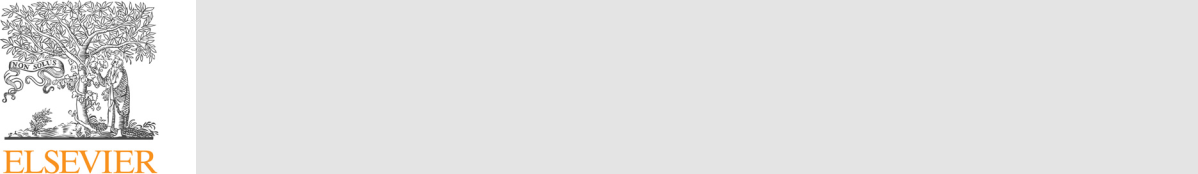
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Cross-competence and aﬀectivity of maize rhizosphere bacteria Bacillus sp. T MT7 in tomato rhizosphere 



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ABSTRACT

Rhizosphere supports the growth and activity of an enormous and diversified microbial network. Certain rhi-zobacteria referred to as ‘plant growth-promoting rhizobacteria’ (PGPR) can contribute towards improved plant growth and stress tolerance and are currently of great interest for sustainable agriculture. A successful PGPR is capable of establishing itself in the rhizosphere of inoculated plants. Root colonization is an essential step in the interaction amongst plant-associated bacteria and host plant and is a multifaceted phenomenon governed by various biotic and abiotic factors.

In the present investigation, an isolate MT7 obtained from maize rhizosphere and identified as Bacillus sp. was tested for its ability to perform in the tomato rhizosphere. It was screened for its survival under various abiotic stresses like salt, drought, heavy metals, and temperature and found to tolerate 10% salt stress, drought stress up to -0.73 MPa (25% PEG), heavy metals like Cr, Cu, and Ni above the permissible limits and grew well from 25 to 45 °C. The bacterial culture was verified for various other plant growth-promoting traits. It expressed chemo-tactic behavior and good biofilm-forming potential in the presence of tomato root exudates. Profuse colonization was observed on the tomato roots. Eﬃcacy of the bacterial culture in the tomato rhizosphere was evaluated under net house conditions. The tomato crop was positively influenced by inoculation of MT7 over the un-inoculated plants. Therefore, it is concluded that competent colonization by MT7 lies in its ability to respond to tomato root exudates, form biofilms, establish and proliferate in the tomato rhizosphere and express various PGP traits that cause an increase in plant growth. The results indicate the potential of MT7 as a bio-inoculant for tomato.

1. INTRODUCTION

As agronomic production is strengthening in recent decades and farmers are becoming more and more dependent on chemical fertili-zers, environmental pollution is becoming one of mankind's significant concerns. The use of chemical fertilizers and pesticides to improve yield and kill pathogens and pests has put the ecosystem stability at higher risk worldwide. An increase in crop productivity and enhanced food security with negligible or no application of chemical fertilizers and pesticides is of major concern these days ([Mahanty et al. 2017](#page8)). Therefore, the interest is shifting towards environment-friendly and sustainable organic farming. One of the indispensable components of organic farming is bio-fertilizers ([Megali et al. 2014](#page8)). Bio-fertilizers, also called plant growth-promoting rhizobacteria (PGPR), are for-mulations of live microorganisms that, when introduced, colonize the rhizosphere and promote plant growth and development by increasing the supply of both macronutrients and micronutrients to host plant



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([Malusa and Vassiley, 2014](#page8)).

PGPRs may either be bacteria, fungi, or algae that are found in the rhizosphere and are actively involved in enhancing soil productivity, stimulating plant growth, and suppression of phytopathogens, resulting in the development of an environmentally friendly sustainable agri-culture. Plant development by PGPRs is possible in either direct or in-direct ways. Indirect stimulation includes suppression of the harmful eﬀects of phytopathogens (mainly fungus) through mechanisms such as antibiosis, activation of host defense mechanisms (ISR), and competi-tion for space and nutrients in the surrounding area of plant roots. Direct promotion of plant growth by PGPRs generally involves me-chanisms like nitrogen fixation, phosphorous solubilization, and iron uptake by the synthesis of siderophores or by altering levels of plant hormones such as cytokinins, auxins, and ethylene ([Gouda et al., 2018](#page8)).

Colonization is an increase in the population of best-adapted mi-croorganisms to a particular ecological niche. Root colonization is an essential step in the interaction amongst plant-associated bacteria and

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host plants. It is a multifaceted phenomenon governed by various biotic and abiotic factors. Bacteria can add value to plant eﬃciency after root colonization. Root colonization by a bacteria involves traits such as chemotaxis, motility, outer membrane proteins, exopolysaccharides and lipopolysaccharides, presence of pili and fimbriae, the ability of biofilm and microcolony formation and the capacity to utilize compo-nents (sometimes specific) present in root exudates as nutrients ([Lugtenberg et al. 2001](#page8), [Rafique et al. 2015](#page8); [Yuan et al. 2015](#page8), [Xiong](#page8) [et al., 2020](#page8)). PGPRs applied on one crop subsequently form the soil microflora of the following crop ([Bhardwaj et al. 2014](#page8)). A PGPR strain of one crop, capable of colonizing the subsequent crop would provide an added advantage to the farmers. However, there are very few studies showing the persistence of PGPRs in the soil and their utilization in subsequent crops. [Walters et al (2018)](#page8) have shown that rhizospheric microflora colonization response to diﬀerent plant genotypes is locally seeded. This may be due to adaptation of the indigenous microbes to root exudate composition of the local plant variety which aﬀects co-lonization and competitiveness of microorganisms in the rhizosphere ([Faure et al. 2009](#page8)). Therefore, selecting PGPRs that can eﬃciently co-lonize multiple host plants would ensure improved germination and growth of the subsequent crop.

Maize (Zea mays) and tomato (Solanum lycopersicum) are two eco-nomically important short duration crops that are grown throughout the year and are often rotated in the same field. Both crops have the potential to influence the diversity and performance of microflora and the rhizosphere microflora of one crop is bound to aﬀect the next crop. However, there is very little work showing the eﬀect and colonization potential of microbes from maize rhizosphere to tomato rhizosphere and vice versa. This study is an attempt to determine the potential of an isolate from the maize rhizosphere to colonize and perform in the to-mato rhizosphere. In the fields usually, tomato seedlings are trans-planted whereas maize seeds are directly sown. It is easier and more common for farmers to apply bio-inoculums to the seed as compared to seedling treatments. Therefore, a maize field applied with bio-fertilizer will build up its population in the soil. The competence of this maize strain to other crops such as tomato will help in the establishment and growth of the plants.

Tomato (Solanum lycopersicum) has universal appeal in terms of its production and industrial value ([Deery 2012](#page8), [Cordero et al., 2018](#page8)). Improved fertilizers and management of tomato crop has become in-creasingly important to overcome large economic losses of up to 90% ([Figueiredo et al. 2010](#page8)). In India, the estimated production of tomato in the year 2018-19 was 20,515,000 MT with 814,000 Ha of the area under cultivation of tomato ([National Horticulture Board, 2019](#page8)). The major challenge faced in tomato cultivation in the Indian scenario is the exposure to various biotic and abiotic stresses (pathogens, heat, and water scarcity), fruit quality, and the need for increased yield. Con-sumer demand is also now directed towards organic produce to mini-mize the deleterious eﬀects on health and environment. Therefore, there is an urgent need to use chemical alternatives and develop bio-fertilizers that can perform eﬀectively in the tomato rhizosphere.

The major step in this direction is to identify the right strain which can eﬃciently colonize the tomato rhizosphere and exhibit beneficial traits resulting in improved plant performance. In this study, the au-thors have screened and selected microbes for application in the tomato rhizosphere acclimatized to the soil and climate type of Punjab, India. This paper deals with assessing the colonization behavior and applic-ability of a potential PGPR strain isolated from maize rhizosphere to the tomato rhizosphere of a locally grown variety.

2. Material and Methods

2.1. Seeds

Seeds of tomato (Solanum lycopersicum) var. SELECTION-22 (wild type) were obtained from the local grain market and surface sterilized

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before use.

2.2. Bacterial culture

The bacterial isolate MT7 was originally isolated from the rhizo-spheric soil of maize plants obtained from an agricultural field in Mullanpur, Chandigarh.

For identification of isolate MT7 16S rRNA gene sequencing was done at MTCC, IMTECH, Chandigarh, using the universal primers 27f and 1492R ([Weisburg et al., 1991](#page8)). The phylogenetic tree was prepared by comparing the 16S rRNA gene sequences of strain MT7 against re-presentative type strains of the genus Bacillus from the EzBioCloud database. Multiple sequence alignment and phylogenetic tree re-construction were done using R programming using the Maximum Likelihood method based on the Kimura two-parameter evolutionary model with 100 bootstrap values.

2.3. Characterization of the bacteria

Gram staining and motility of strain MT7 were studied as per standard protocol ([Aneja, 2003](#page8)).

2.3.1. Growth curve and generation time of strain MT7

The growth of selected isolate at diﬀerent times was analyzed in nutrient broth. Freshly grown culture (108 CFU ml-1) was inoculated @ 1% in 100 ml broth and incubated at 30 °C under stationary conditions. 2 ml sample was withdrawn after regular time intervals and OD was measured at 600 nm. A growth curve was plotted to track the lag phase and calculate the generation time.

2.3.2. Abiotic stress tolerance

MT7 was tested for its tolerance to various abiotic stresses under in vitro conditions. All the tests were performed in three replicates in a 96 well-microtitre plate and data are represented as the mean value of the replicates. To 200 μl of Nutrient broth (NB) with diﬀerent additives to induce stress, 2 μl (1% inoculum) of freshly grown MT7 culture (108 CFU ml-1) was inoculated. Control was maintained with broth having no additives. The plates were incubated at 30 °C for 24 h and assessed for growth by taking OD at 600 nm on a micro ELISA auto reader. Salt stress was induced by adding various concentrations of NaCl (1% to 10%). To induce drought stress diﬀerent concentrations of PEG 6000 varying from 0 to 30% (w/v) were used which created diﬀering water potential ranging from 0 to -1.32 MPa ([Michel and Kaufmann, 1973](#page8)). Drought tolerance was evaluated according to the criteria of [Susilowati](#page8) [et al. (2018)](#page8). Heavy metal tolerance of MT7 was tested against Cd, Cr, Cu, Pb, and Ni. Stock solutions of the heavy metals (1 mg ml-1) were prepared and supplemented in the nutrient broth, viz., Cd @ 0.8 to 2.1 ppm, Cr @ 100-500 ppm, Cu @ 36-116 ppm, Pb @ 85-205 ppm and Ni @ 35-115 ppm.

To test temperature tolerance of MT7, 10 ml of NB was inoculated in triplicate @ 1% with freshly growing bacterial culture and incubated at temperatures 25°, 30°, 35°, 40°, and 45 °C for 24 h under shaking con-ditions. Cell growth was estimated by measuring OD at 600 nm.

2.4. Biochemical analysis of strain MT7

The biochemical analysis of MT7 was carried out to determine the plant growth promotion traits.

2.4.1. Ammonia Excretion

MT7 was grown at 30 °C for10 days in nutrient broth. Every 2 days, the sample was withdrawn and centrifuged at 10000 rpm for 15 min and ammonia excretion was analyzed ([Chaney and Marbach, 1962](#page8)). Briefly, to 1 ml supernatant, 2.5 ml of Reagent A (Phenol 50 g L-1 and Sodium nitroprusside 0.25 g L-1 diluted 5x in water) and 2.5 ml of Re-agent B (Sodium hydroxide 25 g L-1, Sodium hypochlorite 2.1 g/l

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diluted 5x in water) were added and allowed to stand for 30 min with occasional shaking for the development of color. Excreted ammonia was quantified by measuring color intensity at 625 nm using ammo-nium sulfate (100 μgml-1) as a standard ([Bhatia et al. 2008](#page8)).

2.4.2. IAA Production

Quantitative production of IAA was determined by Salkowski’s method ([Tang, 1974](#page8)). MT7wasgrown at 30 °C for 8 days in nutrient broth amended with tryptophan (0.1 gL-1). Every 2 days, a 1 ml sample was withdrawn and centrifuged at 10000 rpm for 15 min and treated with 2 ml Salkowski reagent (50 ml, 35% perchloric acid; 1 ml 0.5 FeCl3), mixed and allowed to stand for 30 min for the development of the color. IAA produced was quantified by measuring the color intensity at 500 nm using IAA (100 μgml-1) as a standard ([Bhatia et al. 2008](#page8)).

2.4.3. Phosphate solubilization

Phosphate solubilization by MT7 was tested by spot inoculation on Pikovskaya’s medium plates and incubation at 30 °C for 48 h. A clear zone of solubilization around the spot indicated solubilization of in-soluble phosphate ([Bhatia et al. 2008](#page8)).

2.4.4. Production of enzymes, biosurfactant, and antifungal assay Ability of MT7 for production of enzymes pectinase, chitinase,

oxidase, and ACC deaminase, and biosurfactants was done by spot in-oculation on respective media under required conditions according to standard methods ([Hitha and Girija, 2014](#page8); [Hsu and Lockwood, 1975](#page8); [Singh et al. 2017](#page8); [Dworkin and Foster, 1958](#page8); [Mulligan et al. 1984](#page8)).

Bacillus sp. strain MT7 was tested for its antifungal activity against 2 fungal strains, Aspergillus niger and Colletotrichum gloeosporioides by the dual culture technique ([Zhao et al. 2010](#page8)). After incubation for 2 days at 30 °C, the plates were checked for growth inhibition.

2.5. Colonization studies

2.5.1. Preparation of root exudates

Tomato (Solanum lycopersicum) seeds var. SELECTION-22 were surface-sterilized by soaking in 70% ethanol for 1 min, followed by 2-3 washing with sterile distilled water, immersion in 5% sodium hypo-chlorite for 5 min, followed by seven washings with sterile water. To initiate the germination process sterilized seeds were transferred to petri plates containing 1% water agar and incubated at 28 °C for 2-3 days. Germinated seeds of tomato were then transferred into sterile collection assemblies as described by [Wadhwa and Narula (2012)](#page8). Twenty such assemblies were maintained. The assemblies were kept in a plant growth chamber for 14 days under ambient conditions of

1. ± 2 °C temperature and a 16-h light/8-h dark photoperiod. After 14 days of growth, the plantlets were removed under aseptic conditions and their roots were gently washed with sterile water to prevent any mixing of nutrient solution.

The tomato plantlets were then collectively transferred into a beaker containing 50 ml sterile distilled water for the collection of root exudates. The beaker was sealed and kept at 28 °C under shaking con-ditions for two days. After two days the root exudates were collected, lyophilized, and sterilized using a 0.22-μm filter and stored at -80 °C for further use ([Tan et al. 2013](#page8); [Yuan et al. 2015](#page8)).

2.5.2. Chemotaxis Assay

Chemotactic response of strain MT7 towards tomato root exudates was assessed by the “drop” assay as described by [de Weert et al. (2002)](#page8) and [Yuan et al. (2015)](#page8) with some modifications. Briefly, strain MT7 was grown overnight and cells were collected by centrifugation and re-suspended in chemotaxis buﬀer (100 mM potassium phosphate [pH 7.0] with 20 μM EDTA) maintaining cell count of 108 CFUml-1. An aqueous solution of 1% hydroxyl-propyl-methylcellulose was added to the cell suspension. The viscous cell suspension was transferred to a 60-mm-diameter Petri-dish. Following this, a 10 μL drop of root exudates

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was added to the center of the Petri dish and incubated for 15-30 min at room temperature. Following this, the plate was inspected for the for-mation of a ring of turbidity near the center. Ring formation indicated the trigger of a positive chemotactic response of bacterial cells.

The chemotactic response of MT7 was quantified by the capillary assay as described by [Adler, (1973)](#page8) and [Yuan et al. (2015)](#page8) with some modifications. The bacterial isolate was grown overnight, centrifuged, collected, and re-suspended in chemotaxis buﬀer to a cell density of 108 CFU ml-1. A standard capillary of size 1 μl was loaded with root exu-dates from one end and sealed at the other end. The loaded capillary was immersed for 1 h in the cell suspension prepared above. Chemo-taxis buﬀer alone was used as control. After the incubation period, the capillary was removed and its exterior ends rinsed with sterile water. The sealed end of the capillary was broken oﬀ over a test tube con-taining nutrient broth and the contents were mixed. Dilutions were made and plated on media plates. Treatment was performed in tripli-cates. The plates were incubated at 30 °C overnight, colonies were counted and CFU determined.

2.5.3. Biofilm formation assay

The eﬀect of concentrated root exudates was checked on growth and biofilm formation by the selected strain MT7in a 96-well microtitre plate. M9 minimal medium supplemented with root exudates was in-oculated @1% with freshly grown bacterial culture in a microtitre plate. Inoculations were done in triplicates and the plate was kept at 30 °C for 48 h. After incubation, the contents of the plate were drawn out by gentle tapping and washed four times with PBS (pH 7.2) to wipe out free-floating cells. Biofilm formed by bacteria adherent on the wells of the plate was stained with crystal violet (1%) for 15 min. Excess stain was removed by rinsing thoroughly with 95% ethanol and the plate was kept for drying. The optical density of stained biofilms was obtained by using a micro ELISA auto reader at wavelength 595 nm. OD595 values above 0.04 were taken as a sign of good biofilm formation ([Jain et al.,](#page8) [2013](#page8)).

2.5.4. Rifampicin tagging

Rifampicin negative strain MT7 was tagged with the rifampicin marker by spontaneous mutation. Briefly, overnight grown MT7 was plated on nutrient agar media plates without rifampicin and supple-mented with 200μgml-1 rifampicin and incubated at 30 °C for 2-3 days. The number of colonies appearing was counted and mutation frequency calculated. 4-5 colonies appearing on the rifampicin containing media plates were picked and re-streaked to recheck the resistance to ri-fampicin. The selected rifampicin-resistant colony of MT7 was grown in nutrient broth for at least 30 generations without rifampicin and sub-sequently grown on rifampicin (200 μgml-1) supplemented nutrient agar plates to confirm the stability of the mutant. ([Nautiyal 1997](#page8))

2.5.5. Colonization assay of MT7 in tomato root exudates

The colonization assay was performed as described by [Simons et al.](#page8) [(1997)](#page8) with slight modifications. Seeds of tomato (Solanum lyco-persicum) were surface sterilized and kept for germination on 1% water agar at 28 °C for 2-3 days. Bacterial strain MT7-Rif was grown in nu-trient broth to a cell density of 108 CFUml-1. 1% carboxymethyl cellu-lose (CMC) was added to the cell broth as an adhesive agent before treating the germinated seedlings. Upon germination, the seedlings were bacterized by soaking them in the cell broth amended with CMC for 30 min. For the control treatment, seedlings were soaked in half-strength Hoagland medium amended with CMC for 30 min. After bac-terization, the seedlings were planted individually in sterile test tubes containing 4 ml of half-strength Hoagland Medium and incubated for 7 days in a growth chamber at 25 ± 2 °C (16/8 h day/nights cycles). Six tubes were prepared for the treatment. After 7 days, the plantlets were removed from the test-tubes (3 replicates) under aseptic conditions and put into 1.0 ml PBS and shaken for 10 min on a vortex, the solution was then plated on Nutrient agar media plates with and without 50μgml-1

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rifampicin. Incubation was given for 24 h at 30 °C and CFU was de-termined.

2.5.6. FE-SEM of plant roots

Plantlets were gently taken out from the tubes after 7 days of in-cubation and roots were aseptically sectioned into 2- 2.5 cm pieces. These root sections were fixed in 100% methanol for 10 min, then transferred to 100% ethanol and kept for 30 min at room temperature. The second step was repeated twice. The root samples were stored at 4 °C until the FE-SEM analysis ([Pathan et al. 2010](#page8)). For analysis, root samples were fixed on metal stubs with double-sided adhesive tape and coated with gold particles and observed under FE-SEM. Micrographs were obtained.

2.6. Competence and PGP activity of MT7 in tomato

Plant studies were conducted in earthen pots (diameter 15 cm, depth 17 cm) in the months of January-March 2019 when the average temperature is around 25 °C. Evaluation of Bacillus sp. strain MT7 in-oculation was done against un-inoculated control plants of tomato. Soil from the fields of Panjab University, Chandigarh, was used for raising the plants. Earthen pots were filled with soil (sandy loam, pH 7.6). The inoculum was prepared by growing the selected bacterial culture in nutrient broth at 30 °C overnight at 100 rpm to a cell density of 108 CFU ml-1. Surface sterilized seeds of tomato were germinated on 1% water agar, bacterized with MT7 for 30 min, and sown in pots filled with soil. Six pots were kept for each treatment. 4-5 seeds were sown in each pot with equal spacing and were thinned to two plants per pot after 14 days of germination. Plants were placed in an open environment in a net-house. Treated and non-treated pots were irrigated with tap water as and when required to maintain the moisture level. After every 7-day interval, 2 ml bacterial culture was added in the respective pots as a booster dose for the initial 3 weeks after sowing. At the pre-bloom stage, (after 70 days) plants were removed from the soil after moist-ening the soil to avoid damaging the roots. Subsequently, the roots were gently washed and plants were dried on a filter paper to remove excess water. Following this, the shoots were cut at the soil surface level and roots were separated from shoots. Root and shoot fresh weight (in-cluding leaves), root and shoot length, and dry weight of shoots and roots (after drying at 80 °C in an oven) were determined. These para-meters were compared between three randomly selected pots (six plants) each from the inoculated and control treatments.

2.7. Statistical analysis

All data were calculated as mean ± SD for at least three replicates. The student's t-test was applied for data analyses using the descriptive statistical analysis tools of windows oﬃce excel 2013. Values with P < 0.05 were considered to be statistically significant.

3. Results

3.1. Identification of bacterial isolate

Gram-positive, motile, isolate MT7 was identified by 16S rRNA gene sequencing and showed a sequence similarity of 100% to Bacillus alti-tudinis and 99.93% to Bacillus xiamenensis. A phylogenetic tree was constructed by the maximum likelihood method ([Fig. 1](#page8)). The tree to-pology confirmed that isolate MT7 is related to members of the genus Bacillus. Therefore the isolate is designated as Bacillus sp. MT7.

3.2. Characterization of the bacteria

3.2.1. Growth curve

Bacillus sp. strain MT7 was tested for its growth pattern by plotting a growth curve at diﬀerent time intervals (Fig S1). The log phase of the

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organism was observed from 5 to 17 h followed by a stationary phase for 5-6 h and a decline thereon. The generation time of strain MT7 was 3.2 h.

3.2.2. Survival under abiotic stress conditions

Strain MT7 could withstand diﬀerent abiotic stress conditions. It could tolerate salt stress up to 10% NaCl (Fig S2). Results also indicated that with an increase in salt concentration, there was reduced bacterial growth. MT7 was tested for its tolerance to induced drought stress varying from 0 to -1.32 MPa and was found to survive up to osmotic pressure of -0.73 MPa ([Fig. 2](#page8)).

The strain demonstrated good tolerance to all the metals tested except lead (Pb). As the concentration of heavy metals increased its growth significantly decreased. In the case of Cr, MT7 tolerated all concentrations up to 500 ppm and grew well. In Cu, it was able to grow up to 56 ppm. In the presence of Ni, MT7 grew up to 95 ppm. In the case of Cd, the bacterial culture was not able to tolerate any of the tested concentrations (Fig S3).

MT7 was grown at diﬀerent temperatures to find its optimum growth temperature (Fig S4). The optimum temperature for its growth was 30 °C. However, MT7 showed considerable growth even at tem-peratures 40 and 45 °C.

3.3. Plant growth-promoting ability of Bacillus sp. MT7

Strain MT7 was tested for various biochemical traits that provide a PGPR competitive advantage over the indigenous microflora. MT7 was found to be an eﬀective solubilizer of phosphate, maximum ammonia excretion was recorded at 83.4 μgml-1 on day 6 and IAA production was 14.44μgml-1 on day 4. Besides these, the strain could produce oxidase enzyme and biosurfactants ([Table 1](#page8)).

3.4. Colonization studies

3.4.1. Chemotactic response and biofilm formation in the presence of tomato root exudates

In the qualitative drop assay concentrated root exudates positively attracted the cells of MT7 as can be seen in [Fig. 3](#page8)(a) and this attraction was more than the control (no root exudates) ([Fig. 3](#page8)b). A ring of tur-bidity was formed near the center of the Petri dish indicating movement of bacterial cells towards the root exudates. As compared to control, a higher number of cells of MT7 got attracted to the root exudates ([Fig. 4](#page8)). This indicates positive chemotaxis of the strain.

Biofilm-forming PGPRs exhibit improved colonization behavior and performance in the rhizosphere than non-biofilm formers. To test this potential of MT7 within the tomato rhizosphere, a microtitre plate assay was performed and good response of strain MT7 was observed in the presence of tomato root exudates ([Fig. 5](#page8)).

3.4.2. Colonization assay

Rifampicin resistance was used to study the competence and colo-nization of MT7 in the tomato rhizosphere. The Rif- tagged strain of MT7 was obtained at a mutation frequency of 5.5 × 105. It was com-pared to its wild type for Gram staining and abiotic stress tolerance and was observed to retain its characteristics.

Colonization behavior of MT7-Rif was studied in the tomato rhizo-sphere after a 7 day inoculation period. CFU of the bacteria on roots was determined on Nutrient agar plates with and without 50 μgml-1 rifampicin. The results showed that MT7 successfully colonized the tomato roots. MT7-Rif was detected on the root tips in higher numbers (1.66 × 105 CFU cm-1) than the root base (8.3 × 104 CFU cm-1) ([Table 2](#page8)). On plates without rifampicin higher CFU was obtained with 1.02 × 106 CFU cm-1 on the root tip and 6.36 × 105 CFU cm-1 on the root base. No bacterial growth was obtained from un-inoculated (con-trol) plants.

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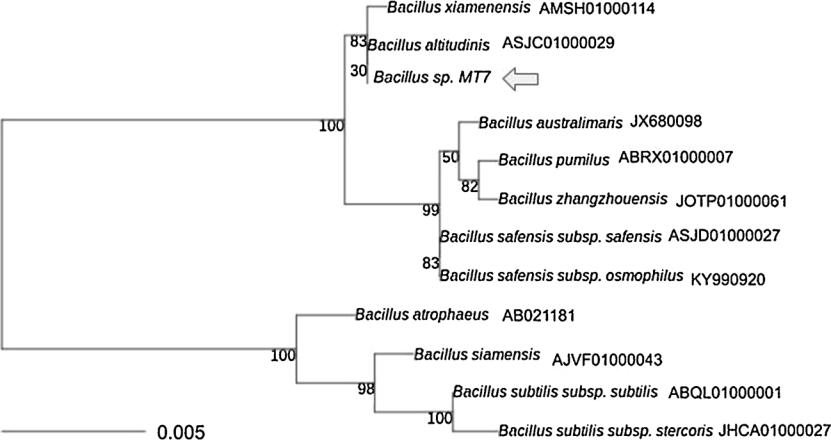
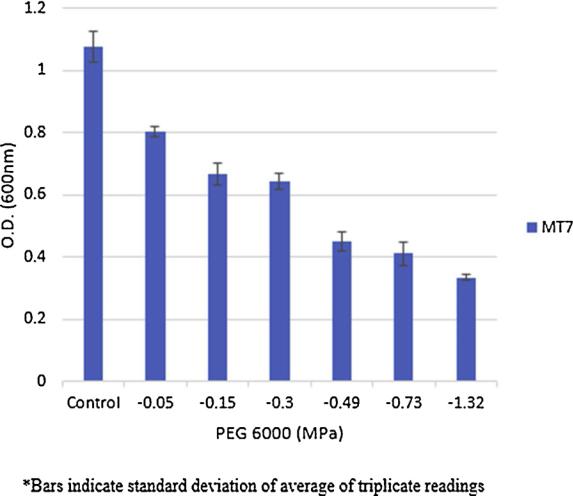


Fig. 1. Phylogenetic tree of strain MT7.

Fig. 2. Eﬀect of drought on growth of MT7 after 24 h.



3.4.3. FE-SEM analysis

The roots were sectioned into two parts, root base and root tips, prepared and analyzed under FE-SEM. Plants inoculated with MT7 (small rod-shaped) showed clustering of the microorganism around the base and root tips though the population of the microorganism was more around the root tip ([Figs. 6](#page8) a, b). No bacterial cells were seen on the root surfaces of the un-inoculated control plants ([Figs. 6](#page8) c, d).

3.5. Eﬀect of MT7 inoculation on growth of tomato under net-house conditions

The performance of PGPR strain MT7 was tested in the tomato (Solanum lycopersicum) rhizosphere under net house conditions. The positive influence of MT7 inoculation was observed on tomato. Overall, healthier growth of plants was observed on inoculation with MT7 as compared to the un-inoculated control. There was no visible incidence of any deficiency or disease. Root and shoot length and dry weight and

Table 1

Various plant growth-promoting properties in strain MT7.

fresh biomass of both roots and shoots of inoculated plants were sta-tistically significant at P < 0.05 as compared to un-inoculated control plants ([Table 3](#page8)) (Fig S5) suggesting the beneficial influence of PGPR strain MT7 on tomato.

4. Discussion

Soil microflora of an agricultural field is majorly dictated by the type of crop cultivated on it ([Edwards et al. 2015](#page8)). For example, fields with leguminous crops are predominantly rich in diazotrophs ([Hartman](#page8) [et al. 2017](#page8)). However, it would be of importance to know how the microbes prevalent in the rhizosphere of one plant aﬀect the growth of the subsequent crop in a diﬀerent cropping season. [Bais et al (2006)](#page8) enlisted several allelopathic molecules found in the root exudates of plants which negatively influence other plants and microflora. For ex-ample, [Wardle et al. (1994)](#page8) reported that secondary metabolites exuded from the roots of musk thistle inhibited the nodulation and nitrogen fixation in white clover leading to its reduced growth and survival in the field conditions. Similarly, [Gharsa et al. (2018)](#page8) reported that 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) secreted by maize inhibited the population of Agrobacterium in its rhizosphere as compared to tomato plants which did not exude this compound.

Tomato and maize due to their distinct exudates profile may have a diﬀerential impact on the microbial population which may lead to adverse implications on their growth and yield. The present study was performed to analyze the potential of a PGPR strain Bacillus sp. MT7 isolated from maize rhizosphere, for its competence and colonization of tomato plants. Bacillus is a bacterial genus commonly found in soil ([Fan](#page8) [et al. 2011](#page8)). Strain MT7 exhibited multiple plant growth-promoting activities such as good phosphate solubilization, production of IAA, ammonia excretion, presence of oxidase enzyme as well as the pro-duction of biosurfactants. These important traits give it a competitive ability over the native microflora.

Tomato is susceptible to various abiotic stresses such as high and low temperatures, salinity, drought, and heavy metals at all stages of its life cycle ([Gerszberg and Hnatuszko-Konka 2017](#page8)). The use of PGPRs which can assist in overcoming these stressed conditions would directly translate to higher production. Strain MT7 showed up to 10% NaCl

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Isolate | Gram’s | Motility | IAA | Ammonia | Phosphate | Pectinase | Chitinase | Oxidase | ACC | Biosurfactant | Antifungal activity against | | | |  |
|  | reaction |  | produ | excretion | solubilization |  |  |  | deaminase | production |  |  |  |  |  |
|  |  |  |  |  | A. niger | | C. gloeosporioides | |  |
|  |  |  | ction |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  | |  |  |  |
| MT7 | +ve rods | Motile | 14.44 | 83.4 | + | - | - | + | - | + | - | | - |  |  |
|  |  |  | μg/ml | μg/ml |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

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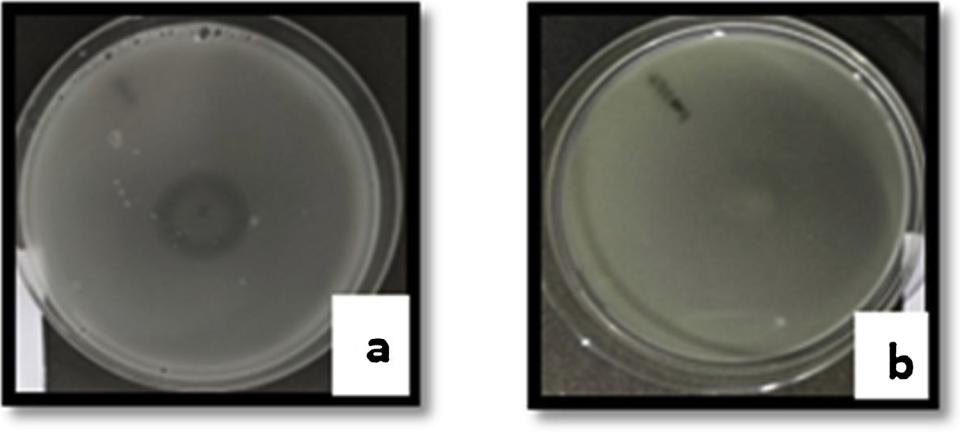


Fig. 3. Chemotactic response towards tomato root exudates, (a) MT7 (b) control.

Fig. 4. Chemotactic response of MT7 towards tomato root exudates.

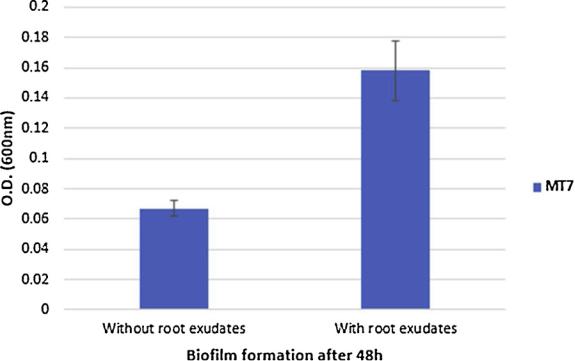
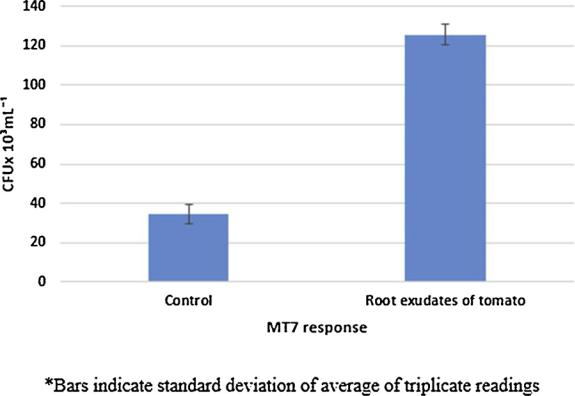


Fig. 5. Biofilm formation by MT7 induced by root exudates of tomato after 48h.

Table 2

Colonization of MT7 on tip and base of tomato roots under in-vitro conditions at 7 days.

Microbial Count (CFU cm-1)

|  |  |  |
| --- | --- | --- |
| Media | Colonization at Root base | Colonization at Root tips |
|  | (CFU cm-1) | (CFU cm-1) |
|  |  |  |
| NA- rif | 8.3 × 104 | 1.66 × 105 |
| NA | 6.36 × 105 | 1.02 × 106 |

\*Values are average of triplicate readings.

tolerance which was higher than [Pindi et al. (2014)](#page8) who reported up to 9% NaCl tolerance by Bacillus sp. [Egamberdieva et al. (2017)](#page8) reported a positive influence of Pseudomonas extremorientalis on the growth of

tomato plants in saline soils. [Azadikhah et al. (2019)](#page8) showed up to 8% NaCl tolerance of bacterial isolates that eﬀectively colonized the roots of barley. However, similar to [Pindi et al. (2014)](#page8) strain MT7 could survive over a wide range of temperatures. Drought tolerance up to -0.73 MPa ([Sandhya et al. 2009](#page8)), and up to -1.5 MPa ([Putrie et al. 2013](#page8)) has been reported earlier. MT7 exhibited the potent capability to combat the destructive drought conditions by surviving up to -0.73 MPa osmotic potential. It could also withstand heavy metal stress of Cr, Ni, Pb, and Cu at concentrations above the permissible limits for heavy metals in soils and plants ([Denneman and Robberse, 1990](#page8); [World](#page8) [Health Organization (WHO, 1996](#page8)). These features indicate the potential of MT7 to eﬀectively survive abiotic stress conditions of a tomato rhi-zosphere.

Root exudates of a plant act as chemoattractants for PGPRs. However, there is no preferred set of chemo-attractants and the re-sponse to particular chemo-attractants may diﬀer among species as well as within strains of a single species ([Yao and Allen, 2006](#page8)). In this study, MT7 which was originally isolated from maize rhizosphere was found to be chemotactically responsive to tomato root exudates. Thus Bacillus sp. MT7 is not host- specific and is not inhibited by any component of tomato root exudates. [Gharsa et al. (2018)](#page8) reported an inhibitory re-sponse of Agrobacterium fabrum genomovar G8 towards maize exudates. This indicates that the nature of a colonizing microbe also influences its colonization behavior in a rhizosphere. MT7 possibly responds to the presence of similar components in the root exudates of both plants. Lactic acid is a strong component found in the root exudates of both maize ([Fan et al. 2012](#page8)) and tomato ([Simons et al. 1997](#page8)) and MT7 possibly gave a positive chemotactic response towards it. A similar observation was made in the cucumber rhizosphere by isolates Bacillus amyloliquefaciens (from cucumber rhizosphere) and B. subtilis (from banana rhizosphere) towards citric acid, a key component of cucumber root exudates ([Zhang et al. 2014](#page8)). [Yuan et al. (2015)](#page8) studied the eﬀect of B. amyloliquefaciens towards root exudates of banana and found that the response was most directed in the presence of malic acid. Similarly, [Rekha et al. (2018)](#page8) also found malic acid in the root exudates of rice and reported the response B. subtilis towards it. Positive chemotaxis suggests that an isolate can get its nutrition from the root exudates and successfully establish itself and colonize in the plant rhizosphere ([Kandaswamy et al. 2019](#page8)).

MT7 also showed good biofilm formation in the presence of tomato root exudates. Biofilm formation is a prerequisite for long-term colo-nization. It reduces microbial competition and provides sustained beneficial eﬀects to host plants ([Kasim et al. 2016](#page8)). [Allard-Massicotte](#page8) [et al. (2016)](#page8) reported the ability of motile B. subtilis cells to diﬀerentiate into biofilm-producing cells within several hours of contact with the plant roots.

Strain MT7 exhibited profuse colonization on the root tips of tomato

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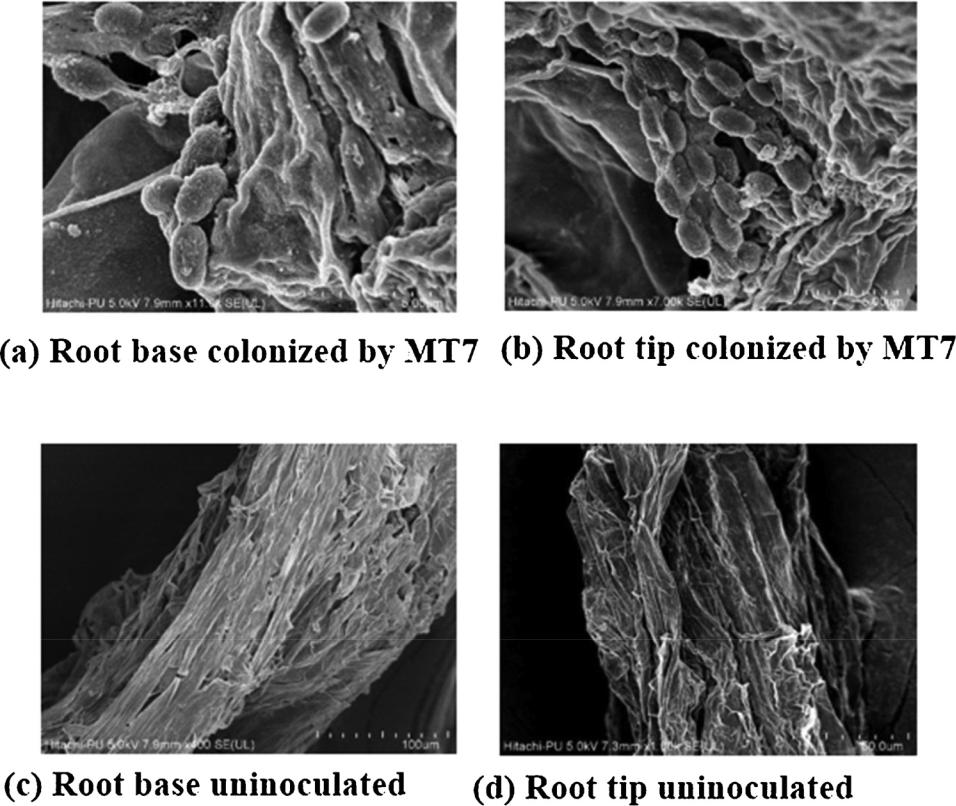


Fig. 6. FE-SEM micrographs depicting colonization behavior of MT7 on tomato roots after 7 days inoculation period (a) and (c) are micrographs of root base colonized by MT7 and control, (b) and (d) are micrographs of root tips colonized by MT7 and control.

Table 3

Eﬀect of MT7 inoculations on tomato crop in earthen pots 70 DAS.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Treatments | Shoot length | | Root length (cm) | | Shoot fresh weight (g) | | Root fresh weight (g) | | Shoot dry weight (g) | | Root dry weight |
|  | (cm) |  |  |  |  |  |  |  |  |  | (g) |
|  |  |  |  |  |  |  |  |  |  |  |  |
| Control | 24.66 | ± 1.15 | 5.26 | ± 0.25 | 1.36 | ± 0.03 | 0.09 | ± 0.009 | 0.61 | ± 0.05 | 0.04 ± 0.025 |
| MT7 | 34.33 | ± 1.52[\*](#page8) | 6.66 | ± 0.28[\*](#page8) | 2.44 | ± 0.03[\*](#page8) | 0.38 | ± 0.05[\*](#page8) | 1.16 | ± 0.05[\*](#page8) | 0.15 ± 0.04[\*](#page8) |

#Values are average of six plants ± SD.

\* Represent a significant diﬀerence (t-test, P < 0.05), ns is no significant diﬀerence.

as visualized by FE-SEM micrographs of the tomato root surface. The secretion of root exudates diﬀers along the longitudinal axis, from the base to the tip of a root, resulting in the spatial distribution of rhizo-bacteria along the root surface. A similar pattern of colonization was seen in Paenibacillus polymyxa, Bacillus amyloliquefaciens, and Bacillus aryabhattai ([Timmusk et al. 2005](#page8); [Fan et al. 2011](#page8); [Park et al. 2017](#page8)). Overall, MT7 responded well to the tomato exudates. Therefore, we can say that tomato rhizosphere can support Bacillus sp. strain MT7 of the maize rhizosphere in a mutualistic relationship.

Tomato crop responses indicate Bacillus sp. MT7 inoculations to be statistically significant (P < 0.05) for all the tested growth parameters over the un-inoculated tomato plants. An increase in the root system of a plant increases its surface area, providing an enhanced ability for the uptake of nutrients like water, nitrogen, and phosphorous, resulting in better growth and health of the plant. MT7 was eﬀective in promoting the root and shoot biomass of tomato plants grown in small pots for 70 days. Other workers have also reported improved plant growth upon bacterial inoculations. [Bhatia et al. (2008)](#page8) reported the positive influ-ence of Azotobacter spp. on cotton crops. Enhancement in growth and biomass yield by using Bacillus spp. was observed for various crops ([Walia et al. 2013](#page8)). [Ahirwar et al. (2015)](#page8) reported a considerable in-crease in length, root and shoot weight, fruit yield per plant, and total fruit yield by application of Pseudomonas fluorescence on tomato plants.

[Rodrigues et al. (2016)](#page8) isolated PGPRs belonging to genera Klebsiella, Enterobacter, and Pantoea from sugarcane and reported them to enhance the growth of maize. Bacillus strains isolated from wheat rhizosphere were reported to increase the growth of corn, soybean, and wheat ([Akinrinlola et al. 2018](#page8)). [Dursun et al. (2019)](#page8) used two diﬀerent bac-terial fertilizers including Azotobacter sp. and mixture of Bacillus subtilis and Bacillus megaterium in two cultivars of tomato and found significant influence on plant growth parameters.

Competent colonization by MT7 lies in its ability to respond to to-mato root exudates, form biofilms, establish and proliferate in the to-mato rhizosphere and express various PGP traits that cause an increase in plant growth. The results indicate the potential of MT7 as a bio-inoculant for tomato.

5. Conclusion

Bio-inoculants can serve as an eﬀective alternative approach for healthy and sustainable agricultural practices. The present investiga-tion promotes Bacillus sp. MT7 as an eﬃcient PGPR strain from the maize rhizosphere that can eﬀectively colonize the tomato rhizosphere, survive under abiotic stress conditions, and influence plant growth. Therefore, it has great potential to be developed as a bio-inoculant for tomato. However, in situ evaluation needs to be done in the future to

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fully establish its eﬃcacy.

CRediT authorship contribution statement

Priyanka Pathania: Investigation, Methodology, Visualization, Formal analysis, Writing - original draft, Writing - review & editing. Ranjana Bhatia: Conceptualization, Methodology, Validation, Writing

* original draft, Writing - review & editing, Supervision. Madhu Khatri: Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influ-ence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.scienta.2020.109480>.

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