improved the P and K uptake in tomato shoots and roots compared to the control. C3 has shown the best results compared to other consortia. Regarding the combinations between different PSB inoculation and PS amendment, C3-inoculated soil amended with 5% PS enabled the highest uptake concentrations of P and K in both shoots and roots. Indeed, this combination enhanced P uptake by 27.89 and 38.57% in shoots and roots, respectively, compared to non-inoculated soil amended with 5% PS. As for K uptake, the said combination increased K by about 38.81% in tomato shoots and 74.67% in roots. In terms of comparison between plant parts, the P and K levels recorded in the shoots are higher than those noted in the roots under control and treated conditions (Table 5).

Flowering percentage in tomato seedlings

Tomato flowering percentage was calculated by referring to the results recorded in PS-deficient soil and not inoculated and presented in Table 6. The flowering percentage increased with the increase in phosphate sludge concentration in the soils enriched with C1 and C3. The highest flowering rate was observed in C3-inoculated soil amended with 5% PS (200%).

Multivariate statistical analysis

Principal component analysis was applied to the parameters studied in tomato seedlings under different treatments. The major part of the cumulative variance (91.47%) was represented by the first two principal components (PC1: 85.25% and PC2: 6.22%). According to 2D space (Fig. 3A) and the matrix of components, PC1 is strongly correlated with all parameters examined in the positive part, mainly with shoot fresh and dry biomass and root dry biomass, while P available, root K content, and shoot P content are most correlated PC2 in the positive side. The distribution of traits studied allowed distinguishing four main treatment groups (Fig. 3B): group 1 comprises the soil amended with 5% PS and inoculated with C1 and C2 as well as soil amended with 1% PS and inoculated with C1 and C3, group 2 includes the soil amended with 1% PS and inoculated with C2 and 1% and

Table 4 Effect of soil enrichment with phosphate sludge and PSB consortia on tomato shoot length during 10 weeks of the growing period

	0 w	1 w	2 w	3 w	4 w	5 w	6 w	7 w	8 w	9 w	10 w
S	5.99 ± 0.60Habc	7.53 ± 1.43GHab	10.82 ± 1.48Ga	16.04 ± 1.58Fa	21.06 ± 2.42Eab	26.60 ± 3.39Da	30.37 ± 3.90CDabc	33.68 ± 3.93Cb	38.65 ± 3.54Bbc	42.60 ± 3.20ABcdef	45.75 ± 2.07Acdef
S + C1	5.99 ± 0.59Jabc	6.09 ± 1.59IJcd	9.20 ± 1.61Ibc	14.69 ± 1.92Hab	20.60 ± 1.43Gabc	26.42 ± 2.75Fab	30.25 ± 3.04Eabc	34.84 ± 2.58Db	39.80 ± 2.44Cb	44.70 ± 2.55Bc	49.00 ± 2.21Acd
S + C2	5.31 ± 0.23Icd	5.20 ± 1.18Id	8.28 ± 1.46Hc	14.98 ± 1.95Gab	21.05 ± 1.91Fab	26.50 ± 1.97Eab	31.39 ± 2.48Dab	34.65 ± 2.57Cb	40.00 ± 2.67Bb	45.15 ± 2.11Abc	47.95 ± 2.30Acde
S + C3	5.49 ± 0.41Jbcd	6.00 ± 0.94Jcd	9.21 ± 0.83Ibc	15.90 ± 1.02Ha	22.00 ± 1.39Ga	26.12 ± 1.82Fab	29.78 ± 1.88Ebc	34.59 ± 2.48Db	39.48 ± 3.05Cbc	44.35 ± 2.73Bcd	47.85 ± 3.00Acde
S + PS1%	5.75 ± 0.46Habcd	7.18 ± 0.74GHabc	9.43 ± 0.90Gabc	13.41 ± 1.28Fbc	17.42 ± 2.04Ede	20.37 ± 2.09Ed	23.78 ± 2.68De	26.65 ± 3.27Dd	30.75 ± 2.74Ce	38.60 ± 2.27Bf	41.88 ± 1.79Af
S + PS1% + C1	5.22 ± 0.32Hd	6.58 ± 0.79GHbcd	9.12 ± 0.89Gbc	13.69 ± 0.87Fbc	18.33 ± 1.05Ecde	23.25 ± 1.54Dbcd	28.14 ± 2.05Cbcd	31.83 ± 2.11Bb	34.15 ± 2.08Bde	43.00 ± 3.59Acde	45.20 ± 3.33Adef
S + PS1% + C2	6.08 ± 0.69Hab	6.85 ± 0.80Habc	8.89 ± 1.02GHbc	12.29 ± 1.86Gc	16.67 ± 2.37Fe	21.19 ± 2.44Ecd	24.75 ± 2.35DEde	27.62 ± 2.56Dcd	35.50 ± 2.42Ccd	40.20 ± 3.58Bdef	44.25 ± 5.77Aef
S + PS1% + C3	5.25 ± 0.25Jcd	6.55 ± 0.55IJbcd	9.64 ± 0.58Iabc	14.76 ± 1.01Hab	20.03 ± 1.84Gabcd	24.82 ± 2.67Fab	29.40 ± 2.29Ebc	33.68 ± 3.14Db	37.90 ± 4.36Cbcd	45.60 ± 3.66Bbc	49.50 ± 3.06Abc
S + PS5%	5.43 ± 0.28Jbcd	6.40 ± 0.46Jbcd	9.04 ± 0.65Ibc	13.96 ± 1.03Habc	18.64 ± 0.94Gbcde	24.01 ± 1.08Fabc	27.35 ± 1.68Ecd	31.19 ± 1.89Dbc	35.65 ± 1.62Ccd	40.00 ± 1.49Bef	44.50 ± 1.78Aef
S + PS5% + C1	6.42 ± 0.71Ja	8.04 ± 0.68IJa	10.35 ± 0.79Iab	15.07 ± 1.20Hab	20.60 ± 1.51Gabc	25.99 ± 1.94Fab	30.30 ± 1.37Eabc	35.15 ± 2.90Db	41.35 ± 1.29Cb	49.00 ± 3.17Bb	53.40 ± 1.74Ab
S + PS5% + C2	5.85 ± 0.60Jabcd	6.95 ± 0.83Jabc	9.57 ± 1.03Iabc	13.74 ± 1.09Hbc	18.98 ± 1.60Gbcde	23.48 ± 1.74Fabcd	28.13 ± 1.62Ebcd	32.25 ± 1.72Db	38.45 ± 1.96Cbc	44.65 ± 3.17Bc	48.85 ± 1.92Acd
S + PS5% + C3	5.88 ± 0.58Jabcd	6.39 ± 0.25Jbcd	10.02 ± 1.02Iab	13.75 ± 1.34Hbc	19.70 ± 1.86Gabcd	25.42 ± 2.04Fab	33.61 ± 1.25Ea	40.15 ± 1.81Da	49.20 ± 2.70Ca	53.40 ± 1.71Ba	59.56 ± 1.88Aa

Values are the mean of $n = 10$ (mean ± standard deviation). Values with different lowercase letters in each column and uppercase letters in each row are significantly different at $p \leq 0.05$

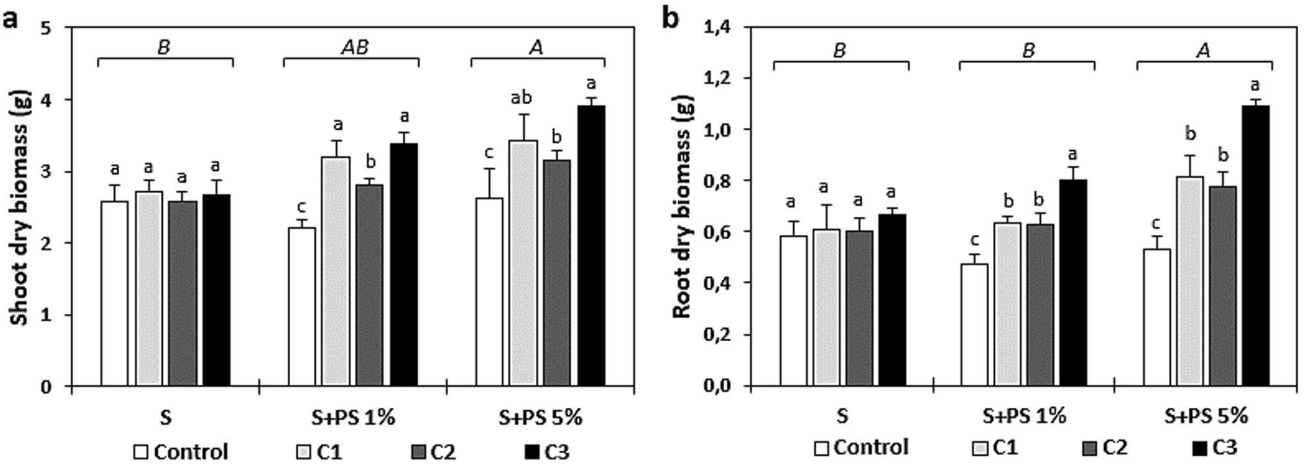

Fig. 1 Shoot (**a**) and root (**b**) dry biomass of tomato seedlings under different combinations (phosphate sludge (PS) and PSB consortia (C)). Values are the mean of $n = 3$ (mean ± standard deviation). Different lowercase and uppercase letters indicate significant differences at $p \leq 0.05$

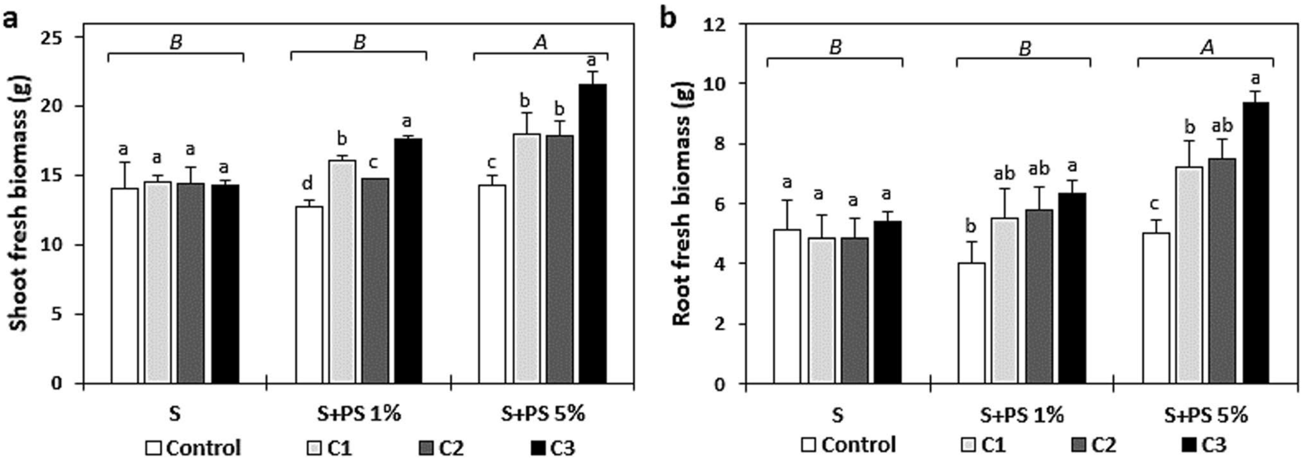

Fig. 2 Shoot (**a**) and root (**b**) fresh biomass of tomato seedlings under different combinations (phosphate sludge (PS) and PSB consortia (C)). Values are the mean of $n = 3$ (mean ± standard deviation). Different lowercase and uppercase letters indicate significant differences at $p \leq 0.05$

5% SP-enriched non-inoculated soil, group 3 includes the PS-deficient soil and the one inoculated with the three PSB consortia, and group 4 comprises only the C3-inoculated soil amended with 5% PS. Among these four groups, the treatment combination of group 4 appeared the most favorable biofertilizing conditions to improve tomato growth and development.

Discussion

Phosphate wash sludge is one of the byproducts generated during the phosphate extraction industry's exploitation and subsequent rock phosphate treatment (Gherghel et al. 2019; Hakkou et al. 2016). It remains unexploited despite their considerable levels of residual phosphates complex, which could constitute at least an important resource of PSB biomass and phosphorus for soils depleted of this mineral as key nutrients for plant growth (Ait-Ouakrim et al. 2023a, 2023b; Benbrik et al. 2020; Di Capua et al. 2022; Gupta et al. 2021). In the present study, the combined inoculation of cultivated tomato seedlings, in soil amended with phosphate sludge, by selected PSB consortia isolated from the phosphate sludge and the rhizosphere of olive trees growing on this phosphate wash sludge showed promising biofertilizer potential to improve plant growth and production. The scientific community is increasingly interested in finding biotechnological approaches to make the residual phosphorus of phosphate sludge and their bacterial biomass available to crop farmers to boost crop production and productivity (Ait-Ouakrim et al. 2023a,

Table 5 Content of phosphorus (P) and potassium (K) in both tomato shoots and roots under different combinations (phosphate sludge (PS) and PSB consortia (C))

Organ	Treatment	P				K			
		Control	C1	C2	C3	Control	C1	C2	C3
Shoot	S	56.43 ± 1.50f	74.41 ± 7.31de	69.74 ± 1.66e	82.46 ± 0.64cd	1197.50 ± 8.08gh	1457.00 ± 36.85d	1184.00 ± 13.86h	1334.00 ± 3.86e
	S + PS 1%	69.46 ± 3.23e	85.71 ± 6.08bcd	75.17 ± 7.82de	97.00 ± 3.80b	1250.00 ± 24.65fg	1435.00 ± 25.06d	1572.50 ± 17.51c	1445.00 ± 10.02d
	S + PS 5%	86.21 ± 1.50bcd	95.04 ± 4.04bc	86.44 ± 1.06bcd	110.25 ± 4.54a	1278.00 ± 12.14ef	1662.50 ± 22.49b	1655.50 ± 12.68b	1774.00 ± 16.08a
Root	S	44.91 ± 2.52h	69.14 ± 2.33de	52.39 ± 2.99gh	67.74 ± 5.70def	211.00 ± 11.07g	477.00 ± 19.85de	426.00 ± 20.08e	517.00 ± 9.89cd
	S + PS 1%	61.07 ± 3.67efg	79.34 ± 5.61cd	56.35 ± 2.02fgh	85.76 ± 4.97c	351.00 ± 29.91f	567.00 ± 35.02c	553.00 ± 10.19c	637.00 ± 20.03b
	S + PS 5%	86.72 ± 5.11c	115.99 ± 4.94ab	107.72 ± 2.49b	120.17 ± 5.21a	429.00 ± 19.79e	638.00 ± 32.18b	570.00 ± 20.44c	749.35 ± 9.99a

Values are the mean of $n = 3$ (mean ± standard deviation). Values with different letters are significantly different at $p \le 0.05$

Table 6 Effect of soil enrichment with phosphate sludge and PSB consortia on tomato flowering percentage

	Flowering rate (%)		
	C1	C2	C3
PS1%	100.00	53.33	120.00
PS5 %	166.67	0.00	200.00

2023b; Aliyat et al. 2022; Azaroual et al. 2022; Benbrik et al. 2020; El Maaloum et al. 2020; Rfaki et al. 2020).

Analysis of soil physicochemical parameters revealed that adding phosphate sludge improves significantly the mineral composition of the soil, including the assimilable phosphorus, total phosphorus, calcium, and sodium. Inoculation with PSB consortia in soil amended by PS allowed for effective and sustainable mobilization of phosphorus from the sludge, compared to the non-inoculated soil. These results are consistent with studies done by Benbrik et al. (2020, 2021) and El Gabardi et al. (2021), which also tested the effect of PSB on P solubilization and release in soil amended with different concentrations of PS. PSB can be effective in making P more available to tomato plants from inorganic P source (PS) by solubilizing and mineralizing insoluble P compounds. The main mechanism of this solubilization involves the excretion of solubilizing agents, including organic acids, protons, and siderophores, which mainly act on P minerals. Organic acids, in particular, enhance P availability in the soil by forming complexes with cations like Al or Fe, or by obstructing P absorption sites on soil particles (Elhaissouf et al. 2022; Ait-Ouakrim et al. 2023a, b), resulting in facilitated and enhanced plant P uptake. P stands as an indispensable nutrient for plant development and growth, considering that P concentration can reach up to 0.5% of the plant's dry weight (Vance et al. 2003). Regarding P dynamic and availability for tomato seedlings, the significant increase in P availability during the 40-day post-inoculation period could be explained by the action of PSB, which solubilize and release P from PS into the soil making it more accessible to plants. After this period, the P availability remained maintained, exhibiting the durable effectiveness of PSB consortia. However, in some treatments, there may be a decrease but it remains significantly high compared to the control. This could be mainly related to the rate of plant growth and development stage, and its growing need to absorb available P (Bargaz et al. 2021). The soil amendment with PS associated with the inoculation with phosphate solubilizing microorganisms revealed a significant beneficial effect on phosphorus bioavailability, increasing the plant growth of some crops, including *Zea mays* (Benbrik et al. 2020) and *Solanum lycopersicum* (El Maaloum et al. 2020). Our findings showed that among all combinations, the "C3 + 5% PS" combination was the best to significantly improve tomato seedlings' growth, where the highest values of plant height and dry and fresh biomass of shoots and roots were recorded. The six strains forming the PSB consortium 3 (C3): *Brevibacterium frigoritolerans* HFBP01, *Bacillus vallismortis* HFBP15, *Streptomyces venezuelae* HFBP26, *Pseudomonas moraviensis* HFBPR01, *Bacillus cereus* HFBPR04, and *Bacillus aryabhattai* HFBPR40 have already shown a synergetic and significant biofertilizing effect on *Phaseolus vulgaris* (Ait-Ouakrim et al. 2023a, 2023b). These PSB strains selected and identified from Moroccan phosphate sludge presented multiple PGP traits, good tolerance to abiotic stresses (salinity, high temperature, pH, and heavy metal), and biocontrol potential against phytopathogens (*Fusarium oxysporum*, *Botrytis cinerea*, and *Verticillium dahliae*) (Ait-Ouakrim et al. 2023a, 2023b). To optimize the reuse of PS as a soil amendment, we tested low proportions in contrast to Benbrik et al. (2020) who used high

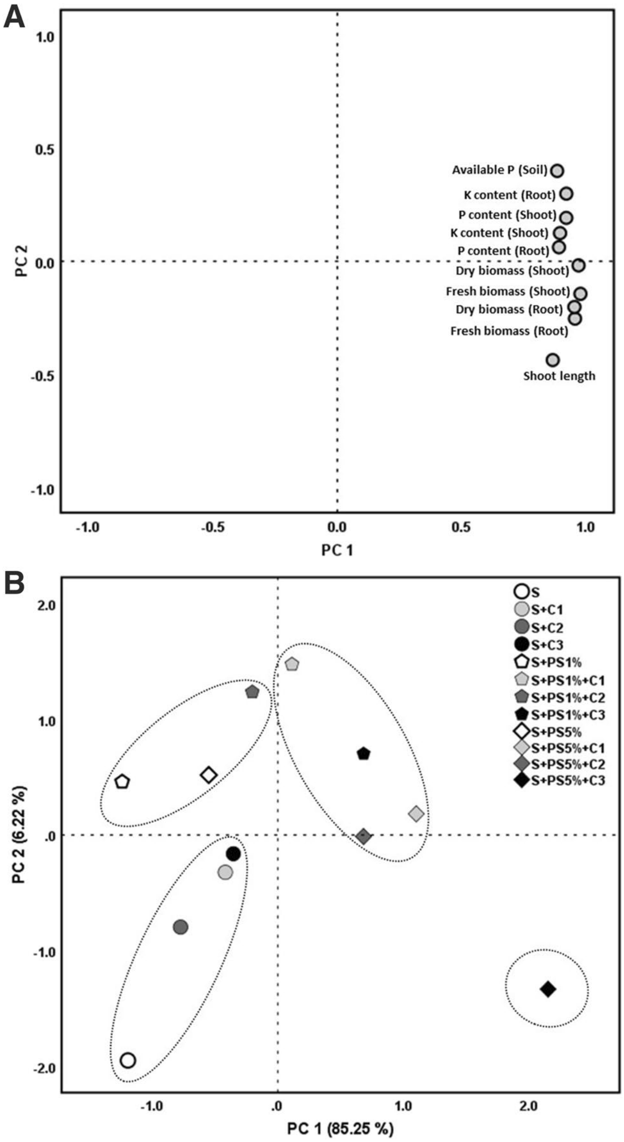

Fig. 3 2D scatterplots illustrating the distribution of the traits studied (**A**) and treatments (**B**) according to the two main principal components obtained by PCA in tomato seedlings

PS percentages (20–100%), whose 40% concentration was the best to increase the growth of *Zea mays* more than the other proportions used. Indeed, the soil amended with 5% PS favored the best plant growth of tomato seedlings, especially with PSB consortium 3. The few studies on soil amendment by direct addition of PS showed a positive effect on vegetative growth in plants inoculated with PSB (Benbrik et al. 2020; El Maaloum et al. 2020). In soil amended with 5% PS, the PSB consortium 3 (C3) significantly increased the shoot and root growth in tomato seedlings compared to the controls and other combinations (C1 and C2); this is mainly due to the tested high and efficient ability of these PSB strains to solubilize the insoluble P contained in the PS and make it available to plants (Ait-Ouakrim et al. 2023a, 2023b). The additive beneficial effect of this consortium (C3) on plant growth is due to the synergistic effect of bacterial strains that compose it and their biocompatibility. Previous studies have reported that the inoculation by bacterial consortia is more effective than the inoculation by single bacterial strains, so that each strain complements the limits of the other, allowing the consortium to provide various functions that a single bacteria may not perform (Ait-Ouakrim et al. 2023a, 2023b; El Maaloum et al. 2020; Gómez-Godínez et al. 2021). According to our findings, the "C3 + 5% PS" combination enabled us to obtain the highest uptake of P and K in both shoots and roots. Several researchers confirm the beneficial contribution of PSB in plant growth promotion through their solubilizing power, which further improves the availability and uptake of certain micronutrients, thereby ensuring the nutritional status and health of plants (Bargaz et al. 2021; Li et al. 2023; Rawat et al. 2021). By synthesizing the indole-3-acetic acid (IAA) hormone, a capability that was observed in the investigated PSB (Ait-Ouakrim et al. 2023a, 2023b), these bacterial consortia have the potential to stimulate the expansion of the plant's root system, thereby enhancing the absorption efficiency of P and K in tomato seedlings and increasing the growth rate. The same combination "C3 + 5% PS" ensured the highest flowering rate of tomato plants compared to the other combinations studied. PSB are characterized by a more diverse set of metabolic skills to improve the bioavailability of different forms of refractory P in the soil. It has been demonstrated that PSB inoculations enhance plant yield by changing the plant's P acquisition strategy and P distribution inside the plant (Li et al. 2023). In addition, the P requirements of plants vary according to the stages of plant development. It is extremely critical during the first weeks of growth and reaches its maximum demand at the flowering stage (Li et al. 2023).

The significant results of tomato plant growth promotion indicated that PSB enhanced the efficiency of PS by promoting its solubilization and providing more P into the mineral P pool (Ait-Ouakrim et al. 2023a, 2023b; Elhaissoufi et al. 2022). Due to its low reactivity against binding and adsorption phenomena of P in soils, PS in combination with PSB could meet tomato plants' higher requirements for P and other minerals, throughout the growth stages. PSB consortia have the ability to provide a readily accessible P fraction to tomato seedlings even during their earliest growth stages, with the assumption that the efficiency of PS utilization intensifies as plant roots expand and more extensively explore the soil rhizosphere. P, an essential nutrient, is requisite for several cellular processes, encompassing photosynthesis, carbohydrate metabolism, energy generation, maintenance of redox homeostasis, and cellular signaling (Siedliska et al. 2021). It assumes also a pivotal role in root development, including the anatomy of root traits and the density of root hairs (Elhaissoufi et al. 2022). If not supplied by soil sufficiently, PS amendment in combination with PSB consortia can therefore ensure significant amounts of soluble and available P to improve plant growth.

It is widely accepted that organic acids are initially secreted by PSB, which then chelate with phosphate-related cations through their carboxyl and hydroxyl groups or by releasing H^+ to decrease the pH of the rhizosphere, and the fixed P can then be transformed into an available form for plants. This rhizosphere bioacidification in the vicinity of the root system allows the release of phosphate from PS to plants and the use of soil nutrients (Ait-Ouakrim et al. 2023a, 2023b; Li et al. 2023). Mobilization of insoluble P by PSB is a key trait contributing to the formulation of effective and robust microbial biofertilizers. By maintaining their P-solubilizing ability, PSB strains have gained widespread acceptance as eco-friendly resources readily available for biofertilizer agents to replace chemical fertilizers. The improvement of plant growth and nutrition as well as the regulation of biotic and abiotic stresses tolerance could be effectively conferred by certain multi-trait PSB strains isolated from phosphate sludge (Ait-Ouakrim et al. 2023a, 2023b). Nonetheless, further studies would be necessary to rigorously assess the long-term impacts of using this bioformulation on soil health and fertility, carefully examining the potential influence of its repeated application on soil properties and microbial communities, and its effectiveness across various crop species to evaluate its versatility and suitability in diverse agricultural environments.

Conclusion

The present study has highlighted the ability of soil amendment by the combination of phosphate sludge and PSB consortium to improve the vegetative growth and flowering rate of tomato plants. The soil amended with 5% PS and associated with C3 inoculation showed the optimal performance of the P and K uptake in both tomato plants' shoots and roots. This bioformulation (C3 + 5% PS) could be the most appropriate eco-friendly and circular economy strategy to significantly improve P solubilization and mobilization, nutrient uptake, and crop growth and yield. The optimization of this bioformulation and the elucidation of the mechanisms underlying the P bioavailability could be of potential economic importance in improving crop yields to meet the rising food demand driven by an increasing human population.

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Declarations

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