Isolation Of Bacterial Strains From Compost Teas And Screening Of Their PGPR Properties

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Research Article

Keywords: PGPR, Compost, Potato, Field, Yield, Tuber quality

Posted Date: February 15th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1286369/v1>

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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 were affiliated with *Serratia plymuthica* (B.AT), *Serratia grimesii* (B.A10) and *Achromobacter piechaudii* (B.A30) species. A characterization of the PGPR traits of these three bacteria was carried out *in vitro* (Siderophore, Exopolysaccharides, IAA production...) and the study of their effect on potato plants growth, yields and tuber quality was studied under field culture conditions.

Intoduction

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 et al., 1999 ; Mengesha et al., 2017 ; De Corato et al 2020). Furthermore, CTs microbial population and soluble nutrients play an important role in suppressing disease and promoting plant growth (De Corato et al., 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 a 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 (Rodríguez 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 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 (Etesami et al., 2015; Ma et al., 2011; Ullah et al., 2015). The main plant hormones produced by PGPR, we can cite indol 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 (Ma et al., 2016; Rajkumar et al., 2012 ; Min Ren et al., 2019). Many PGPR play 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; Diáñez et al., 2005), antibiosis (Ulloa-Ogaz et al., 2015), hydrolytic enzymes production (Goswami et al., 2016), secondary metabolites production like hydrogen cyanide (Voisard et al., 1989) and aminocyclopropane-carboxylic acid (ACC) deaminase production which reduce the level of ethylene (Glick et al., 2010, Jacobson et al., 1994). These mechanisms, protect plants against biotic stress (Glick, 2010, Ma et al., 2011, Rajkumar et al. 2012).

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

The use of PGPRs to improve plant growth by inoculating or coating seeds, roots or tubers is a very old practice (Brown 1974; Gaskins et al. 1985); and it has been largely focused on legumes and cereals (Sessitsch and Mitter, 2015). Even today, there is still a growing interest in the application of artificial bacterial soil. However, the efficacy of PGPR *in vitro* does not mean there efficacy under field conditions. In the same time, many strains may have mechanisms to promote plant growth that are difficult to screen for under laboratory conditions. These strains are discarded based on poor performance of classical *in vitro* PGPR screening methods (Cardinale et al., 2015).

One of the major problems associated with the use of microbial inocula is their limited persistence in field (Martínez-Viveros et al., 2010; Rani and Goel, 2012), which is linked to the fact that the interaction between the PGPR bacteria and the plant also involves other interactions with living organisms, possibly competitors, and the involvement of variable abiotic factors (Dutta and Podile, 2010; Rani and Goel, 2012). To promote plant growth, introduced bacteria must colonize the roots, survive and proliferate in the rhizosphere (Lugtenberg and Kamilova, 2009).

The aim of the present study is firstly the characterization of bacterial strains isolated from three different compost teas and secondly to study their plant growth promoting effect in field condition, these compost teas, previously prepared, 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

1. Isolation of bacterial strains from compost teas

Composts were prepared by mixing olive mill waste water (OMW) olive pomace (P), coffee grounds (G), and phosphogypsum (PG).

AT compost: 25% G +25% OMW + 50% P+ 0% PG

A10 compost: 22,5% G + 22,5% OMW + 45% P + 10% PG

A30 compost: 17,5%G + 17,5%OMW+ 35%P + 30%PG

Compost teas were then prepared by soaking composts in water with daily mechanic aeration for 7 days at 1:5 w/v (compost:water) ratio.

Three bacteria were isolated from these AT, A10 and A30 compost teas. Isolation was performed by the multiple streak method, on nutrient agar medium containing triclosan, a broad spectrum antibiotic inactive against several gram-negative PGPR genera such as *Pseudomonas*, *Serratia* and *Achromobacter* (Welsch and Gillock, 2011).

2. Phylogenic identification of the bacterial strains

a) Genomic DNA extraction

Genomic DNA extraction was performed for pure strains already isolated using the Wizard Genomic DNA Purification Kit (Promega) from 3 ml of culture, following the protocol provided by the manufacturer. After suspending the cells in EDTA (40 mM), digestion of cell membranes with lysozyme at a final concentration of 2 mg/ml was performed. After incubation for 1 h at 37 °C and centrifugation at 13.000 g for 2 min, the pellet was solubilized in 600 µl of cell lysis solution. The mixture was incubated at 80 °C for 5 min. After cooling to room temperature, 3 µl of RNase (stock solution: 4 mg / ml) were added and the mixture was incubated for 1 h at 37 °C, then 200 µl of a protein precipitating solution was added and the mixture was

placed for 5 min on ice. Subsequently, the mixture was centrifuged and the supernatant was taken up in 600 µl of isopropanol in order to precipitate the extracted DNA. To recover DNA, a third centrifugation was performed and the pellet was washed with 70% ethanol.

b) Amplification of the 16s rDNA

The DNA sequence that encodes for 16S rRNA was amplified by PCR using specific primers, FD1 and 1492r (Table 1). The PCR program began with 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 PCR products purified 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 positioning 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 500 replicas.

3. Determination of PGPRs traits In Vitro

a) Growth of 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 (CdCl₂) (10, 40, 200, 300 and 400 mg/L) and incubated at 30 °C for 24 hours.

b) Phosphorus solubilisation

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 cultured on the surface of the medium and then incubated at 30 °C for 7 days. Phosphate solubilization is indicated by the formation of a clear zone around the colonies.

c) Production of siderophores

The bacteria were cultured on the surface of the Chrome Azurol S (CAS) medium. The dishes were incubated 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).

d) Exopolysaccharids Production

The EPS was extracted by adding ethanol to the supernatant of cellular culture (3:1). The precipitated EPS was then washed three times (Mody et al., 1989)

e) 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 hours. The supernatants were obtained by centrifuging the bacterial cultures at 600 rpm for 10 min. Colorimetric detection of AIA was performed according to the method of Bric et al., (1991). Two ml 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 AIA production (Ahmad et al., 2008; Tarnawski et al., 2006). The

intensity of the resulting coloration was measured by absorbance at 530 nm. The concentration of AIA / ml in each culture was determined by comparison to the standard curve, established from a series of dilutions of AIA (Sigma-Aldrich) (0; 5; 10; 25; 50 and 100 µg / ml).

f) Production of volatile substances (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.

g) Nitrogen fixation

Atmospheric nitrogen fixation was tested on a solid nitrogen-free medium. The NFM 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.

h) Production of enzymes

The capacity of the strains to produce proteases was qualitatively evaluated on a solid medium containing skimmed milk. The dishes were incubated at 30 ° C for 7 days. The presence of the enzyme activity appeared as halos around the colonies.

4. Plant growth parameters

Bacterial treatments were prepared as follow: BAT (10³ cfu/ml) and BAT (10⁷ cfu/ml); BA10 (10³ cfu/ml) and BA10 (10⁷ cfu/ml); BA30 (10³ cfu/ml) and BA30 (10⁷ cfu/ml).

Thirteen tuber seeds from potato cultivar were soaked in the bacterial suspensions for 15 minutes 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.

a) Determination of chlorophyll content

The chlorophyll a and b contents were determined 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 80% acetone and 1 ml of pure acetone. The extract was centrifuged at 4 ° C for 10 min at 12,000 rpm and then the supernatant was adjusted to a volume of 2 ml with 80% acetone. An optical density reading was performed taken at two different wavelengths: 645 nm and 663 nm. The chlorophyll a and b contents are calculated in µg / g MF as follows:

$$- Ca (\mu\text{g/g FW}) = \text{Chlorophylle a} = (12,7 \times DO_{663} \text{ nm}) - (2,69 \times DO_{645} \text{ nm}) \times V/\text{FW}.$$

$$- Cb (\mu\text{g/g FW}) = \text{Chlorophylle b} = (22,9 \times DO_{645} \text{ nm}) - (4,68 \times DO_{663} \text{ nm}) \times V/\text{FW}.$$

where : V = Volume of adjustment acetone added to the supernatant ; MF = Fresh weight.

b) Stomatal activity

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

c) Chemical characterization of tubers

Determination of dry matter (DM)

The percentage of dry matter content was determined after drying the fresh sample at 105 ° C for 48 hours (Afnord, 1982).

Determination of reducing sugars

The dry sample (1g) was dispersed in distilled water (10ml). The mixture was brought to boil for 10 minutes 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 is centrifuged at 1000 rpm for 5 minutes. The reducing sugars recovered in the supernatant were then determined with dinitrosalicylic acid (DNS): 0.1 ml of supernatant diluted in 0.9 ml of distilled water were treated with 3 ml of DNS at the boil for 10 minutes. The optical density was 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).

Starch dosage

The starch content was determined based on the enzymatic method described by Ellouz-Ghorbel (1996). The sample dried at 50 ° C and ground into a powder (1g) was dispersed in 10 ml of distilled water. The mixture was treated with 10 µl of α-amylase (Termamyl 120L, Novozyme) for 2 hours at 90 ° C. After cooling, 20 µl of amyloglycosidase (AMG 300, Novozyme) are added and the solution was incubated for 6 hours at 60 ° C then centrifuged at 1000 rpm for 5 minutes. The reducing sugars released in the supernatant were determined with dinitrosalicylic acid (DNS): 20 µl of the supernatant diluted in 980 µl of distilled water were treated with 3 ml of DNS, at the boil for 10 minutes. The optical density 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).

5. Statistical analysis

All data are expressed as the mean ± the standard deviation, from three biological replicates. Statistical analyzes of the ANOVA type: Analyzes of variance (Pvalue <0.05) were carried out with the statistical software SPSS (version 17.0, SPSS Inc., Chicago, IL, USA).

Results

Three Composts were prepared previously 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 physico-chemical characterization of these compost teas showed that they harbor plant growth promoting bacterial strains such as *Pseudomonas* (Samet et al., 2019). One Bacterial strain was isolated from each compost tea using a selective medium. The microscopic observation of the different isolated bacteria showed that they are motile gram-negative bacilli (Figure 1). These bacteria were also identified by 16s rDNA sequencing and were affiliated with *Serratia plymuthica* (B.AT), *Serratia grimesii* (B.A10) and *Achromobacter piechaudii* (B.A30) species (Figure 2). A characterization of the PGPR traits of these three bacteria was carried out and the study of their effect on the growth and yields of potato plants grown in fields was envisaged.

1. Evaluation of PGPR traits of the different isolated strains

The biological properties of the three isolated strains were analyzed in order to determine the various secondary metabolites and enzymatic activities related to a PGPR effect that they may exhibit.

1. Nitrogen Fixation

Nitrogen-Free Medium was used to evaluate nitrogen fixation ability. Results show that B.AT and B.A10 can fix atmospheric nitrogen. Nitrogen-fixing bacteria associated with the rhizosphere are increasingly used of non-leguminous plants such as sugar beet, sugar cane, rice, corn and wheat. These results are in agreement with those of Etminani and Harighi. (2018) and

Ullah et al. (2015) who showed that the *Serratia plymuthica* and *Serratia Grimesii* bacteria are able to fix atmospheric nitrogen.

2. Phosphorus solubilization

The ability of the isolated strains to dissolve tricalcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$) has shown that the three bacteria are able to dissolve phosphate. These results are in agreement with those of Etminani and Harighi (2018) and Ullah et al. (2015) who showed that the bacteria *Serratia plymuthica* and *Serratia Grimesii* are phosphate solubilizers. Likewise, Ma et al. (2016) have shown that the bacterium *Achromobacter pieaunchedii* is able to solubilize phosphate.

3. IAA Production

The production of IAA has been shown in all three strains B.AT, B.A10 and B.A30. The quantification of these compounds in the different strains suggests that the B.A10 strain is the most productive (Table 2). These results are in agreement with those described by Vleeschauwer et al. (2007) who showed that *S. plymuthica* can produce IAA and with those of Ullah et al. (2015) who showed that *S. grimesii* is IAA productive. Ma et al. (2016) also showed the ability of *A. pieaunchedii* bacteria to produce IAA.

4. Enzymatic and antimicrobial activities

Bacteria isolated from the different compost teas didn't show any amylase, pectinase, glucanase, cellulase, laccase, lipase or chitinase activity *in vitro*. This explains the lack of antimicrobial activity against the fungal plant pathogens *Botrytis cinerea* and *Fusarium solani* tested.

Both B.A10 and B.AT strains showed proteolytic activity. The latter appeared to exhibit the greatest activity with an inhibitory halo of approximately 4 cm in diameter. While that of the B.A10 strain was of 1.8 cm (Figure 3). These results corroborate those of Neupane et al. (2014) and Nascimento et al. (2018) who showed that *S. plymuthica* and *S. grimesii* are protease producers.

5. HCN production

The production of hydrogen cyanide (HCN) was tested in all three strains B.AT, B.A10 and B.A30 since a number of PGPR bacteria produce this type of volatile antibiotic. None of the strains studied show such production. Cyanide production hydrogen 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 the *Serratia* genus, showed a distinct yellow-orange halo on the medium O-CAS indicating the production of hydroxamate-type siderophores (Schwyn and Neilands, 1987) while the strain from A30 compost tea didn't give any response (Figure 4).

These results are in agreement with those of Etminani and Harighi. (2018) and Ullah et al., (2015) who have shown that the *S. Plymuthica* and *S. grimesii* bacteria are producers of siderophores. These results are also in agreement with Ma et al. (2016) who showed that the bacterium *Achromobacter piechaudii* does not produce siderophores.

7. Exopolysaccharides Production

The synthesis of exopolysaccharides was evaluated by precipitation with ethanol. Results showed that all the bacterial strains are able to produce exopolysaccharides (EPS) especially the B.AT strain (Table 3).

All of the results obtained showed that the three strains isolated exhibited different PGPR characteristics. The bacteria B.AT and B.A10 of the genus *Serratia* exhibited PGPR traits (nitrogen fixation, solubilization of P, production of AIA, siderophores and exopolysaccharides), they also produced proteases. The bacterium from B.A30 of the genus *Achromobacter* solubilized phosphate and produced AIA and exopolysaccharides.

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 Cd and NaCl. The three isolated strains were capable to grow in media supplemented with NaCl (15g/l) and CdCl₂ (400mg/l). Bacteria from B.A10 seemed to be more tolerant to NaCl (40g/l) than the others, while the B.A30 exhibited higher tolerance to Cd (Table 4). 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 CdCl₂. These results are in agreement with several studies which have shown that bacteria of the genus *Serratia* and *Achromobacter* are tolerant to salinity and heavy metals (Barra et al., 2016, Ma et al., 2011).

The presence of *Achromobacter* bacteria in A30 compost tea may be associated with the presence of phosphogypsum. The metal content (Cd) of the phosphogypsum had an effect on the microbial biodiversity in the 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 (Sarathambal et al., 2017, Pramanik et al., 2017; Sinha et al., 2008; Lin et al. , 2016).

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).

II. Effect of the three isolated strains on the growth and yields of potato

Potato tubers of the Spunta variety were soaked for 15 minutes in bacterial suspensions at 10³ CFU/ml and 10⁷ CFU/ml of B.AT, B.A10 or B. A30. 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 inoculation on the growth of potato plants

Regular observations of potato plants from bacteria treated tubers showed the positive effect of the bacterial inoculation on plant vigor in comparison with the control plants (Fig. 5).

Plants growth parameters (Figure 6) showed that tuber inoculation with the strains at a concentration of 10³ cfu/ml had no significant effect on the elongation of the main stem. However, the treatment with 10⁷ cfu/ml with B.AT and B.A10 promoted the elongation of plants after 45 days of culture compared to the control plants. Inoculation with the B.A30 strain at the 10⁷ concentration resulted in better elongation after 95 days of culture.

Significantly higher leaf number and area in the inoculated plants compared to the control plants (Figure. 7). Determination of plants fresh weight confirmed these results. Indeed, after 45 days of cultivation plants from tubers treated with 10⁷ cfu/ml bacterial concentration 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 10³ cfu/ml of the different strains. The best roots fresh weight was obtained with B.A30 treatment.

Plants inoculated with B.AT show an increase in leaf and stem fresh weight. The FW of the stems of the plants inoculated with B.A10 and B.A30 and of the control plants were almost equal (Figure 8).

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 content

The pre-treatment of the tubers with B.AT bacteria resulted to the highest chlorophyll gain in the potato plant leaves (Figure 9).

Plants from tubers treated with strains B.A10 and B.A30 showed also higher levels of chlorophyll in the leaves compared to the controls. The increase of chlorophyll content in plants obtained from the treated tubers suggests more photosynthetic activity than that of the control plants. The B.AT strain appears to be the best performing (Figure 10 and 11).

The evaluation of the carotenoid content in leaves showed higher level in plants inoculated by B.AT strain in comparison to 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.

These results are in agreement with other studies which have shown that *Serratia* and *Achromobacter* are used as PGPRs to improve growth of several plant species (Zahir et al., 2011; Zhang et al., 2002; Belimov et al., 2009; Jha and Kumar., 2009). Several endophyte strains of *S. plymuthica* (Benhamou et al. 2000; Berg et al. 2005) increase the growth and yield of cucumber (Egamberdieva et al. 2011).

Koo and Cho (2009) have shown that *Serratia plymuthica* SY5 isolated from soils contaminated with heavy metals increased the growth of roots of maize plants through the production of IAA. De Vleeschauwer and Höfte. (2007) also showed that *S. plymuthica* is an endophyte associated with several plants including potato. Tavares et al., (2018) showed that *Serratia grimesi* bacteria are able to increase the growth of bean plants. Likewise, *Achromobacter piechaudii* is able to increase the growth of sedum plants in the presence of heavy metals (Ma et al., 2016).

A reduction of stomatal activity was observed in leaves of plants coming from with B.AT, B.A10 and B.A30 treatments, after 45 days of culture compared to control plants. Stomatal closure following inoculation with PGPRs represents a structural barrier that may delay disease progression. (Pieterse et al., 2014, Rudrappa et al., 2008). After 95 days of culture, at the end of the vegetative cycle an increase of stomatal activity was observed in B.A10 and B.A30 treated plants (Figure 11).

b) Effect of inoculation on plant nutrient absorption

Analysis of the mineral element 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). The P accumulation increased 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 from plants treated with B.A30 (10^3 cfu/ml) showed an increase of Zn accumulation in addition to the improved uptake of Fe for 10^3 and 10^7 cfu/ml bacterial treatment. Roots from B.AT and B.A10 treated plants, exhibited higher K, Mg and Ca accumulation in comparison to control. The Fe content increase was observed 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).

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 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 5), these values remain within the range of concentrations found by Walworth et al. (1993) who studied the variations in the concentrations of mineral elements in potato plant tissues during the period of vegetative growth.

Several studies reported that inoculation with PGPRs increases the bioavailability of nutrients for several crops such as chickpea (Elkoca et al. 2008), barley (Cakmakci 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 indicated that increased nutrient uptake by plants is related to increased root area (Yildirim et al. 2011; Adesemoye et al. 2008; Ndakidemi et al., 2011)

c) Bacterial concentration in plants tissues

The determination of bacterial concentration in leaves, stems and roots showed that for the control plants, bacterial concentration was very low in stems and roots (Table 6). The plants treated with the B.AT, B.A10 and B.A30 bacteria and (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 bacterial treatment of tubers (10^7 cfu/ml) led to bacterial accumulation in leaves and stems.

For plants inoculated with the strain B.A10 (10^7 cfu/ml), the bacteria were detected in the stems (Table 6). 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 interior plant tissues without causing symptoms while improving their growth.

2. Effect of inoculation on yield and quality of tubers

Tuber yields were determined in term of weight and number of tubers per plant. Tuber size were also measured (Table 7).

The results showed that all the bacterial inoculations with B.AT, B.A10 and B.A30 increased 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 7, Fig 12). These results are in agreement with those of Kurze et al. (2001) who showed that inoculation of strawberry plants with *Serratia plymuthica* bacteria increased yield by 60%. Likewise, the use of *Serratia grimesii* inoculation increased the yield of wheat plants and the Zn content of grains (Ullah et al., 2015). Abdel-Rahman et al. (2017) also showed that *Achromobacter* Sp. Bacteria increased the yield of tomato plants. The use of B.AT bacteria for seed tuber inoculation didn't have significant effect on tuber caliber.

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 8) showed that treatment 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 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; Abbas 2013)

Conclusion

In conclusion, the bacterial strains isolated from AT, A10 and A30 compost teas can be characterized as PGPR. Bacteria isolated from AT and A10 compost teas belong to *Serratia* genus they are nitrogen fixers, phosphate solubilizers, and phytohormone AIA and siderophore producers, which explains their beneficial effect on potato plant the growth and yield. The B.A30 strain of the genus *Achromobacter* is a phosphate solubilizer, exopolysaccharide producer and AIA producer. It also has a high tolerance to Cd, so it can be used as a PGPR in media contaminated with heavy metals.

Declarations

Ethical Approval

Not applicable

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.

Funding:

This work was financially supported by the Tunisian Ministry of High Education and Scientific Research

Competing Interests

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

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Mariem Samet and Imen Ghazala. 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.

Availability of data and materials

Not applicable

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Tables

Table 1: primers Sequences used for 16s rDNA amplification

		References
FD1	AGAGTTTGATCCTGGCTCAG	Weisburg et al., 1991
1492R	GGTTACCTTGTTACGACTT	Lane, 1991

Table 2: Indole-3-acetic acid content production

	Microbial strain		
	B.AT	B.A10	B.A30
[IAA] (µg/ml)	46.222	57.786	42.561

Table 3 : EPS Production

	Microbial strain		
	B.AT	B.A10	B.A30
[EPS] (g/L)	2.4	1.84	0.4

Table 4: Growth capacity of three bacterial strains isolated on LB medium supplemented with NaCL or CdCl₂

	Medium + NaCl					Medium + CdCl ₂				
Concentration	5g/l	15g/l	35g/l	40g/l	50g/l	10mg/l	40mg/l	200mg/l	300mg/l	400mg/l
B. AT	+++	+++	++	+/-	+/-	+++	+++	++	+/-	-
B. A10	+++	+++	++	+	+/-	+++	+++	++	-	-
B A30	+++	+++	+/-	+/-	-	+++	+++	+++	+	+/-

Table 5: Effect of inoculation with the different bacteria B.AT, B.A10 and B.A30 (10³ and 10⁷ CFU/ml) on the accumulation of mineral elements in 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

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

Tableau 6: 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 (10³ and 10⁷ CFU/ml) and control plants after 45 days of culture.

	Leaves		Stems		Roots	
Control (UFC/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 (UFC/g FW)	353.982	666.666	236.973	100.738	1490.384	2971.887
B.A10 (UFC/g FW)	0	0	0	70.365	864.695	1114.427
B.A30 (UFC/g FW)	0	802.469	0	311.086	2259.887	4984.930

Table 7: Effect of inoculation with B.AT, B.A10 and B.A30 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)	103	107	103	107	103	107
B.AT	206.6ab	281.33b	4.5b	4a	4.5a	4.75a
	±26.839	±44.736	±0.57	±0.81	±0.408	±0.645
B.A10	236.66b	304.33b	3.25a	3.25a	8.25b	8b
	±8.082	±29.091	±0.95	±0.957	±0.645	±0.707
B.A30	223.33b	251.66b	3.75ab	4.25b	7.875b	7.875b
	±32.005	±39.004	±0.5	±0.95	±1.108	±0.75

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

Table 8: Effect of inoculation with the different bacteria B.AT, B.A10 and B.A30 (103 and 107 CFU / ml) on the quality 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	24.041d	21.472bc	22.47c	20.104a	20.617ab	19.736a
	±1.152	±0.883	±1.604	±0.147	±0.38	±1.162	±0.395
%Starch	70.438ab	68.019a	76.846c	76.377c	65.275a	74.829bc	67.214a
	±3.233	±3.385	±3.986	±3.464	±2.472	±2.364	±2.945
% reducing sugar	7.436d	4.856b	3.482a	4.346ab	12.199e	7.335d	6.212c
	±0.454	±0.811	±0.358	±0.244	±1.146	±0.15	±0.32
K (mg/g MS)	13.210a	17.095b	18.325bc	17.845bc	18.52c	17.8bc	13.503a
	±0.826	±0.654	±1.049	±0.407	±0.165	±0.706	±0.324
Fe (mg/g MS)	0.007a	0.0139b	0.0128b	0.0229d	0.0187c	0.017c	0.0311e
	±0.001	±0.002	±0.001	±0.001	±0.001	±0.001	±0.002
Ca (mg/g MS)	8.297ab	9.499b	8.253ab	7.960a	11.006c	15.341d	12.189c
	±0.491	±1.478	±0.089	±0.473	±0.44	±0.318	±0.962
Zn (mg/g MS)	0.056c	0.057c	0.0433b	0.029a	0.064d	0.0612c	0.0563c
	±0.001	±0.005	±0.001	±0.003	±0.003	±0.007	±0.002
Mg (mg/g MS)	0.889a	1.145cd	0.915a	0.823a	1.028b	1.208d	1.095bc
	±0.074	±0.061	±0.047	±0.056	±0.001	±0.005	±0.08
P (mg/g MS)	0.0457a	0.187e	0.072bc	0.057ab	0.085c	0.11d	0.079bc
	±0.002	±0.034	±0.002	±0.002	±0.001	±0.004	±0.001

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

Figures

Figure 1

Microscopic observation of the isolated bacteria B.AT, B.A10 and B.A30 (magnification: 100 × 100)

Figure 2

Phylogenetic tree based on the sequence of the gene encoding 16S rRNA showing the positioning of bacterial strains isolated from different compost teas

Figure 3

Evaluation of the proteolytic activity of B.AT, B.A10 and B.A30

Figure 4

Synthesis of siderophores by B.AT, B.A10 and B.A30

Figure 5

Morphology 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 cultivation in fields

Figure 6

Elongation of the stems of plants from tubers treated with B.AT, B.A10 and B.A30 (10^3 and 10^7 CFU / ml) and control plants after 45 and 95 days of culture

Figure 7

Number of leaves (A) and leaf area (B) of plants inoculated with B.AT, B.A10 and B.A30 and control plants after 45 days of treatment

Figure 8

Fresh weight of leaves, stems and roots of plants inoculated with B.AT, B.A10 and B.A30 (10^3 and 10^7 CFU / ml) and control plants after 45 of culture

Figure 9

Chlorophyll content in leaves of plants inoculated with B.AT, B.A10 and B.A30 (10^3 and 10^7 CFU / ml) and control plants after 45 and 95 days of cultivation in fields

Figure 10

Carotenoid content in leaves of plants inoculated with B.AT, B.A10 and B.A30 (10^3 and 10^7 CFU / ml) and control plants after 45 and 95 days of culture

Figure 11

stomatal activity in leaves of plants inoculated with B.AT, B.A10 and B.A30 (10^3 and 10^7 CFU / ml) and control plants after 45 and 95 days of culture

Figure 12

Tubers morphology from control, B.AT, B.A10 and B.A30 (10^3 and 10^7 CFU / ml) treated plants