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Bacillus sp. Strain Fo03, a Phosphate Solubilizing Bacterial Strain, Promotes Potato Growth and Decrease Inorganic Fertilizer

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Abstract

Biofertilizers are a sustainable biotechnological alternative for decreasing use of inorganic fertilizer. Some bacterial strains, such as *Bacillus pumilus*, can solubilize phosphates, making them available to crops such as potato (*Solanum tuberosum* L. cv. Citlali). In this paper was studied the effect of *Bacillus* sp. strain Fo03, a phosphate solubilizer bacterial strain, on the growth promotion, production, and sprouting of potatoes under greenhouse conditions, compared with inorganic fertilizer. Five treatments were assayed: NPK 100% (T1), NK 100% (T2), *Bacillus* sp. strain Fo03, BP (T3), BP + NPK 50% (T4), and BP + NK 50% (T5). The parameters evaluated were plant height, fresh and dry weights, number of internodes, SPAD values, fresh weight of tubers, total number of tubers, number of tubers with diameters greater than or equal to 15 mm, length and width of sprouts. Treatments T1 and T4 showed a similarity in plant height (21.65 cm, 20.95 cm), number of internodes (12.42, 13.11), fresh weight (9.97 g, 5.53 g), and dry weight (1.87 g, 1.08 g), respectively. The tuber fresh weight (g), number of total tubers, tuber diameter ≥ 15 mm, and sprout width were statistically significant in T4 compared to the treatment with *Bacillus* sp. strain Fo03. The highlight result was the effect combined of *Bacillus* sp. strain Fo03 and the half dose of mineral fertilizer (NPK 50%) for having good yield of potato cultivation (growth, production, and sprouting), providing to farmers an alternative response for decreasing use of inorganic fertilizers. The use of *Bacillus* sp. strain Fo03 as biofertilizer may have a promising effect on the quality of potatoes, particularly in sprout length and width.

Introduction

Potato (*Solanum tuberosum* L.) is the third most important food crop globally after rice and wheat. In 2021, the global yield of potatoes was 376,119,974 tons of tuber. The same year, Mexico produced 1,947,760 tons of potatoes harvested from 61,293 ha [1]. This crop has several nutritional characteristics, including health-promoting components such as vitamin C, phenolic compounds, and iron. Potato production requires relatively high fertilizer levels, the recommended amounts are 80 to 120 kg/ha of nitrogen (N), 50 to 80 kg/ha of phosphorus (P) and 125 to 160 kg/ha of potassium (K). In addition, potatoes require a porous soil with a pH of 5 to 6 [2]. It is essential to apply these nutrients in the form of mineral fertilizers to promote optimum foliage and tuber growth.

During the early stages of potato cultivation, approximately 30 to 40 days after plant emergence, the greatest uptake of N and K occurs. Phosphorus is necessary in both early and late stages of potato cultivation, as it plays an important role in the maturation of tubers [3]. Nitrogen promotes foliar development, which increases surface area for photosynthesis. This, in turn, leads to starch production and directly affects the translocation of starch from leaves to tubers. Therefore, N supply influences yield, plant height, and number of tubers per unit area [3]. Potassium

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is an essential nutrient for plant productivity, influencing photosynthesis, chlorophyll content, chloroplast structure, leaf anatomy; it fulfils an important function in the translocation and storage of assimilates, since the mobilization of sugars to the tubers allows achieving high production and quality [4]. Phosphorus (P) is crucial for good root and aerial development during seedling establishment, as well as for various metabolic processes such as energy transfer, nucleic acid and starch synthesis, respiration, membrane synthesis and stability, enzyme activation and deactivation, redox reactions and carbohydrate metabolism [5]. On the other hand, P has also been implicated in tuber starch synthesis and in increasing tuber specific gravity/dry matter [6].

For the above reasons, potatoes require large amounts of nutrients to increase yield (tuber filling), which requires proper management of the chemical fertilizers applied [7]. However, overuse of agrochemicals can contribute to soil acidification and soil crust, reduce the content of organic matter and humus, alter pH of soil, promote pests, and even lead to the release of greenhouse gases. Soil acidity reduces crop phosphate intake, increases the concentration of harmful ions in the soil, and inhibits crop growth [8]. According to the FAO, the total agricultural use of inorganic fertilizers in 2019 was 189 million tons. This consisted of 108 million tons of N (57% of the total), 37 million tons of K (20%), and 43 million tons of P (23%), which is 40% higher than in 2000 (33% higher for N, 73% higher for K and 34% higher for P) [9]. Phosphorus has a relatively high demand in potato crops compared to other crops. According to Nawara et al. [10], a soil P concentration of about 76 mg/kg of P is required for potatoes to achieve 95% of their yield potential, which is four times more than that needed for crops like cereals.

P uptake by the plant can be hindered when this nutrient is accumulated in the soil through adsorption processes with metallic cations, such as Ca, Mg, Fe, and Al, rendering it unavailable for plant growth. According to estimates, 70% of inorganic P from agrochemical applications is rapidly transformed into insoluble complexes with the aforementioned metal cations [11]. Some soil microorganisms have the ability to convert Pi into forms that can be assimilated by plants. These microorganisms are known as phosphate solubilizers [12] and could be potentially applied as biofertilizers, in order to restore P content in soil. Biofertilizers are liquid or solid formulations based on non-harmful microorganisms, pure or in consortia that increase the nutrient availability for plants uptake by means of N fixation and solubilization of P and K, and therefore serve for soil restoration [13]. Several biofertilizers are formulated with strains of rhizobacteria from different genera, including *Azotobacter*, *Azospirillum*, *Pseudomonas*, *Acetobacter*, *Burkholderia*, *Enterobacter*, *Erwinia*, and *Bacillus*, due to possessing some of traits above mentioned [12].

In this context, previous studies have shown that several *Bacillus* species are model organisms in research on associative plant–microbe interactions [14]. In particular, *Bacillus pumilus* a Gram-positive rod, spore-forming bacterium, is commonly found in various environments such as marine water, deep-sea sediments, and soil. After *B. subtilis*, *B. pumilus* is the second most studied bacterial strain from the genus *Bacillus* in terms of its plant growth promotion properties. Indeed, previous research showed that *B. pumilus* enhances salt tolerance in culture rice (*Oryza sativa* L.) [15], and together with P, it can enhance the uptake of boron by rapeseed [16]. Importantly, *B. pumilus* can have a positive effect on native soil bacterial communities. This bacterium species can remain in the soil for an extended period and alter the bacterial community [17]. Studies have shown that *B. pumilus* can enhance bacterial biodiversity, including Shannon diversity index, in the rhizosphere and root endosphere of rice [18]. Besides these beneficial characteristics, the effect of *B. pumilus* as a biofertilizer capable of reducing the amount of NPK fertilizer on potato remains to be investigated, as does its efficacy in enhancing both plant growth and soil quality. Therefore, in this study we aimed to evaluate the performance of *Bacillus* sp. strain Fo03, previously isolated from potato cultivars, selected, and characterized as an efficient P solubilizing bacterium in vitro [19], to increase the potato plant production (growth, production, and sprouting) in different mineral fertilizer conditions (NPK) in a greenhouse experiment.

Materials and Methods

Phosphate-Solubilizing Bacterial Strain

The bacterial strain *Bacillus* sp. strain Fo03 (GenBank Accession Number: MN100586) belongs to phosphate solubilizing bacteria collection of the Edaphology and Environment Laboratory of Sciences Faculty, Autonomous University of Mexico State. It was previously isolated from potato cultivars, purified, selected and characterized on its solubilization capacity of $Ca_3(PO_4)_2$ in Pikovskaya liquid medium (PVK) [19, 20].

Bacillus sp. Strain Fo03 Characterization

The bacterial strain was characterized morphological and biochemical by means of different tests: Grams' staining, osmotolerance, production of catalase, oxidase, growth on Simmons Citrate agar, Triple Sugar Iron agar (sucrose, lactose, glucose fermentation, and H_2S production), MIO agar (motility, indole, ornithine), capacity of starch hydrolysis, and nitrate reduction. Tests were performed with Bioxon® differential culture media, according to

manufacturer's protocols and conditions. Also, methyl red and Voges–Proskauer tests were performed according to McDevitt [21].

Also, the bacterial strain was tested for auxin production and its ability for solubilizing other insoluble inorganic P sources such as aluminum and iron phosphates. For IAA production, *Bacillus* sp. strain Fo03 was cultured for 48 h at 28 °C on Tryptophan broth Bioxon©. IAA concentration produced was measured by Salkowski's method on a Thermo Spectronic™ Genesys 20 spectrophotometer at 530 nm wavelength. IAA concentrations were calculated using a standard curve of IAA (0–10 µg/mL). For quantification of soluble P, *Bacillus* sp. strain Fo03 was inoculated in flasks with PVK broth (with three Pi sources separately at 5 g/L of, tricalcium, aluminum and iron P, respectively) for 7 days at 28 °C in an oscillatory shaker (100 rpm). The soluble P concentration was determined spectrophotometrically by blue molybdenum method at 830 nm wavelength according to [19], all assays were performed by three replicates.

Bacillus sp. Strain Fo03 Culture Conditions

The bacterial inoculum was prepared from bacterial colonies preserved in PVK agar at 4 °C. In a seed tube with 7 mL of 0.85% NaCl, bacterial biomass was added until obtaining 0.20 absorbance units at a wavelength of 600 nm using a UV–VIS spectrophotometer (GENESIS 20), which corresponds approximately to 1×10^6 CFU/mL. Next, 2 mL were added to 500 mL Erlenmeyer flasks containing 200 mL of nutrient broth in triplicate. The flasks were then incubated for 4 days at 28 ± 2 °C. After 4 days, the biomass was transferred to 12 mL centrifuge tubes and centrifuged at 2500 rpm for 15 min. The collected biomass was washed with 0.85% NaCl, and then transferred to a 50 mL Erlenmeyer flask. To determine the population density of bacteria in the collected biomass, the viable-cell count method was performed by surface seeding [22]. This procedure was carried out in triplicate for each dilution and Petri dishes were incubated at 27 °C for 24 h. The colony-forming units (CFU) on the surface of Petri dishes were counted and expressed as CFU/mL.

Plant Material and Growth Conditions

Virus-free microplants of potato (*Solanum tuberosum* cv. Citlali), obtained from the Germplasm Bank of the Programa Nacional de Papa del Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias (INIFAP) in Toluca, México, were propagated as nodal cuttings in planting tubes [23] containing Murashige and Skoog medium [24] modified [25]. The medium contained per liter: NH_4NO_3, 17.5 g; KNO, 20 g; $CaCl_2 \cdot 2H_2O$, 4.5 g; KH_2PO_4, 1.75 g; H_3BO_3, 50 mg; $MnSO_4$, 200 mg; $ZnSO_4$ $7H_2O$, 100 mg; KI, 10 mg; Na_2MoO_4, 2.5 mg; $CuSO_4$ $5H_2O$, 5.0 mg; $CoCl_2 \cdot 6H_2O$, 0.5 mg, and it was supplemented with Myo-Inositol 0.1 g; Fe, 0.065 g; thiamine, 0.0004 g; calcium pantothenate, 0.002 g; glycine, 0.00005 g; GA3, 0.0001 g; and sucrose, 30%. The microplants were incubated at 20 ± 1 °C under a 16 h photoperiod (fluorescent lights: 35 µmol/m^2/s, 400–700 nm) and in sterile conditions.

Soil Physicochemical Characterization

The physicochemical characteristics from soil used in the greenhouse experiment were organic carbon (3.96%), organic matter (6.80%), available phosphorus (189.78 mg/kg of soil), electrical conductivity (1103 µS/cm), pH 5.76, texture Clay loam. Soil analyses were performed according to [26].

Experimental Location and Climatic Conditions

Experiment was conducted from May to August 2022, during the spring–summer season, with a 16 h photoperiod. It took place in a non-technified greenhouse at the facilities of the Laboratorio de Fisiología-Biotecnología. Programa Nacional de Papa, INIFAP Estado de México, México, with geographic coordinates of 19° 24′ 51″ north latitude and 99° 58′ 44″ west longitude [27]. The climate is temperate and sub-humid with rainfall occurring in the summer, and precipitation ranging between 700 and 1000 mm. The altitude is between 2500 and 2800 m [28].

During the potato crop's growing season under greenhouse conditions, air temperature and relative humidity were recorded using the Elitech GSP-6 Digital Temperature and Humidity Data Logger®. In 2022, from May 10 to June 10 (temperatures maximum and minimum 35.6 to 9.0 and relative humidity maximum and minimum 88% to 15.7%); from June 11 to July 11 (temperatures maximum and minimum 40 to 9.1 and relative humidity maximum and minimum 95.7% to 26.4%); from July 12 to August 12 (temperatures maximum and minimum 33.8 to 9.8 and relative humidity maximum and minimum 94.5% to 71.9%).

Experimental Design and Treatments

The experimental design was completely randomized. It consisted of 15 replicates per treatment, with one plant per pot as a replicate. The treatments were as follows: T1: NPK 17/17/17, full dose of 100% NPK chemical fertilizer, T2: $(NH_4)_2SO_4$ (NPK 21/00/00/24S) and KNO_3 (NPK 13/00/46) fertilizer without phosphorus with a full dose of 100% NK chemical fertilizer, T3: *Bacillus* sp. strain Fo03 inoculant, T4: *Bacillus* sp. strain Fo03 inoculant and 50% NPK chemical fertilizer, and T5: *Bacillus* sp. strain Fo03 inoculant and 50% NK chemical fertilizer $(NH_4)_2SO_4$ and KNO_3.

The crop was fertilized with 180 kg/ha of N, distributed as 90 kg at sowing and 90 kg at 50 days after sowing (DAS); 60 kg of P only at sowing, and 350 kg of K, split into 70 kg at sowing and 280 kg at 60 DAS. In the treatments where fertilizer was at 50%, the following fertilizer doses were applied: 90 kg of N, distributed as 45 kg at sowing and 45 kg at 50 DAS; 30 kg of P only at sowing and 175 kg of K, distributed as 35 kg at sowing and 140 kg at 60 DAS. The roots of potato plants were immersed for 20 min in a suspension containing *Bacillus* sp. strain Fo03 at a concentration of 10^8 CFU/mL.

The plants were planted in 1 kg pots containing unsterilized soil from potato crops, horticultural grade perlite, and peat moss in a 1:1:1 (*w/v*) ratio. After 50 DAS, a re-inoculation with *Bacillus* sp. strain Fo03 strain at a concentration of 10^3 CFU/mL was performed. Crop management and harvest consisted of manual irrigation of plants every 3rd day with potable water. To control *Trialeurodes vaporariorum* (whitefly), potassium soap was sprayed on the leaves of the plants once a week for 15 days, 30 DAS. The plants were harvested after 90 DAS and potato mini tubers were harvested 7 days after cutting the potato plants.

Data Collection

The study recorded plant height, number of internodes per plant, fresh weight of leaves and stem, and dry weight of leaves and stem. SPAD values were also measured using a Konica Minolta® SPAD-502. The measurements were taken in the leaflets of the third and fourth leaf, in the middle portion of the leaflets, at 8:00 am, 50 DAS, according to the methodology described by Degan et al. [29]. For DW, the whole plant biomass was oven-dried at 70 °C for 72 h. During the harvest, number of tubers smaller than 15 mm in diameter per plant, number of tubers equal to or larger than 15 mm in diameter per plant, total tubers per plant and total fresh weight of tubers per plant were evaluated [30]. The mini tubers were stored in paper bags, individually for each treatment, at a temperature between 15 and 16 °C and with diffuse light on the tubers. Afterwards, the sprout length and width of mini tubers were evaluated.

Statistical Analysis

All results presented were statistically analyzed using IBM SPSS statistics 21. Analysis of variance (one-way ANOVA) was performed for data that were normally distributed, while for data that did not meet this normal distribution a Kruskal–Wallis one-way ANOVA was performed. Statistically significant differences were compared using Duncan's multiple range test ($P < 0.05$) for parameters with a normal distribution. For parameters that did not meet a normal distribution, Dunn's test was performed for each pair of groups, with adjustment of the degree of significance (p) by Bonferroni.

Results

Biochemical Characterization of *Bacillus* sp. Strain Fo03

Bacillus sp. strain Fo03 colonies are circular in shape with an irregular margin, and opaque and whitish in color (Fig. S1). The results of biochemical and physiological characterization of the bacterial strain showed that *Bacillus* sp. strain Fo03 is a Gram-positive rod, positive for catalase and oxidase, without gas formation, with a response acid/acid for carbohydrate fermentation indicating a heterotrophic fermentative metabolism for triple sugars (glucose, lactose, and sucrose). Also, MR/VP and OA tests resulted positives, indicating sugars fermentation by the mixed acid and butanediol pathways, respectively. This growth response produces a decrease in medium pH, generally due by the aerobic release of organic acids, which is a key mechanism for P solubilization. This strain also showed a positive response for starch hydrolysis (Table 1). The assay MIO agar test confirmed indole production.

The strain *Bacillus* sp. strain Fo03 was able to grow under osmotolerant conditions with glucose at 10 and 30 g/L and presented a solubilization halo on PVK solid medium with tricalcium phosphate as non-soluble P source (Fig. S2). By other side this bacterial strain, grew and solubilized three Pi sources in PVK liquid medium (calcium > iron > aluminum). Additionally, this bacterial strain showed being able to produce auxins in a Trp broth (4 µg/mL) (Table 2, Fig. S2).

Table 1 *Bacillus* sp. strain Fo03 biochemical characteristics

Biochemical characteristic														
Bacterial strain	G	C	O	CF	GP	H_2S	SH	Cit	Mot	Or	I	MR/VP	OA	NR
Bacillus sp. strain Fo03	+	+	+	Acid/acid	−	−	+	+	−	+	+	+	+	−

G gram, *C* catalase, *O* oxidase, *CF* carbohydrates fermentation, *GP* gas production, *H_2S* H_2S production, *SH* starch hydrolysis, *Cit* citrate, *Mot* motility, *Or* ornithyine, *I* indole, *MR/VP* methyl red/Voges–Proskauer, *OA* organic acid, *NR* nitrate reduction

Table 2 Phosphate solubilization and auxin production by the bacterial strain

Bacterial Strain	P solubilization PVK agar 7 days at 28 °C		P solubilization (µg/mL) in PVK liquid medium, 7 days at 28 °C			Auxin production in Trp broth, 48 h at 28 °C
	10 g/L glucose	30 g/L glucose	$Ca_3(PO_4)_2$	$FePO_4$	$AlPO_4$	Indole acetic acid (µg/mL)
Bacillus sp. strain Fo03	+	+	350 ± 1.97*	0.19 ± 0.03*	0.03 ± 0.01*	4.0 ± 0.2*

*Data are expressed as mean ± standard deviation of three replicates

Table 3 Growth parameters of potato plants treated with *Bacillus* sp. strain Fo03 strain and/or NPK doses

Treatments	Plant height (cm)[1]	Number of internodes/plant[1]
T1: NPK 100%	21.65 ± 5.71[a]	12.42 ± 2.93[a]
T2: NK 100%	15.97 ± 2.18[ab]	10.85 ± 1.95[a]
T3: *Bacillus* sp. Fo03	13.42 ± 4.71[b]	10.07 ± 3.01[a]
T4: *Bacillus* sp. Fo03 + NPK 50%	20.95 ± 8.23[a]	13.11 ± 3.75[a]
T5: *Bacillus* sp. Fo03 + NK 50%	18.94 ± 8.87[ab]	12.00 ± 4.94[a]

[1]Data are expressed as mean ± standard deviation. Different letters in each column represent statistically significant differences by Duncan $P < 0.05$

Effect of *Bacillus* sp. Strain Fo03 and NPK Fertilizer on Plant Growth Parameters of Potato Plants

The application of *Bacillus* sp. strain Fo03 with a NPK 50% dose had a similar effect on plant growth compared to NPK 100% dose (Table 3). The addition of *Bacillus* sp. strain Fo03 in combination with NPK 50% and NK 50% (T4 and T5) did not present significant differences ($P > 0.05$) in plant height of potato plants compared to NPK 100% and NK 100% (T1 and T2). Its effect on height plant and number of internodes of potato is evident and although statistically there are no differences, its efficiency is equal to treatment T4: BP + NPK 50% with phosphorus.

The SPAD values indirectly indicate chlorophyll and photosynthates content in plant leaves. It is evident from Fig. 1 that there are significant differences ($P < 0.05$) in SPAD values in treatments T2 and T5 with T1. Treatment with lowest SPAD values was *Bacillus* sp. strain Fo03 without the addition of NPK.

Table 4 shows measurements of fresh and dry weight of stem and leaves of potato plants. There is clear evidence that treatment with *Bacillus* sp. strain Fo03 had statistically the lowest value. In contrast, treatments T4 and T5, the effect on fresh and dry weight are statistically equals to treatment with 100% NK, indicating a support nutrient by N and K.

Production of Mini Tubers of Potato Plants

The potato tubers production had no significant differences in fresh weight of tubers between T2 and T4 compared to T1 ($P > 0.05$). The inoculation with *Bacillus* sp. strain Fo03 with NPK 50% and NK 50% (T4 and T5) improved tuber fresh weight by 75% and 67%, respectively, compared to T3. Treatments T4 and T5, increased total tubers (TT) per plant significantly ($P < 0.05$) compared to T3 (Table 5).

The treatment with *Bacillus* sp. strain Fo03 and NPK 50% (T4) increased the number of tubers with a diameter ≥ 15 mm compared to treatment with *Bacillus* sp. strain Fo03 alone (T3). There were no significant differences ($P > 0.05$) in number of tubers with diameter ≥ 15 mm between treatments T2 and compared to treatment T1. Additionally, number of tubers with diameter < 15 mm, did not differ significantly ($P > 0.05$) between treatments (Table 6).

After 180 days of storage, tuber shoot length in the treatment with *Bacillus* sp. strain Fo03 (T3) increased significantly ($P < 0.05$) compared to treatment NPK 100% (T1). Treatments T2, T4, and T5 did not show any significant differences ($P > 0.05$) compared to treatment T1 (Fig. 2).

The length and width of potato tubers provide information for agricultural producers in order to predict the field performance of tubers crop. The treatment *Bacillus* sp. strain Fo03 showed the lowest sprout width ($P < 0.05$) compared to other treatments (Table 7).

Discussion

Biofertilizers are a sustainable biotechnological alternative for decreasing use of inorganic fertilizer, and *Bacillus pumilus* is a promising bacterium that could be used for this purpose while improving plant production. This work assesses the impact of *Bacillus* sp. strain Fo03, a P solubilizing bacterium, on the growth of potato under different chemical fertilizer regimes in a greenhouse experiment. Also, this strain was biochemical and physiological characterized.

Several characteristics are desirable for biofertilizers, such as in vitro culturable, non-pathogenic for human and plants, biocompatible with others beneficial microorganisms, being genetically stable, to grow with culture media by using broad spectrum of carbon sources as raw material for

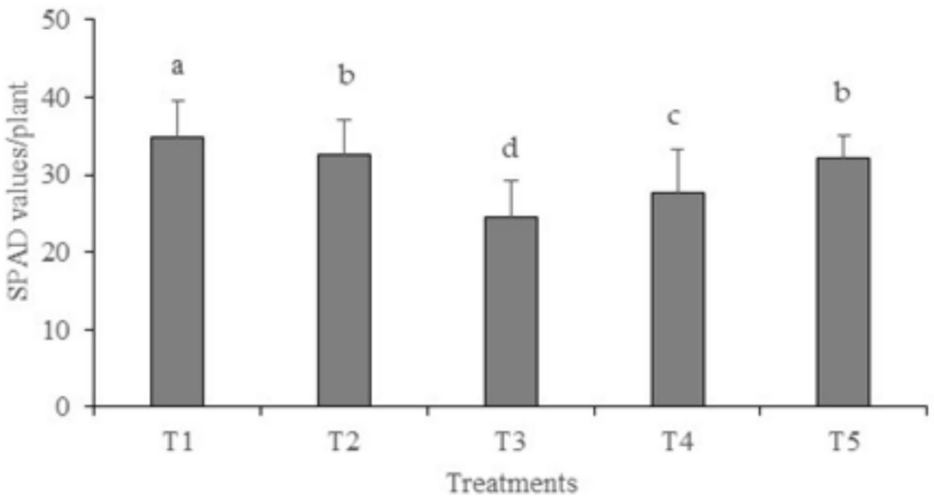

Fig. 1 Value SPAD in potato cv. Citlali at 90 DAS. Results are expressed as means of four replicates and vertical bars shown in the figure represent standard deviations and different letters indicate significant differences at $P < 0.05$ by Duncan

Table 4 Fresh and dry weight of potato plants 90 DAS, treated with *Bacillus* sp. strain Fo03 strain and/or NPK doses

Treatments	Fresh weight of stem and leaves (g)/plant[1]	Dry weight of stem and leaves (g)/plant[1]
T1: NPK 100%	9.97 ± 8.54^{a}	1.87 ± 0.94^{a}
T2: NK 100%	3.68 ± 1.28^{ab}	0.84 ± 0.37^{bc}
T3: *Bacillus* sp. Fo03	1.30 ± 0.81^{b}	0.23 ± 0.11^{c}
T4: *Bacillus* sp. Fo03 + NPK 50%	5.53 ± 4.49^{ab}	1.08 ± 0.72^{ab}
T5: *Bacillus* sp. Fo03 + NK 50%	5.23 ± 3.13^{ab}	1.38 ± 0.52^{ab}

[1]Data are expressed as mean ± standard deviation. Different letters in each column represent statistically significant differences Dunn's test $P < 0.05$

Table 5 Tuber fresh weight and number of total tubers per plant 90 DAS

Treatments	Fresh weight of tubers (g)/plant[1]	Total tubers/plant[1]
T1: NPK 100%	22.12 ± 5.59^{a}	6.57 ± 2.57^{a}
T2: NK 100%	17.82 ± 3.39^{ab}	6.00 ± 2.44^{a}
T3: *Bacillus* sp. Fo03	4.86 ± 2.49^{c}	2.93 ± 1.43^{b}
T4: *Bacillus* sp. Fo03 + NPK 50%	19.42 ± 10.50^{ab}	5.22 ± 1.85^{a}
T5: *Bacillus* sp. Fo03 + NK 50%	14.87 ± 4.82^{b}	4.66 ± 2.87^{ab}

[1]Data are expressed as mean ± standard deviation. Different letters in each column represent statistically significant differences Duncan $P < 0.05$

organic acids production and their scale up, finally keep up activity and viability under environmental field conditions [31]. In this work, the strain *Bacillus* sp. strain Fo03 showed several promising characteristics such as the non-selective use of carbon sources (citrate, starch, fructose, glucose, and lactose) which is an advantage for the industrial scaling, culture and cheaper medium formulation. Also, *B. pumilus* showed osmotolerance, a characteristic that is advantage for the application on degraded soils subjected to abiotic stresses. Interestingly, this strain was able to solubilize P even under these adverse conditions. Furthermore, *Bacillus* sp. strain Fo03 could solubilize in vitro three different sources of insoluble P (aluminium, iron, and calcium P). This is another advantageous property that provides useful information, because in soils, chemically applied P is rapidly insolubilized by interaction with divalent cations and precipitated as calcium, aluminium and/or iron P. In fact, according to the acid/acid response in Triple Sugar Iron medium, *Bacillus* sp. strain Fo03 produces organic acids that leads to the lowering of pH.

Previous research showed that release of extracellular low molecular weight organic acids is a P solubilization mechanism shared by bacterial and fungi biofertilizers [32]. These organic acids have one or more carboxyl groups (negative charged) that can chelate the cations of insoluble P sources, resulting in a release of orthophosphate, which is bioavailable for plants [33]. The organic acids are produced by bacteria in both aerobic (for example, citric, succinic, oxalic, and malic acids are produced in tricarboxylic acids cycle), and anaerobic conditions (by example acetic acid is produced by incomplete oxidation of sugars, acidogenesis and acetogenesis, lactic acid in homolactic fermentation, butyric acid by anaerobic oxidation of pyruvate) [34]. Chawngthu et al. [35] demonstrated that bacterial strains *Bacillus cereus* and *Bacillus subtilis* produce oxalic acid, malic acid, formic acid, acetic acid tartaric acid, and gluconic acid. In other study, it was demonstrated that different organic acid patterns are involved in the solubilization of different inorganic P sources [36]. In this work, we showed that *Bacillus* sp. strain Fo03 solubilizes different P sources, but more studies should be done to determine the pattern of organic acid release by this strain, to fully understand the mechanisms involved in this solubilization.

Bacillus is a genus that contains several species considered good biofertilizers, not only for the capacity to increase the N, P, and K by atmospheric N_2 fixation and P and K solubilization, but also due to its ability to stimulate the plant growth through the production of phytohormones [37]. In this work, the auxin production of *Bacillus* sp. strain Fo03 was confirmed, in addition to the ability to solubilize different P sources. Auxin plays an important role in plant growth promotion processes such as gametogenesis, embryogenesis, vascular formation and flower development [38]. In a study conducted by Cruz-Martin et al. [39], *B. pumilus* was found to produce 28.9 µg/mL of IAA, showing an increase in banana plants growth, such as the stem height and thickness, the root architecture, and fresh and dry weight. These results are similar to those obtained in this work, where the growth parameters of potato were improved by the bacteria addition.

Phosphorus can promote root growth and accelerate tuber formation, making it a critical element in the initial period of plant development and tuber formation [40]. Studies have shown that adding P can increase the proportion of large tubers harvested [41, 42], although some have observed that increase of number of small tubers was offset with a decrease in number of large tubers [43]. P solubilizing bacteria can

Table 6 Number of tubers with diameter greater than/equal 15 mm per plant and less than 15 mm per plant

Treatments	Number of tubers with diameter ≥ 15 mm/plant[1]	Number of tubers with diameter < 15 mm/plant[1]
T1: NPK 100%	4.00 ± 3.40^{ab}	3.28 ± 2.92^{a}
T2: NK 100%	3.14 ± 2.03^{ab}	2.85 ± 2.03^{a}
T3: *Bacillus* sp. Fo03	1.45 ± 0.93^{b}	2.54 ± 2.06^{a}
T4: *Bacillus* sp. Fo03 + NPK 50%	4.66 ± 2.33^{a}	3.16 ± 1.32^{a}
T5: *Bacillus* sp. Fo03 + NK 50%	2.66 ± 1.00^{ab}	2.37 ± 1.59^{a}

[1]Data are expressed as mean ± standard deviation. Different letters in each column represent statistically significant differences Dunn's test $P < 0.05$

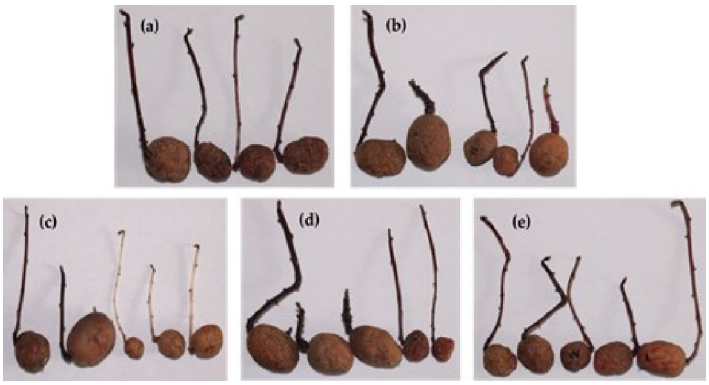

Fig. 2 Sprouts of potato tubers cv. Citlali, 180 days after harvest: **a** T1: NPK 100%, **b**T2: NK 100%, **c** T3: *Bacillus* sp. strain Fo03, **d** T4: *Bacillus* sp. strain Fo03 + NPK 50% and **e** T5: *Bacillus* sp. strain Fo03 + NK 50%

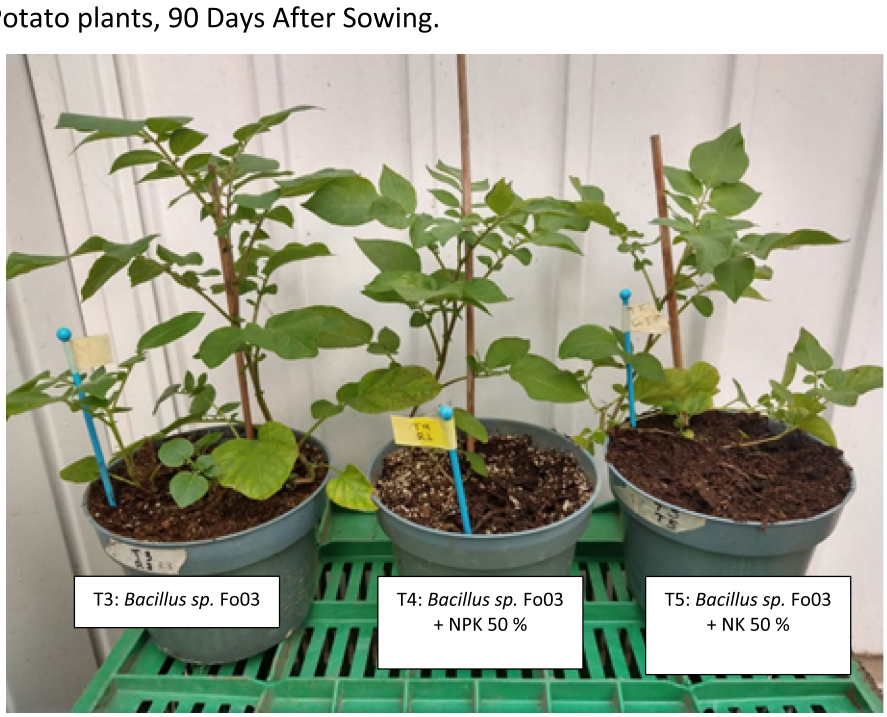

Table 7 Length and width sprouts of mini potato tubers

Treatments	Sprout length (cm)/plant[1]	Sprout width (cm)/plant[1]
T1: NPK 100%	3.00 ± 1.88^{b}	0.14 ± 0.06^{a}
T2: NK 100%	3.53 ± 2.41^{ab}	0.16 ± 0.06^{a}
T3: *Bacillus* sp. Fo03	4.72 ± 2.45^{a}	0.10 ± 0.02^{b}
T4: *Bacillus* sp. Fo03 + NPK 50%	3.71 ± 2.08^{ab}	0.17 ± 0.09^{a}
T5: *Bacillus* sp. Fo03 + NK 50%	3.54 ± 1.90^{ab}	0.14 ± 0.07^{a}

[1]Data are expressed as mean ± standard deviation. Different letters in each column represent statistically significant differences Dunn's test $P < 0.05$

mineralize and solubilize phosphorus from organic and inorganic fertilizers, ensuring adequate availability of P content in soils during cultivation [44]. According to Kostenko et al. [45], the use of biological preparations together with mineral fertilizers can reduce the required dose by half. This is due to the bacteria's ability to increase absorption of microelements by plants, which in turn increases the volume of root system and its adsorbing activity. Furthermore, there is a close relationship between P solubilization and IAA production in promoting plant growth. Essential plant nutrients are taken up from the soil by the roots. In this sense, good root growth is a characteristic effect of auxins such as IAA [46].

Some biofertilizers can also stimulate root growth either by inducing plant phytohormone production or by directly releasing these compounds [46]. In this work, when *Bacillus* sp. strain Fo03 was inoculated in potato plants under different regimes of fertilizer, an increase of plant growth parameters was found, and both mechanisms could be participating in the potato growth promotion.

The inoculation with *Bacillus* sp. strain Fo03 and NPK 50% improved the plant height, fresh and dry weight of

stem and leaves and SPAD values compared to the treatment with *Bacillus* sp. strain Fo03 alone. It is known that the full dose of NPK chemical fertilizer has a direct effect on plant growth parameters and the physiological status of crops, but it has a negative ecological impact [47].

Masood et al. [32], found that the inoculation of *B. pumilus* improved growth of tomato (*Solanum lycopersicum* L.) with addition of fertilizer. Overall, these results confirm that biofertilizers such as *B. pumilus* can be used effectively to reduce chemical fertilizer without sacrificing productivity.

Considering that the edible part of the potato crop is the tubers, it was interesting to observe the effect of *Bacillus* sp. strain Fo03 on this yield. As for the total number of tubers with a diameter greater than 15 mm, this yield was maintained even when the NPK fertilizer rate was reduced by half and *Bacillus* sp. strain Fo03 was added. Regarding the yield of fresh weight of tuber, similar results were obtained by El-Sayed et al. [40]. They obtained a yield of tubers (11.9 t/ha) from plots that received a full rate of mineral fertilizer plus compost was statistically equal to a treatment with biofertilizers (*Azospirillum brasilense*, *Azotobacter chroococcum*, *Bacillus megaterium*, vesicular–arbuscular mycorrhiza, and *Bacillus cereus*) in combination with a compost-type organic amendment and 50% mineral fertilizer. These results together with ours suggest the feasibility of applying fewer chemical fertilizers with the inoculation of *B. pumilus* as biofertilizer for potato production.

Potato cultivation uses seed tubers as planting material. The physiological status of seed potato has a great impact on sprouting and may depend on nutrient management on field [47]. P also plays a crucial physiological role in sustaining sprouts, since it is an essential component for synthesis, transport and storage of starches, carbohydrates necessary for the growth of new sprouts [47]. However, the impact of biofertilizers on the quality of potato sprouts in terms of length, width and number is still a relative unexplored topic. In our experiment, we observed that application of *Bacillus* sp. strain Fo03 has an effect on length and width of potato sprouts, showing that this strain can modulate the potato sprouting. For potato producers this information is important, because tubers greater than/equal to 15 mm per plant means they are good quality and marketable [48].

To the best of our knowledge, this work is the first to evaluate the ability of *Bacillus* sp. strain Fo03 to promote plant growth on a half fertilizer regime. In this first approach, the beneficial effect that this bacterial strain can exert, thus reducing the need for full dose inorganic fertilizer can be observed. Furthermore, it is important to note that the inoculation of *Bacillus* sp. strain Fo03 with NK 50% (without P) also produces a plant growth rate similar to the full dose fertilizer (with P). These results could be due to the bacterial ability to solubilize the naturally precipitated P sources from the soil, highlighting its potential use as a biofertilizer in potato crops.

Conclusions

In this study, *Bacillus* sp. strain Fo03, is a bacterial strain with biofertilizer potential that has been characterized through its biochemical and physiological on its ability to use in vitro different C sources, to solubilize aluminum, iron and calcium P and produce auxins, as well as its ability to acidify the medium and grow under osmotic stress environment. The application of *B. pumilus* under different chemical fertilizer regimes on potato was studied and the highlight result was the effect combined of *Bacillus* sp. strain Fo03 and the half dose of mineral fertilizer (NPK 50%) for having good yield of potato cultivation (growth, production and sprouting), providing to farmers an alternative response for decreasing use of inorganic fertilizers. Further research should be encouraged to determine the compatibility of *Bacillus* sp. strain Fo03 with other beneficial organisms and whether they can contribute synergistically to the growth and production of potato cv. Citlali under field conditions.

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Data Availability The data used to support the findings of this study are available from the corresponding author upon request.

Code Availability Not applicable.

Declarations

Competing Interests The authors have no relevant financial or non-financial interests to disclose.

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

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