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RESEARCH ARTICLE

Isolation of bacterial strains from compost teas and screening of their PGPR properties on potato plants

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Abstract

The beneficial effect of compost and compost tea on plant growth and protection is mainly associated with the microbial diversity and the presence of bacteria with plant growth–promoting effect. PGPR are considered as eco-friendly bio-fertilizers that may reduce the use of chemical pesticides and fertilizers. Three composts (AT, A10, and A30) were previously prepared from industrial wastes (olive mill wastewater, olive pomace, coffee ground, and phosphogypsum). In the present study, we isolated three bacterial strains from the compost teas. The phylogenetic identification of these bacterial strains (B.AT, B.A10, and B.A30) showed that they correspond to *Serratia liquefaciens* (B.AT and B.A10) and *Achromobacter spanius* (B.A30) species. A further characterization of the PGPR traits of these bacteria showed that they produce siderophore, exopolysaccharides, and IAA. Their effect on potato plant growth, yields, and tuber quality was performed under field culture conditions. Results showed that these strains can be characterized as PGPR, the best effect on potato plant growth was observed with *Serratia liquefaciens* (B.AT), the best yield and tuber quality was observed with *Serratia liquefaciens* (B.A10) while bacterial treatment with *Achromobacter spanius* (B.A30) is a Cd-tolerant PGPR.

Keywords Compost · Field · PGPR · Potato · Tuber quality · Yield

Introduction

Compost teas (CTs) are the liquid organic product obtained from mixing compost with tap water for a known ratio and incubation period (Morales-Corts et al. 2018). The CT quality is related to several factors, such as compost to water ratio, compost type, and aeration, which determine the development of specific groups of microorganisms (Ingham 1999; Mengesha et al. 2017; De Corato 2020). Furthermore, CTs microbial population and soluble nutrients play an important role in suppressing disease and promoting plant growth (De Corato 2020; Castano et al. 2011).

Plant growth–promoting rhizobacteria (PGPR) are the most abundant microorganisms in compost teas. They promote plant growth through different mechanisms that make plants more resistant to different biotic and abiotic stresses (Hamid et al. 2021). From these mechanisms, we can mention nitrogen fixation (Beijerinck 1901), phosphorus, and potassium solubilization by producing organic acids. The application of these PGPR as bio-fertilizers can reduce the use of agrochemicals (Setiawati and Mutmainnah 2016). Field trials in India have shown that the use of phosphate-solubilizing microorganisms (PSM) can increase yields of tomatoes, lettuce, potatoes, and rice (Rodrìguez and Fraga 1999).

Bacterial siderophores also play a significant role in enhancing plant growth and protection (Aznar and Dellagi 2015; Khan et al. 2016). Indeed, given their ability to sequester iron, they are able to deprive

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pathogenic fungi of this essential element since fungal siderophores have a lower affinity than PGPR (Kumar et al. 2017; Meena et al. 2017).

The production of phytohormones is considered as one of the most important mechanisms, underlying the beneficial effect of PGPRs on plant growth and nutrition. It has been reported that they stimulate plant growth and improve their stress response (Ma et al. 2011; Etesami et al. 2015; Ullah et al. 2015). The main plant hormone produced by PGPR is indole acetic acid (IAA) (Notununu et al. 2022).

The role of PGPRs to alleviate plant abiotic stress has been confirmed in many reviews (Yang et al. 2009; Sessitsch et al. 2013; Meena et al. 2017; Backer et al. 2018; Ilangumaran and smith 2017). Resistance of PGPRs to heavy metals is associated with several mechanisms such as biosorption, bioaccumulation, precipitation, complexation, and enzymatic transformation of heavy metals, thus reducing their toxicity towards the plant (Rajkumar et al. 2012; Ma et al. 2016). Many PGPRs play also an important role in improving plant-water relations, ion homeostasis, and photosynthetic efficiency in plants under salt stress (Sati et al. 2022).

Such beneficial microorganisms can act also as pathogen antagonists by several mechanisms such as competition (Hoitink and Changa 2004; Diánez et al. 2005), hydrolytic enzyme production (Goswami et al. 2016), secondary metabolites production like hydrogen cyanide (HCN) (Voisard et al. 1989), and aminocyclopropane-carboxylic acid (ACC) deaminase production which reduce the level of ethylene (Jacobson et al. 1994; Glick 2010). These mechanisms protect plants against biotic stress (Glick 2010; Ma et al. 2011; Rajkumar et al. 2012).

PGPRs can elicit molecular and biochemical defense responses within the plant (Lugtenberg and Kamilova 2009). Indeed, to prime plant resistance against pathogen attack, PGPR trigger the induced systemic resistance (ISR) and activate pathogenesis-related genes expression (Pieterse et al. 2014).

The inoculation of plants with PGPRs by coating seeds, roots, or tubers is a very old practice used to improve plant growth (Brown 1974; Gaskins et al. 1985), mainly in legumes and cereals (Sessitsch and Mitter 2015).

The aim of the present study is firstly the identification and the characterization of bacterial strains isolated from three different compost teas by determining their in vitro PGPR proprieties and secondly to study their effect on potato plant growth yield and tuber quality in field condition. These compost teas, previously prepared (Samet et al. 2018), showed a positive effect on potato plant growth in greenhouse condition and their microbial characterization showed that they contain several plant growth–promoting bacterial strains (Samet et al 2018, 2019).

Material and methods

Isolation of bacterial strains from compost teas Three different Composts (AT, A10, and A30) were previously prepared by mixing olive mill waste water (OMW) olive pomace (P), coffee grounds (G), and phosphogypsum (PG) (Samet et al. 2018; 2019).

Three bacteria were isolated from AT, A10, and A30 compost teas. Isolation was performed by the multiple streak method, sample/inoculum is diluted by streaking it across the surface of the agar plate, isolated colonies were picked, and re-streaked on fresh agar plates. Nutrient agar medium containing 0.025 g/l triclosan was used. Triclosan is a broad-spectrum antibiotic inactive against several gram-negative PGPR genera such as *Pseudomonas, Serratia*, and *Achromobacter* (Welsch and Gillock 2011).

Phylogenetic identification of the bacterial strains

a) **Genomic DNA extraction**

Genomic DNA extraction was performed from pure strains already isolated and cultivated on LB medium using the Wizard Genomic DNA Purification Kit (Promega) from 3 ml of culture, following the protocol provided by the manufacturer.

b) **Amplification of the 16S rDNA**

The DNA sequence (1.5 kb) that encodes for 16S rRNA was amplified by PCR using, Taq DNA polymerase (Fermentas) and specific primers, FD1 (sequence: AGAGTTTGATCCTGGCTCAG; Weisburg et al. 1991), and 1492r (sequence: GGTTACCTTGTTACGACTT; Lane 1991). The amplification was carried out as follow: a denaturation phase (5 min at 94 °C) followed by 30 cycles of: denaturation (45 s at 94 °C), hybridization (45 s at 55 °C) and elongation (1 min 45 s at 72 °C). For each test, a negative control was added comprising all the components except DNA which was replaced by ultra-pure water.

c) **Sequencing and phylogenetic analysis**

The sequencing of the purified PCR products was performed using the primers FD1 and 1492R in an automatic sequencer of the ABI PRISM 3100-Avant Genetic Analyzer type (Applied Biosystems) using the BigDye® Terminator v3.1 Cycle Sequencing kit. From the results, the phylogenetic analysis of the strains was carried out.

Similarity search was performed to find the closest sequences using NCBI's Blast program. The phylogenetic trees were constructed according to the method of the nearest neighbor-joining method (Saitou and Nei 1987) using the software Mega 7 (Tamura et al. 2013).

The robustness of the tree was tested by a bootstrap analysis obtained on 1000 replicas. The obtained sequences were deposited in GenBank under accession numbers ON210808 (B.AT), ON210809 (B.A10), and ON210810 (B.A30).

In vitro *determination of PGPRs traits*

1. **Growth of bacterial strains in the presence of salt or cadmium**

   Each bacterial strain was inoculated as a surface streak on solid LB medium in the presence of salt (NaCl) at different concentrations (5, 15, 35, 40, 50 g/L) or of cadmium ($CdCl_2$) at 10, 40, 200, 300, and 400 mg/L and incubated at 30 °C for 24 h.
2. **Phosphate solubilization**

   The solubilization of the phosphate was qualitatively evaluated on Pikovskaya medium, according to the method described by Mehta and Nautiyal (2001). A colony of each bacterial strain was cultivated on the surface of the medium at 30 °C for 7 days. Phosphate solubilization is indicated by the formation of a clear zone around the colonies.
3. **Production of siderophores**

   The bacteria were cultured on the surface of the Chrome Azurol S (CAS) medium, for 1 to 3 days at 30° C. The production of siderophores is indicated by the appearance of a yellow-orange halo around the colonies (Husen 2003).
4. **Exopolysaccharids EPS production**

   The EPS production was determined quantitatively. The extraction was carried on by adding ethanol to the supernatant of cellular culture (3:1). After 48 h of precipitation at 4 °C, the precipitated EPS were washed three times, lyophilized, and weighted (Meneses et al. 2011).
5. **Production of indole 3 acetic acid (IAA)**

   The bacterial isolates were cultured in liquid LB medium supplemented with L-tryptophan (100 mg/l), with stirring at 200 rpm, at 30 °C for 24 h. The supernatants were obtained by centrifuging the bacterial cultures at 6000 rpm for 10 min. Colorimetric detection of IAA was performed according to the method of Bric et al. (1991). Two millimeters of the supernatant were mixed with 2 ml of Salkowski's reagent and 2 drops of O-phosphoric acid. After incubation in the dark at room temperature for 30 min, the development of a pink color is indicative of IAA production (Tarnawski et al. 2006; Ahmad et al. 2008). The absorbance at 530 nm was measured to determine the intensity of the resulting coloration. The concentration of IAA (µg/ml) was determined by comparison to the standard curve, established from a standard range of IAA (Sigma-Aldrich) (0, 5, 10, 25, 50, and 100 µg/ml).
6. **Production of hydrogen cyanide (HCN)**

   The bacteria were cultured on solid LB medium supplemented with glycine (4.4 g/l). A Whatman paper saturated with alkaline picrate was placed in the lid of each box. The dishes were sealed with parafilm and incubated at 30° C for 4 days (Ahmad et al. 2008). The appearance of a red–orange color confirms the production of HCN.
7. **Nitrogen fixation**

   Atmospheric nitrogen fixation was tested on a solid nitrogen-free medium (NFM). This medium was inoculated with streaks from the bacterial culture and then incubated at 30 °C for 48 h. Any growth on this medium reflects the bacteria ability to fix nitrogen (Ding et al. 2005).
8. **Production of hydrolytic enzymes**

   The capacity of each strains to produce hydrolytic enzymes was qualitatively evaluated separately on solid medium containing the appropriate substrate for each hydrolytic enzyme activity: cellulase (Verma et al. 2007), glucanases, chitinases (Naik & Sakthivel 2006), pectinases (Mefteh et al. 2017), amylases (Saleem and Ebrahim 2014), laccases (Mefteh et al. 2017,) and proteases (Naik & Sakthivel 2006). The dishes were incubated at 30° C for 7 days. The presence of the enzyme activity appeared as halos around the colonies.

Plant growth parameters

Bacterial inoculum was prepared as follow: BAT (10^3 cfu/ml and 10^7 cfu/ml); BA10 (10^3 cfu/ml and 10^7 cfu/ml); BA30 (10^3 cfu/ml and 10^7 cfu/ml).

Thirteen potato seed tubers of the Spunta variety were soaked in the bacterial suspensions for 15 min before plantation in soil (without fertilizer). Control tubers were soaked in sterile distilled water. During the period of potato plants cultivation, the stem elongation, leaf, stem, and root fresh weight and leaf number were measured after 45 and 95 days of plantation.

1. **Determination of leaf chlorophyll and carotenoid content**

   The chlorophyll a and b and carotenoid contents were extracted according to the method of Arnon (1949). About 100 mg of fresh leaves were weighed and crushed in a mortar in the presence of 500 µl of pure acetone, then 1 ml of 80% acetone was added. The extract was centrifuged at 4 °C for 10 min at 12,000 rpm, and the supernatant was adjusted to a volume of 2 ml with 80% acetone. The absorbance at 663 and 645 nm was measured. The chlorophyll a and b contents were calculated in µg/g FW as follows:

$$Ca\ (\exists \imath g/g\ FW) = Chlorophyll\ a = (12,7 \times OD663\ nm) - (2,69 \times OD645\ nm) \times V/FW$$

$$Cb\ (\exists \imath g/g\ FW) = Chlorophyll\ b = (22,9 \times OD645\ nm) - (4.68 \times OD663\ nm) \times V/FW$$

where V = volume of adjustment acetone added to the supernatant; FW = fresh weight.

Carotenoid concentration was determined by spectrophotometry at 450 nm using a molar extinction coefficient of 2500 mol^{-1} L^{-1} cm^{-1} as reported by Morris et al. (2004). Results were expressed in terms of µg/g FW.

2. **Stomatal activity**

The conductance of the stomata was measured with a Leaf Porometer (Model SC-1; DecaGon Devices).

3. **Chemical characterization of tubers**

a. Determination of dry weight (DW)

The percentage of dry weight was determined after drying the fresh sample at 105 °C for 48 h (AFNOR 1991).

b. Determination of reducing sugars

The dry sample (1 g) was dispersed in distilled water (10 ml). The mixture was boiled for 10 min then cooled. The solution (1 ml) was defecated by adding 0.1 ml of potassium ferrocyanide (15%) and 0.2 ml of zinc acetate (30%). The mixture was centrifuged at 1000 rpm for 5 min. The reducing sugars recovered in the supernatant were then determined using dinitrosalycilic acid (DNS): A volume of 0.1 ml of supernatant diluted in 0.9 ml of distilled water was mixed with 3 ml of DNS and boiled for 10 min. The absorbance was then determined at 550 nm. The sugar content reducing agents was determined based on a standard range of glucose (0, 0.1, 0.2, 0.4, 0.8, 1.2, 1.6, 2 g/l); Miller (1959).

c. Starch dosage

The starch content was determined based on the enzymatic method described by Khabou et al. (1996). The sample, dried at 50 °C and ground into a powder (1 g) was dispersed in 10 ml of distilled water. The mixture was supplemented with 10 µl of α-amylase (Termamyl 120L, Novozyme) and incubated for 2 h at 90 °C. After cooling, 20 µl of amyloglycosidase (AMG 300, Novozyme) were added and the solution was incubated for 6 h at 60 °C, then centrifuged at 1000 rpm for 5 min. The reducing sugars released in the supernatant were determined with dinitrosalycilic acid (DNS): 20 µl of the supernatant diluted in 980 µl of distilled water were treated with 3 ml of DNS, and boiled for 10 min. The absorbance was finally measured at 550 nm. A control was prepared by following the same steps but without adding enzymes. The glucose content of the sample was determined based on a standard glucose range (0.2, 0.4, 0.6, 0.8, 1, 1.2, 1.4, 1.6, 1.8, 2 g/l).

Statistical analyses

All data are expressed as the mean ± the standard deviation, from three independent biological replicates. Statistical analyzes of variance (ANOVA) were carried out with the statistical software SPSS (version 17.0, SPSS Inc., Chicago, IL, USA). Significant means were separated using the Duncan procedure. Significant differences between means were determined at P value < 0.05.

Results and discussion

In a previous work, we prepared three composts by mixing olive mill waste water (OMW), olive pomace, coffee grounds, and phosphogypsum (0, 10, and 30%). Their derived compost teas showed a beneficial effect on potato plant growth and protection against *Fusarium solani* infection (Samet et al 2018). The characterization of these compost teas showed that they harbor plant growth–promoting bacterial strains such as *Pseudomonas* (Samet et al. 2019). In this study, we isolated one bacterial strain from each compost tea using a selective medium. The microscopic observation of the different isolated bacteria showed that they were motile gram-negative bacilli. These bacteria were also identified by 16 s rDNA sequencing and were affiliated with *Serratia liquefaciens* (B.AT and B.A10) and *Achromobacter spanius* (B.A30) species (Fig. 1). A characterization of the PGPR traits of these three bacteria was carried out and the study of their effect on potato plant growth and yields under field culture conditions were investigated.

Evaluation of PGPR traits of the different isolated strains

The biological properties of the isolated strains were analyzed in order to determine the various secondary metabolites and enzymatic activities related to a plant growth promotion they may exhibit.

1. **Nitrogen fixation**

Nitrogen-free medium was used to evaluate nitrogen fixation ability. Results showed that B.AT and B.A10 strains can fix atmospheric nitrogen. Nitrogen-fixing bacteria associated with the rhizosphere are increasingly used on non-leguminous plants such as sugar beet, sugar cane, rice, corn, and

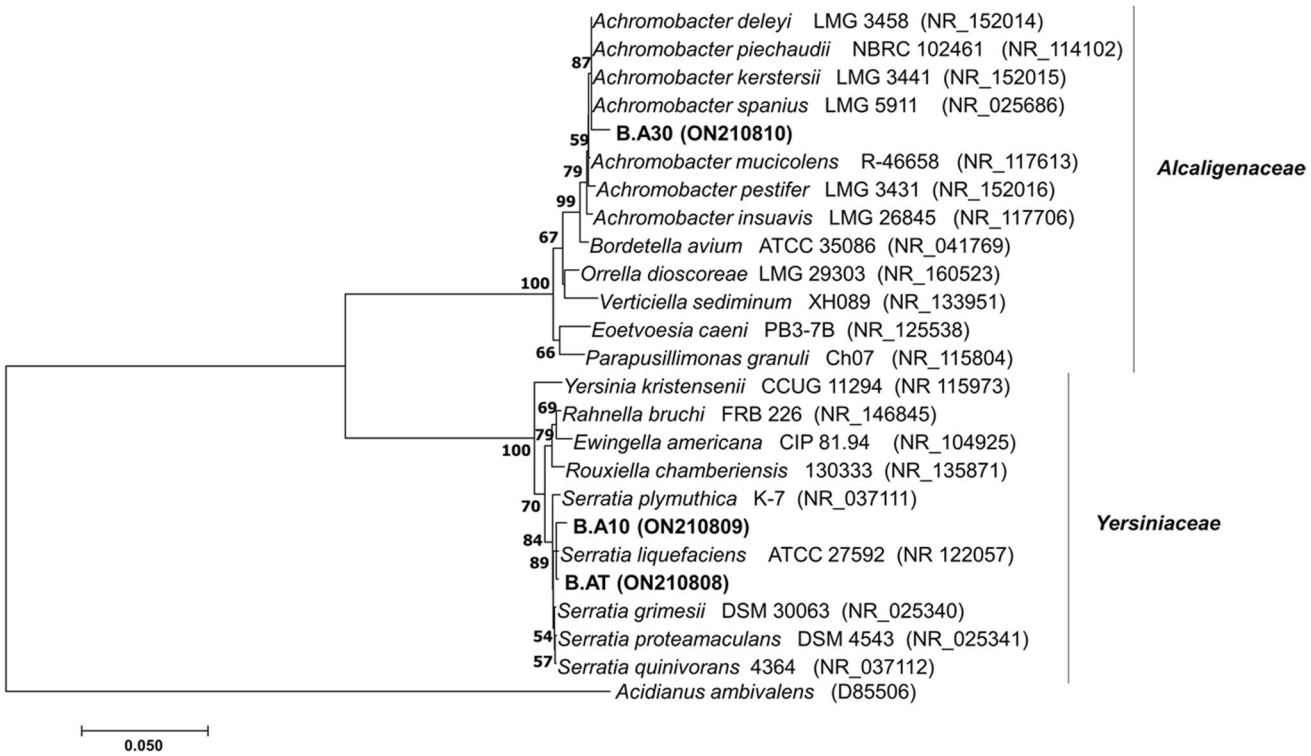

Fig. 1 Phylogenetic tree based on the sequence of the gene encoding 16S rRNA of the bacterial strains isolated from different compost teas. The tree is based on the Juke-Cantor model and the neighbor-joining method. The sequence of *Acidianus ambivalens* was used as the outgroup

wheat (Basu et al. 2021). Similar results were reported by Zelaya-Molina et al. (2016) who showed that the *Serratia liquefaciens* bacteria are able to fix atmospheric nitrogen.

2. Phosphorus solubilization

The isolated bacteria were capable to dissolve tricalcium phosphate (Ca_3 PO_4) as reported by Zelaya-Molina et al., (2016) who showed that the bacteria *Serratia liquefaciens* was phosphate solubilizer. Likewise, Santos and Rigobelo (2021) have shown that the bacterium *Achromobacter spanius* was able to solubilize phosphate.

Successful applications of phosphate-solubilizing bacteria were carried out for many important crops such as wheat (Kumar et al. 2014), mung bean (Biswas et al. 2018), rapeseed (Valetti et al. 2018), tomatoes (Nassal et al. 2018), and potato (Aloo et al. 2020).

3. IAA production

The production of IAA was noticed for all strains (B.AT, B.A10, and B.A30). The quantification of these compounds suggested that the B.A10 strain is the most productive (Table 1). Similarly, Aloo et al. (2020) showed that *S. liquefaciens* can produce IAA and Ahmad et al. (2008) showed the ability of *Achromobacter spanius* bacteria to produce IAA.

Table 1 Indole-3-acetic acid and exopolysaccharides production

	Microbial strain		
	B.AT	B.A10	B.A30
[IAA] (µg/ml)	46.222 ± 4.152 a	57.786 ± 3.598 b	42.561 ± 1.503 a
[EPS] (g/L)	2.4 ± 0.2 c	1.84 ± 0.052 b	0.4 ± 0.043 a

Values with different superscripts (a, b, c) are significantly different (means of three replicates)

4. Enzymatic and antifungal activities

Bacteria isolated from the different compost teas didn't show any amylase, pectinase, glucanase, cellulase, laccase, or chitinase activity in vitro. This may explain the lack of antifungal activity against *Botrytis cinerea* and *Fusarium solani* (data not shown). However, both B.A10 and B.AT strains showed proteolytic activity. The higher activity noticed for B.AT strain with an inhibitory halo of approximately 4 cm in diameter, while that of the B.A10 strain was of 1.8 cm. Previous studies described such protease production for *S. liquefaciens* (Baglinière et al. 2017).

5. **HCN production**

Although the production of hydrogen cyanide (HCN) measured in a number of PGPR bacteria, none of the strains studied here (B.AT, B.A10, and B.A30) showed such production. Cyanide hydrogen production can limit the growth of plant pathogens in the soil, but it can also affect the growth of beneficial microorganisms in the rhizosphere and therefore indirectly plant growth (Zdor 2015).

6. **Siderophores production**

The B.AT and B.A10 bacteria of *Serratia* genus showed a distinct yellow-orange halo on the O-CAS medium, indicating the production of hydroxamate-type siderophores (Schwyn and Neilands 1987) while B.A30 strain did not give any response. These results are in agreement with those of Zelaya-Molina et al. (2016) who have shown that the *S. liquefaciens* bacteria are producers of siderophores *Enterobacter* is the main *Enterobacteriaceae siderophore*. Two of the four enterobacterin synthase genes (entE and entF) are annotated in the complete genome of *S. liquefaciens* ATCC 27,592 (Zelaya-Molina et al. 2016). In contrast, Abo et al. (2019) showed that *Achromobacter spanius* does not produce siderophores which corroborated our results.

7. **Exopolysaccharide production**

The synthesis of exopolysaccharides was evaluated by precipitation with ethanol. Results showed that all the bacterial strains were able to produce exopolysaccharides (EPS) especially the B.AT strain (Table 1). Similarly, Abaidullah et al. (2015) showed that *S. liquefaciens* can produce EPS. The exopolysaccharide contains many negative charges that can efficiently sequester metal cations (van Hullebush et al. 2003).

8. **Assessment of the tolerance of bacteria to NaCl and Cd**

The tolerance of the different strains (B.AT, B.A10, and B.A30) to Cd and NaCl was studied by culture on LB medium containing different concentrations of $CdCl_2$ and NaCl. All isolated strains were capable to grow in media supplemented with NaCl (15 g/l) and $CdCl_2$ (400 mg/l). B.A10 seemed to be more tolerant to NaCl (40 g/l) than the others, while the B.A30 exhibited higher tolerance to Cd (Table 2). In fact, bacteria of the *Serratia* genus (B.AT and B.A10) continue to grow in the presence of 35 g/l NaCl. In contrast, *Achromobacter* (B.A30) were capable to grow in a medium supplemented with 200 mg/l $CdCl_2$. These results corroborate several studies which have shown that bacteria of the genus *Serratia* and *Achromobacter* are tolerant to salinity and heavy metals (Ma et al. 2011; Barra et al. 2016).

The presence of *Achromobacter* bacteria in A30 compost tea may be associated with the presence of phosphogypsum with high Cd concentration (15 ppm) as reported by Kammoun et al. (2017). The Cd content of the phosphogypsum seems to affect the microbial biodiversity in the A30 compost tea (Chen et al. 2015; Sarathambal et al. 2017). Cd tolerance and PGPR traits of these bacterial strain may improve plant growth under Cd stress (Sinha and Mukherjee 2008; Sarathambal et al. 2017; Pramanik et al. 2017).

The capacity of *Achromobacter* (B.A30) to solubilize phosphate and its tolerance to Cd may minimize Cd mobilization to plants by increasing soluble phosphorus in the soil, thereby promoting plant growth under stress conditions (Pramanik et al. 2018). In addition, the polysaccharide coating of this bacteria can provide numerous sites for adsorption and scavenging of toxic metals from the soil (Bruins et al. 2000; Rajkumar et al. 2010).

Effect of the isolated bacterial strains on the growth and yields of potato

Potato tubers of the Spunta variety were soaked for 15 min in B.AT, B.A10, or B. A30 bacterial suspensions at 10^3 CFU/ml or 10^7 CFU/ml. Control tubers were soaked in sterile distilled water. The treated and control tubers were then planted in the soil and irrigated regularly with tap water.

1. **Effect of bacterial inoculation on potato plant growth**

Regular observations of potato plants from bacteria treated tubers showed the positive effect of the inoculation on plant vigor in comparison to the control plants (Fig. 2).

Table 2 Growth capacity of B.AT, B.A10, and B.A30 on LB medium supplemented with NaCL or $CdCl_2$

	Medium + NaCl				Medium + $CdCl_2$					
Concentration	5 g/l	15 g/l	35 g/l	40 g/l	50 g/l	10 mg/l	40 mg/l	200 mg/l	300 mg/l	400 mg/l
B. AT	+++	+++	++	+/−	+/−	+++	+++	++	+/−	-
B. A10	+++	+++	++	+	+/−	+++	+++	++	-	-
B A30	+++	+++	±	+/−	-	+++	+++	+++	+	+/−

(+++) Very high; (++) High; (+) moderate; (±) low; (-) absent

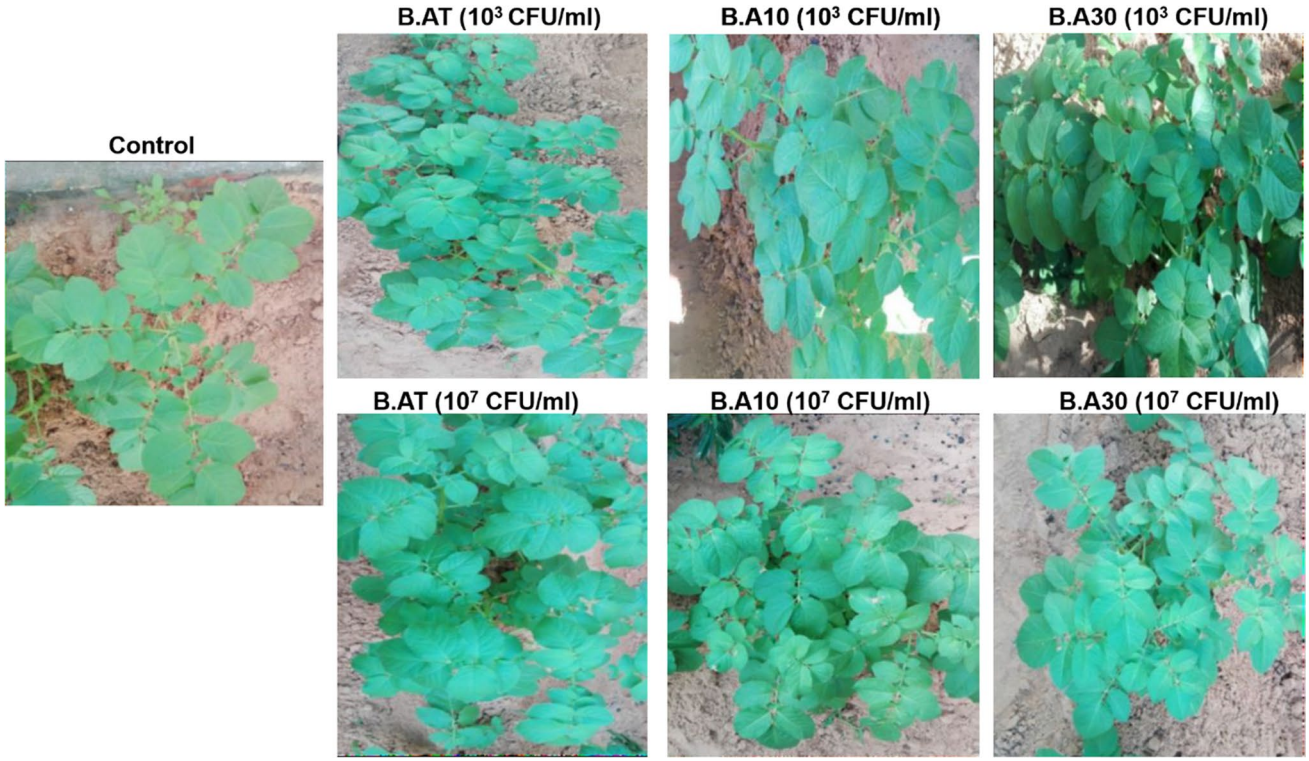

Fig. 2 Potato plants morphology after 45 days of culture

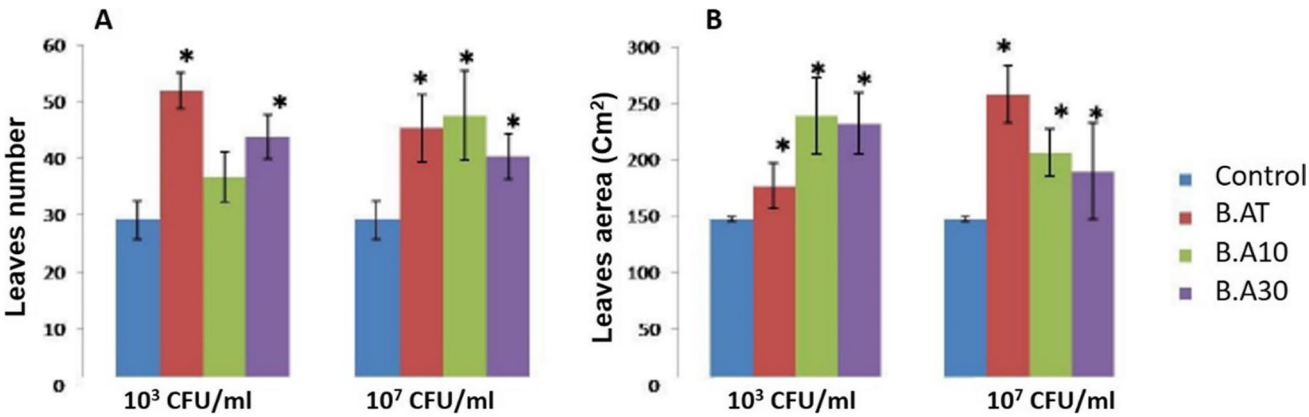

Fig. 3 Stems elongation of control and treated potato plants after 45 and 95 days of culture. Values with a single asterisk (a single asterisk (*) is significantly different to the control (at p value ≤ 0.05)

Plant growth parameters (Fig. 3) showed that tuber inoculation with the strains at a concentration of 10^3 cfu/ml had no significant effect on the elongation of the main stem. However, the treatment with 10^7 cfu/ml with B.AT and B.A10 promoted the elongation of plant stem after 45 days of culture compared to the control plants. Inoculation with the B.A30 strain at the 10^7 concentration resulted in a better elongation after 95 days of culture in comparison to control (Vejan et al. 2016).

Significantly, higher leaf number and area were observed in the inoculated plants compared to the control ones (Fig. 4). Determination of plants fresh weight confirmed these results. Indeed, after 45 days of cultivation plants from tubers treated with 10^7 cfu/ml bacterial suspension displayed higher leaf fresh weight than the others. The roots were more developed after 45 days of culture in the plants from tubers inoculated by 10^3 cfu/ml of the different strains. The best root fresh weight was obtained with

B.A30 treatment. Plants inoculated with B.AT showed higher leaf and stem fresh weight than control. Similar, stem FW of plants inoculated with B.A10 and B.A30 and of the control plants were noticed (Fig. 5).

These results confirm that all the tested strains isolated from compost tea had a beneficial effect on potato plant vigor in term of leaf size and number.

a) Chlorophyll and carotenoid contents

The pre-treatment of the tubers with the bacterial strains resulted in a chlorophyll gain in the potato plant leaves in comparison to control ones (Fig. 6). Plants from tubers treated with B.AT strain showed the best chlorophyll content. Such increase of chlorophyll content in plants obtained from the treated tubers suggests a more important photosynthetic activity than that of the control plants (Fig. 6).

The evaluation of the carotenoid content in leaves showed higher level in plants inoculated by B.AT strain in comparison to the others. B.A10 and B.A30 strains seem to have a less marked effect since the carotenoids contents in the leaves increased slightly after 95 days of cultivation (Fig. 7).

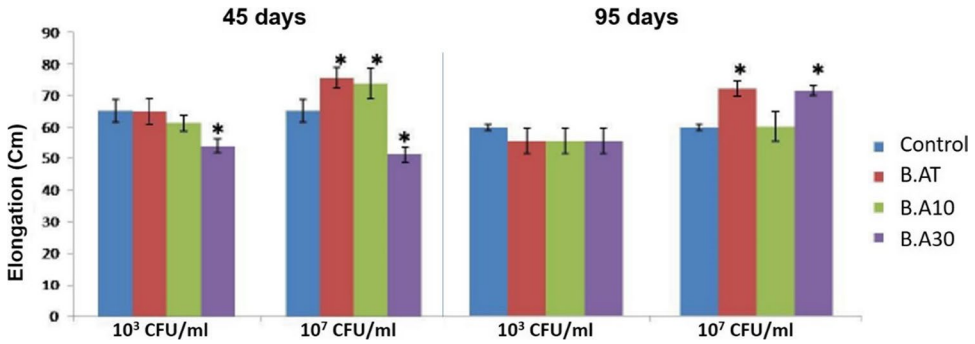

Fig. 4 Leaf number (**A**) and area (**B**) of control and treated potato plants after 45 days of culture. Values with a single asterisk (*) are significantly different to the control (at *p value* ≤ 0.05)

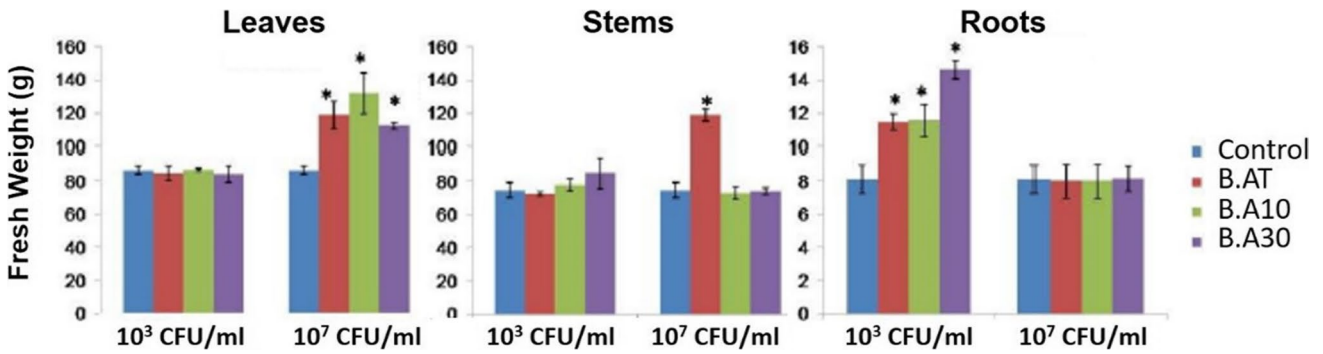

Fig. 5 Leaf, stem, and root fresh weight of control and treated potato plants after 45 days of culture. Values with a single asterisk (*) are significantly different to the control (at *p* value ≤ 0.05)

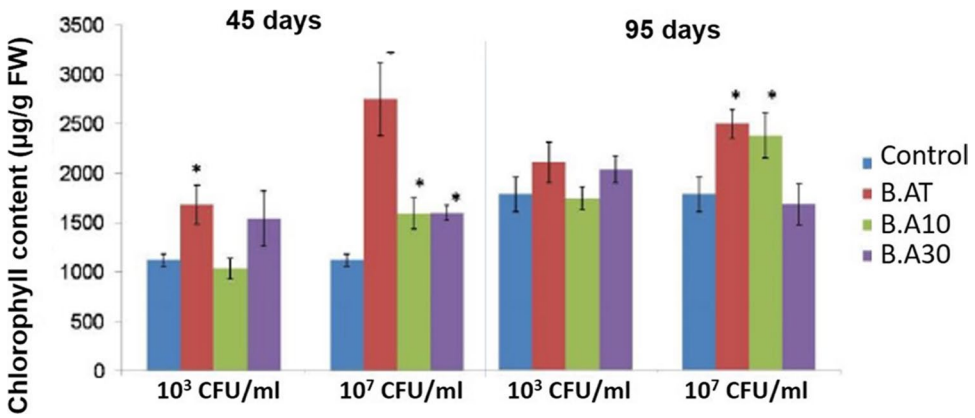

Fig. 6 Chlorophyll content in leaves of control and treated potato plants after 45 and 95 days of culture. Values with a single asterisk (*) are significantly different to the control (at *p* value ≤ 0.05)

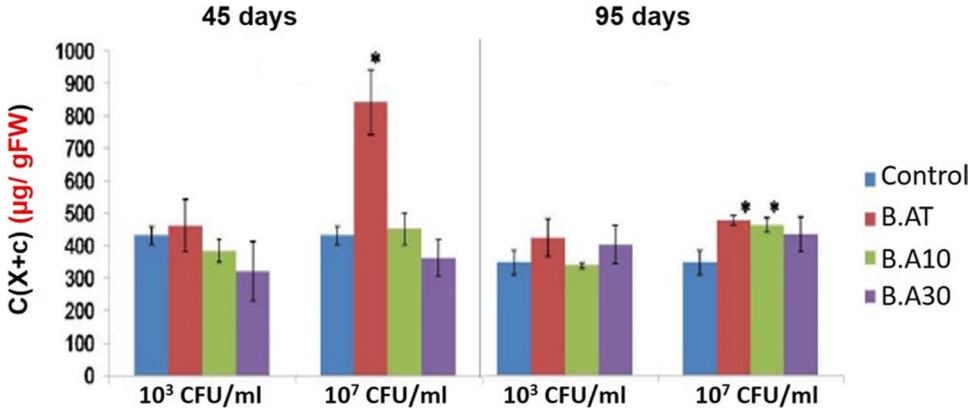

Fig. 7 Carotenoid content in leaves of control and treated potato plants after 45 and 95 days of culture. Values with a single asterisk (*) are significantly different to the control (at p value ≤ 0.05)

All these results confirm that *Serratia* and *Achromobacter* can be used as PGPRs to improve plant growth as reported by other reports (Zhang et al. 2002; Belimov et al. 2009; Jha and Kumar 2009; Zahir et al. 2011). Moreover, El-Esawi et al. (2018) showed that *Serratia liquefaciens* could enhance maize growth under normal and high salinity conditions. Aloo et al. (2020) also showed that *S. liquefaciens* isolated from potato rhizosphere is a PGPR which can be exploited as biofertilizer. Likewise, *Achromobacter spanius* inoculation was able to increase the growth of sugarcane plants under greenhouse conditions (Santos and Rigobelo 2021).

A reduction of stomatal activity was observed in leaves of plants coming from B.AT-, B.A10-, and B.A30-treated tubers, after 45 days of culture in comparison to control plants (Fig. 8). Stomatal closure following inoculation with PGPRs represents a structural barrier that may delay disease progression (Rudrappa et al. 2008; Pieterse et al. 2014). After 95 days of culture, at the end of the vegetative potato cycle, an increase of stomatal activity was observed in B.A10- and B.A30-treated plants (10^7 cfu/ml) (Fig. 8).

b) Effect of bacterial inoculation on plant nutrient absorption

Analysis of the mineral content in the leaves and roots of plants after 45 days of cultivation showed a significant increase in the K, Mg, and Zn contents in leaves of plants from tubers treated with B.AT (10^7) (Table 3). The P accumulation increased significantly in leaves of all plants treated by bacterial strains. An increase in Fe uptake was also observed in leaves of plants treated by B.A10 (10^7 cfu/ml) and B.A30 (10^3 and 10^7 cfu/ml). Leaves of plants treated with B.A30 (10^3 cfu/ml) showed a significant increase of Zn accumulation in addition to the improved uptake of Fe for 10^3 and 10^7 cfu/ml bacterial treatment in comparison to control. Roots from B.AT- and B.A10-treated plants exhibited higher K, Mg, and Ca accumulation than control. The Fe content increase significantly in roots of plants treated by B.A10 (10^3 and 10^7 cfu/ml) and B.A30 (10^3 cfu/ml), while Zn content increased in roots of plants treated by B.AT (10^3 cfu/ml), B.A10 (10^3 and 10^7 cfu/ml), and B.A30 (10^3 cfu/ml) (Table 3).

The nutrient uptake increase can be attributed to the production of growth regulators by the bacteria, which stimulate root development and allows a better uptake of water and plant nutrients (Lifshitz et al. 1987; Höflich et al. 1997). Even though a decrease of Mg, Zn, and

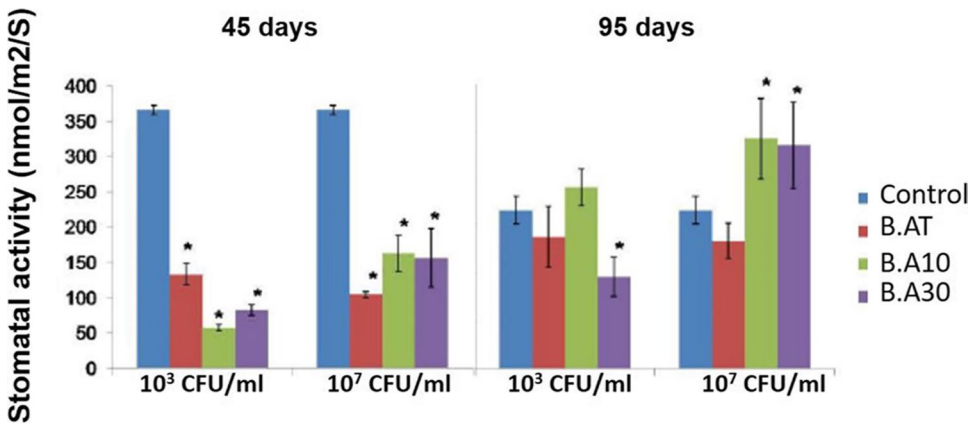

Fig. 8 Stomatal activity in leaves of control and treated potato plants after 45 and 95 days of culture. Values with a single asterisk (*) are significantly different to the control (at p value ≤ 0.05)

Table 3 Effect of inoculation with the different bacteria B.AT, B.A10, and B.A30 (10^3 and $10.^7$ CFU/ml) on the accumulation of mineral elements in plant leaves and roots (mg/g DW)

		Control	B. AT (10^3)	B. AT (10^7)	B. A10 (10^3)	B. A10 (10^7)	B. A30 (10^3)	B. A30 (10^7)
Leaves	K	48.537 a ± 6.495	78.332 b ± 5.712	84.248 b ± 15.337	49.165 a ± 14.467	71.086 b ± 2.897	47.383 a ± 14.771	30.802 a ± 17.074
	Mg	9.219 b ± 0.078	9.295 b ± 0.239	11.063 c ± 0.326	5.040 a ± 1.072	9.286 b ± 1.204	8.590 b ± 0.629	5.842 a ± 0.94
	Zn	0.078b ± 0.014	0.045a ± 0.002	0.262c ± 0.023	0.04 a ± 0.002	0.077b ± 0.008	0.239c ± 0.02	0.045a ± 0.002
	Ca	56.881 b ± 4.193	28.619 a ± 5.158	52.135 b ± 0.422	25.889 a ± 1.56	32.527 a ± 6.689	77.892 c ± 5.11	33.646 a ± 5.452
	Fe	0.027 b ± 0.001	0.022 a ± 0.004	0.021 a ± 0.0008	0.059 d ± 0.004	0.025 ab ± 0.0004	0.053 c ± 0.001	0.081 e ± 0.009
	P	0.111 a ± 0.032	0.262 b ± 0.053	0.254 b ± 0.092	0.279 b ± 0.049	0.516 c ± 0.082	0.791 c ± 0.02	0.297 b ± 0.018
Roots	K	11.959ab ± 6.323	19.252c ± 1.331	44.213d ± 7.202	15.607bc ± 2.782	12.675b ± 2.988	8.054a ± 6.229	8.645a ± 2.605
	Mg	4.323a ± 1.297	6.859b ± 1.428	10.189c ± 0.171	5,813 b ± 0.744	6.654b ± 0.462	6.180 b ± 0.095	4.303a ± 0.572
	Zn	0.105b ± 0.023	0.132b ± 0.073	0.037a ± 0.002	0.283c ± 0.005	0.112b ± 0.034	0.119b ± 0.091	0.028a ± 0.007
	Ca	44.213a ± 1.433	78.526d ± 0.424	64.507c ± 0.008	52.542b ± 5.941	62.458c ± 4.114	63.387c ± 0.412	41.033a ± 6.66
	Fe	1.571a ± 0.144	3.382c ± 0.272	1.274a ± 0.27	3.379c ± 0.363	3.357c ± 0.059	2.350b ± 0.131	0.867a ± 0.067
	P	0.357 b ± 0.009	0.412 b ± 0.065	0.398 b ± 0.16	0.366 b ± 0.012	0.397 b ± 0.07	0.267a ± 0.022	0.317 b ± 0.21

Statistical analysis were performed separately for leaves and roots and for each concentration (10^3 and$10.^7$). Values with different superscripts (a, b, c, d, e) are significantly different (means of three replicates)

Ca contents was observed in leaves of plants treated by 10^3 cfu/ml B.A10 and P contents in roots of plants treated with B.A30 (10^3 cfu/ml) (Table 3), these values remain within the range of concentrations found by Walworth and Muniz (1993) who studied the variations in the concentrations of mineral elements in potato plant tissues during the vegetative growth period.

El-Esawi et al. (2018) showed that maize plants inoculated with *Serratia liquefaciens* KM4 exhibited significant improvements in K + and Ca2 + contents as compared with non-inoculated plants. Several studies reported that the inoculation with PGPRs increases the bioavailability of nutrients for several crops such as chickpea (Elkoca et al. 2008), barley (Cakmakc et al. 2007), tomato (Adesemoye et al. 2010), strawberries (Günes et al. 2009), and broccoli (Yildirim et al. 2011). Other reports suggested that improved nutrient uptake by PGPRs is attributed to the increased water uptake by plants (Dey et al. 2004). Other studies suggested that increased nutrient uptake by plants is related to increased root area (Adesemoye et al. 2008; Yildirim et al. 2011; Ndakidemi et al. 2011).

c) Bacterial concentration in plants tissues

The determination of bacterial concentration in leaves, stems and roots showed that plants treated with the B.AT, B.A10, and B.A30 bacteria (10^3 and 10^7 cfu/ml) exhibited high bacterial concentration in roots. Similarly, high bacterial concentrations were measured in leaves and stems of plants inoculated with B.AT. B.A30 bacteria (10^7 cfu/ml) led to bacterial accumulation in leaves and stems. For plants inoculated with B.A10 strain (10^7 cfu/ml), high bacterial concentration was detected in the stems (Table 4). These results are in agreement with those of Devi et al. (2016) and Mukherjee et al. (2017) who showed that *Serratia* and *Achromobacter* bacteria are endophytes that colonize the plant tissues without causing symptoms while improving their growth.

2. Effect of inoculation on tuber yield and quality

Table 4 Counting of bacteria in CFU/g using a PCA medium in the various organs of plants inoculated with B.AT, B.A10, and B.A30 (10^3 and $10.^7$ CFU/ml) and control plants after 45 days of culture

	Leaves		Stems		Roots	
Control (CFU/g FW)	0		86,474		99,447	
Bacterial inoculum (cfu/ml)	10^3	10^7	10^3	10^7	10^3	10^7
B.AT (CFU/g FW)	353.982	666.666	236.973	100.738	1490.384	2971.887
B.A10 (CFU/g FW)	0	0	0	70.365	864.695	1114.427
B.A30 (CFU/g FW)	0	802.469	0	311.086	2259.887	4984.930

Plants yields were determined in term of weight and number of tubers per plant. Tuber size were also measured (Table 5). The results showed that all the inoculations with B.AT, B.A10, and B.A30 increased significantly the plant yield in term of number of tuber/plant by 20.81%, 38.39%, and 30.6% respectively. A significant increase in tuber size with 53.488% and 46.511% was also observed in B.A10- and B.A30-treated plants, respectively. (Table 5, Fig. 9). These results are in agreement with those of El-Esawi et al. (2018) who showed that maize seed inoculation by *Serratia liquefaciens* KM4 significantly improved maize plant growth and yield biomass. Abdel-Rahman et al. (2017) also showed that *Achromobacter* sp. bacteria increased the yield of inoculated tomato plants.

The evaluation of the bacterial treatment on tuber quality was carried out by measuring dry matter, starch, reducing sugars, and minerals content. The results (Table 6) showed that with B.AT (10^7 cfu/ml) and B.A10 (10^3 cfu/ml) increased tuber DW and starch contents and reduced reducing sugars levels compared to the tubers of control plants. In addition, the P, K, and Fe contents increased in almost all tubers from treated plants.

Tubers of plants treated with B.A30 showed a significant increase in the Ca and Mg contents. Similar Zn contents were measured in the tubers obtained from treated or control

Table 5 Effect of inoculation with B.AT, B.A10, and B.A30 (10^3 and $10.^7$ CFU/ml) bacteria on the yield of potato plants

	Yield (g/plant)		Tuber number/plant		Caliber (cm)	
Control	171a ± 31.432		2.75a ± 0.5		5.375a ± 0.853	
Bacterial inoculum (cfu/ml)	10^3	10^7	10^3	10^7	10^3	10^7
B.AT	206.6ab ± 26.839	281.33b ± 44.736	4.5b ± 0.57	4a ± 0.81	4.5a ± 0.408	4.75a ± 0.645
B.A10	236.66b ± 8.082	304.33b ± 29.091	3.25a ± 0.95	3.25a ± 0.957	8.25b ± 0.645	8b ± 0.707
B.A30	223.33b ± 32.005	251.66b ± 39.004	3.75ab ± 0.5	4.25b ± 0.95	7.875b ± 1.108	7.875b ± 0.75

Statistical analysis were performed separately for leaves and roots and for each concentration (10^3 and$10.^7$). Values with different superscripts (a, b) are significantly different (means of three replicates)

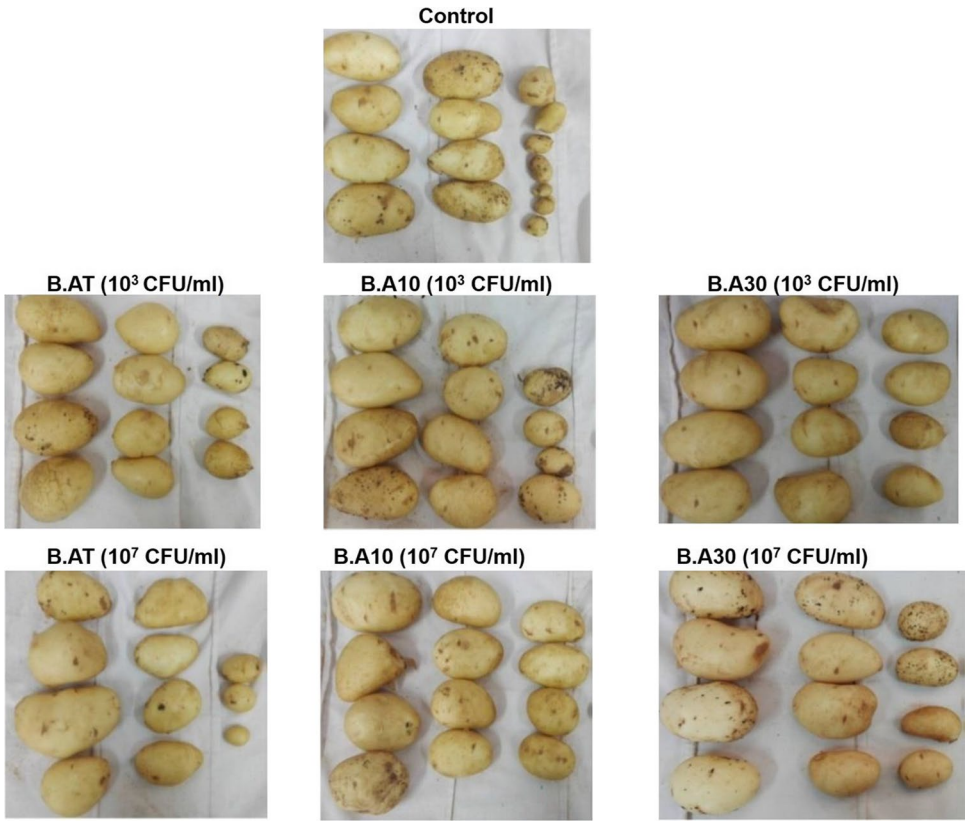

Fig. 9 Morphology of potato tubers from control and PGPR inoculated plants

Table 6 Effect of inoculation with the different bacteria B.AT, B.A10, and B.A30 (10^3 and $10.^7$ CFU/ml) on the composition of potato tubers

	Control	B. AT		B. A10		B. A30	
		10^3	10^7	10^3	10^7	10^3	10^7
% DW	21.458bc ± 1.152	24.041d ± 0.883	21.472bc ± 1.604	22.47c ± 0.147	20.104a ± 0.38	20.617ab ± 1.162	19.736a ± 0.395
%Starch	70.438ab ± 3.233	68.019a ± 3.385	76.846c ± 3.986	76.377c ± 3.464	65.275a ± 2.472	74.829bc ± 2.364	67.214a ± 2.945
% reducing sugar	7.436d ± 0.454	4.856b ± 0.811	3.482a ± 0.358	4.346ab ± 0.244	12.199e ± 1.146	7.335d ± 0.15	6.212c ± 0.32
K (mg/g DW)	13.210a ± 0.826	17.095b ± 0.654	18.325bc ± 1.049	17.845bc ± 0.407	18.52c ± 0.165	17.8bc ± 0.706	13.503a ± 0.324
Fe (mg/g DW)	0.007a ± 0.001	0.0139b ± 0.002	0.0128b ± 0.001	0.0229d ± 0.001	0.0187c ± 0.001	0.017c ± 0.001	0.0311e ± 0.002
Ca (mg/g DW)	8.297ab ± 0.491	9.499b ± 1.478	8.253ab ± 0.089	7.960a ± 0.473	11.006c ± 0.44	15.341d ± 0.318	12.189c ± 0.962
Zn (mg/g DW)	0.056c ± 0.001	0.057c ± 0.005	0.0433b ± 0.001	0.029a ± 0.003	0.0644d ± 0.003	0.0612c ± 0.007	0.0563c ± 0.002
Mg (mg/g DW)	0.889a ± 0.074	1.145 cd ± 0.061	0.915a ± 0.047	0.823a ± 0.056	1.028b ± 0.001	1.208d ± 0.005	1.095bc ± 0.08
P (mg/g DW)	0.0457a ± 0.002	0.187e ± 0.034	0.072bc ± 0.002	0.057ab ± 0.002	0.085c ± 0.001	0.11d ± 0.004	0.079bc ± 0.001

Statistical analysis were performed separately for leaves and roots and for each concentration (10^3 and$10.^7$). Values with different superscripts (a, b, c, d, e) are significantly different (means of three replicates)

plants except for those obtained from plants treated with the B.A10 (10^7 cfu/ml) where the Zn contents decreased significantly.

These results are in agreement with several studies which have shown that inoculation with PGPRs increased the nutritional quality of plant products such as broccoli, tomato, and beans (Yildirim et al 2011; Sirichaiwetchakul et al. 2011).

Conclusion

This study showed that the bacterial strains isolated from AT, A10, and A30 compost teas can be characterized as PGPR. *Serratia liquefaciens* strains isolated from AT and A10 compost teas exhibited higher PGPR traits (nitrogen fixation, solubilization of P, production of AIA, siderophores, and exopolysaccharides production) than *Achromobacter spanius* (B.A30). However, this latter species showed higher Cd tolerance than *S. liquefaciens* allowing using it in contaminated soils. Field trial results showed that all the isolated strains improved potato plant growth by increasing stem elongation, leaves (at 10^7 CFU/ml) and roots (at10^3 CFU/ml) fresh weight, and chlorophyll and carotenoids content. The best plant growth parameters were observed in *S. liquefaciens* (B.AT)-inoculated plants. These results can be related to by the best nutrient (K and P) uptake observed in these plants. The CFU counting in plant tissues show also that *S. liquefaciens* (B.AT) can colonize all plant tissues. Moreover, the use of treatment 10^7 cfu/ml *S. liquefaciens* (B.A10) as inoculum seems to be more efficient in increasing tuber yield and quality than the other treatments. Therefore, we can conclude that *S. liquefaciens* (BAT and BA10) acts as PGPR for potato plants under standard conditions and it can be used as biofertilizer.

Author contribution All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Mariem Samet and Imen Ghazala. Dr. Fatma Karray carried out the phylogenetic analysis, Cyrine Abid contributed to the in vitro analysis of the PGPR proprieties, Nour Chiab and Oumèma Nouri-Ellouz contributed to the field trials, Sami Sayadi helped to draft the manuscript and Radhia Gargouri-Bouzid participated in the design of the study and its revision. The first draft of the manuscript was written by Mariem Samet and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Declarations

Consent to Participate Informed consent was obtained from all individual participants included in the study.

Consent to Publish The participant has consented to the submission of the case report to the journal.

Competing interests The authors declare no competing interests.

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