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Beneficial effect of plant growth promoting bacteria isolated from rice rhizosphere

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ABSTRACT

Rhizosphere refers to the environment influenced by plant roots in which elevated microbial activity is observed. Plant growth promoting rhizobacteria (PGPRs), which are adapted to this ecosystem, increase plant health and productivity based on integrated plant-bacteria systems. Since the condition of microbial combination is altered by root exudates, isolated bacteria, including *Pseudomonas fluorescens* UTSP50, *Bacillus subtilis* UTSP40, *Pseudomonas mosselii* UTSP6 and *Ochrobactrum anthropi* UTSP24, triggered growth in two varieties of rice, differently. To probe how the isolated bacteria affect rice growth, phosphate solubilizing and auxin production abilities were examined *in vitro* and phyto chamber conditions. *P. fluorescens* UTSP50 and *P. mosselii* UTSP6 showed phosphate solubilizing activity on Pikovskaya's agar medium. *P. mosselii* UTSP6 and *O. anthropi* UTSP24 *in vitro* and, almost, all the tested bacteria in transgenic *DR5::GUS* rice plants, were found to produce auxin and/or alter auxin maxima. This may indicate the hypothesis that rhizosphere is necessary to produce indole acetic acid (IAA) by beneficial bacteria or the presence of PGPRs regulates transportation and gradients of auxin in plants.

INTRODUCTION

Rhizosphere is a dynamic system in which physical interactions between the root and microorganisms play an important role to maintain plant growth and productivity. Plant growth promoting rhizobacteria (PGPRs), which are adapted to this ecosystem, increase plant fitness and are important in biotechnological applications based on integrated plant–bacteria systems (Barahona et al., 2010). PGPRs influence plant productivity and health manifested as increased shoot and root growth, nutrient content, tolerance to drought stress, resistance to pathogens (Dodd and Perez-Alfocea, 2012; Nie et al., 2015). They apply direct and indirect mechanisms such as enhancing the availability of inorganic phosphate to plants (Qureshi et al., 2012; Souza et al., 2013; Nouri et al., 2014; Pande et al., 2017) and production or altering plant hormones economy, for example, auxin (Zamioudid et al., 2012; Iqbal and Hasnain, 2013; Reetha et al., 2014) to trigger plant growth. In return, microbial combinations in the rhizosphere benefit from the plant-derived carbohydrates generated from photosynthesis (Schubler et al., 2006).

Rice (*Oryza sativa*) is a globally significant crop which feeds over three billion people around the world (Hosseyni-Moghaddam and Soltani, 2013; Muthayya et al., 2014). Future demand for rice is expected to increase, considering the fact that there is an upward trend in the world population size. On the other hand, increasing the use of chemical fertilizers creates environmental problems (Fattahi et al., 2015). Therefore, PGPRs can be considered as the environmentally friendly heritage of the rhizosphere zone to enhance plant yield. The PGPRs isolated from roots of rice increased root length, shoot length, fresh and dry weights, and also, they were capable of producing phytohormones, for example, indole-3-acetic acid (IAA) (Hasan et al., 2014). Rice plants inoculated with PGPRs recorded an improved plant growth and higher photosynthetic capacity (Sharma et al., 2014; Saengsanga, 2018).

The present study aimed to isolate naturally occurring and closely associated rice bacteria to evaluate their plant growth promoting abilities in two different varieties of *O. sativa* cv. Nipponbare and Sariceltik, and also characterize two mechanisms, including phosphate-solubilizing and auxin producing, applied by testing PGPRs.

MATERIALS AND METHODS

Isolation and screening of bacteria

In this study rice plants (*O. sativa* cv. Dylamani) took from the fields in Mazandaran province, Tonekabon, Iran and bacteria were isolated from root samples using soil dilution method.

Bacterial selection (a total of 100 isolates) was carried out according to their antagonistic potential against *Magnaporthe oryzae*, considering the future studies, using dual culture assay (data not shown) (Oldenburg et al., 1996).

Sequencing of 16s rRNA gene

Bacterial DNA was extracted and sequenced based on 16srRNA region with M13 Universal primers (F: 5´-d(CGCCAGGGTTTTCCCAGTCACGAC)-3´ and R: 5´-d(TCACACAGGAAACAGCTATGAC)-3´, by sequencing center at Biophore building, University of Lausanne. Databases at the National Center for Biological Information (NCBI) were used to compare sequence homology to other DNA sources.

Rice growth conditions, inoculation with PGPRs and growth assay

O. sativa cv. Nipponbare and Sariceltik were grown in phyto chambers with a 12 h day/12 h night cycle at 28°C/23°C. The soil of each pot was applied upon planting with 1 mL of bacterial suspension containing 10^8 CFU/mL (Suslow and Schroth, 1981). Growth assay was carried out after four weeks of inoculation by testing bacteria. Weights of shoot and root were measured after drying the samples at 65°C in oven. Ten plants were used for each treatment.

Evaluation of bacterial population

Rhizosphere samples were obtained, after 6 weeks, by collecting the soil adhering to the roots. The numbers of colony forming bacterial cells were calculated using 10 g of each soil sample and Ten-fold series dilution method.

Phosphate-solubilizing activity and auxin-producing ability in PGPRs

To detect the phosphate solubilizing bacteria, strains were streaked onto Pikovskaya's agar medium (Katznelson and Bose, 1959), and after three days of incubation the plates that showed clear zone around the colonies were considered as positive. Measurement of phosphate solubilization activity was carried out following standard method (King, 1936). Production of IAA was determined following the standard method (Bric and Bostock, 1991), and the production of auxin was identified by the formation of a red halo on the paper, immediately, surrounding the bacterial colonies. IAA response assay in *O. sativa DR5::GUS* seeds was carried out in phyto chamber with a 12 h day/night cycle at 28°C/23°C. After four weeks *DR5::GUS* seedlings were submerged in GUS staining buffer for 12-18 h (Jefferson et al., 1987). Water and 10 µM naphthalene acetic acid (NAA) were used as negative and positive controls, respectively. Also, genotyping of seven segregating seedlings was performed by polymerase chain reaction (PCR) to identify homozygous transgenic plants (data not shown) (Berendzen et al., 2005).

Statistical analysis

Results were analyzed statistically using ANOVA Genstat 5 release 4.1 (2.15.0), fourth edition.

RESULTS AND DISCUSSION

The experiment was performed with four identified bacteria and the region sequence of 16s rRNA revealed 98% homology to *P. fluorescens*, *P. mosselii*, *B. subtilis* and *O. anthropi*. Sequences were deposited in Genbank with accession numbers KM974652 for *P. fluorescens* UTSP50, KM974649 for *P. mosselii* UTSP6, KM974651 for *B. subtilis* UTSP40, KM974653 for *O. anthropi* UTSP24 (Figure 1).

After six weeks of inoculation, none of the tested bacteria seemed to have a deleterious effect on the rice growth. In this study, *P. fluorescens* UTS50 showed a significant PGPR activity on shoot and root of Nipponbare in comparison with control. In contrast, the same isolate could not exhibit good performance on Sariceltik growth in comparison with other bacteria. A noticeable increment of root dry weight was, only just, observed in Sariceltik infected by *P. mosselii* UTSP6 and *O. anthropi* UTSP24 (Figures 2 and 3). On the other hand, population of *P. fluorescens* was higher than the other bacteria in both varieties of rice, including Nipponbare and Sariceltik, while *B. subtilis*, only, showed a better stability in the rhizosphere of Nipponbare (Figure 4). The same strain of

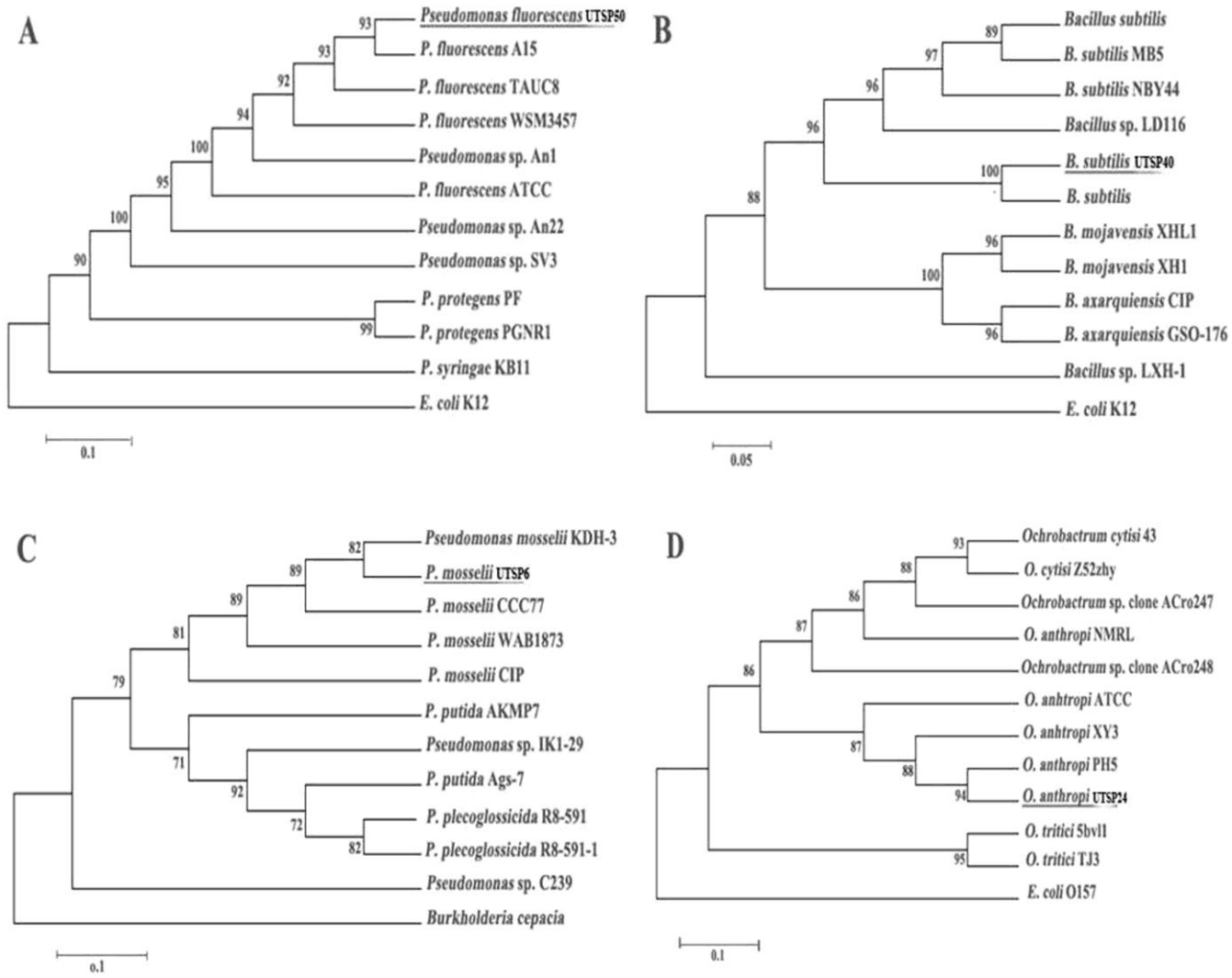


Figure 1. Phylogeny of the isolated bacteria based on Neighbor-Joining (NJ). **A,** *P. fluorescens* UTSP50; **B,** *B. subtilis* UTSP40; **C,** *P. mosselii* UTSP6; **D,** *O. anthropi* UTSP24. Assessment with Bootstrap (100 value).

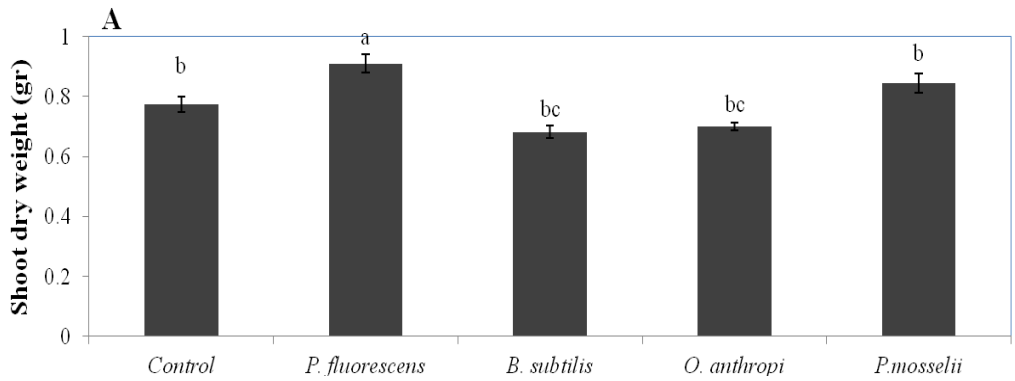
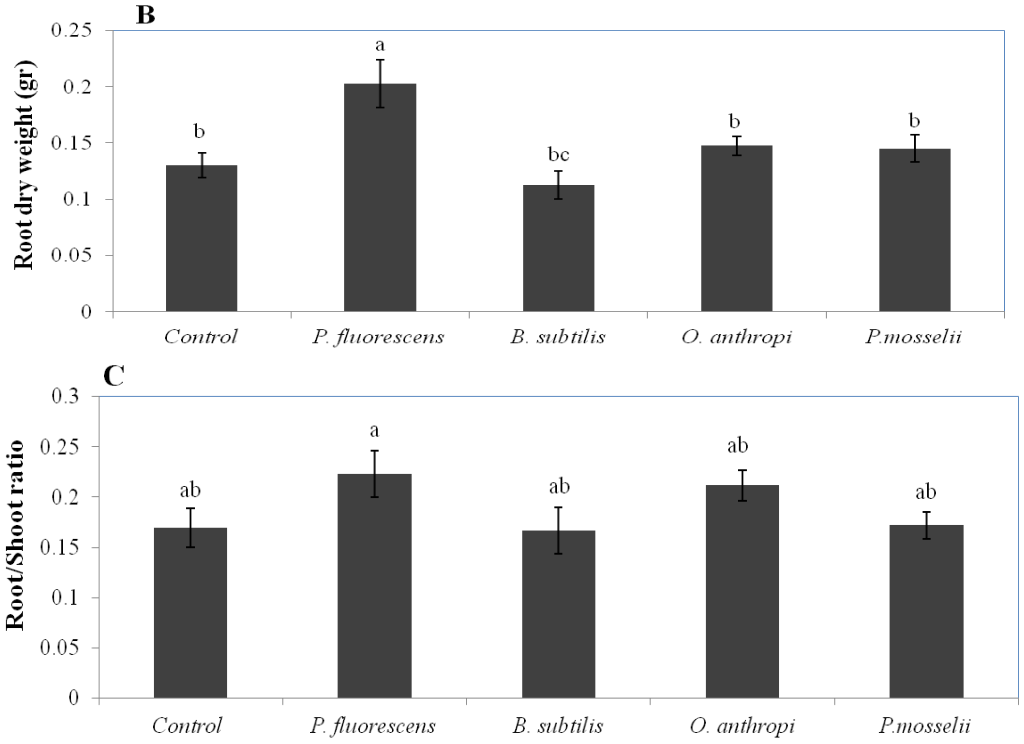


Figure 2. Effects of *P. fluorescens* UTSP50, *B. subtilis* UTSP40, *O. anthrupi* UTSP24 and *P. mosselii* UTSP6 on rice (*O. sativa* cv. Nipponbare) growth. **A,** shoot dry weight; **B,** root dry weight; **C,** root/shoot ratio. Bars represent LSD (least significant differences, P<0.05) for comparisons between treatments with ten replicates.



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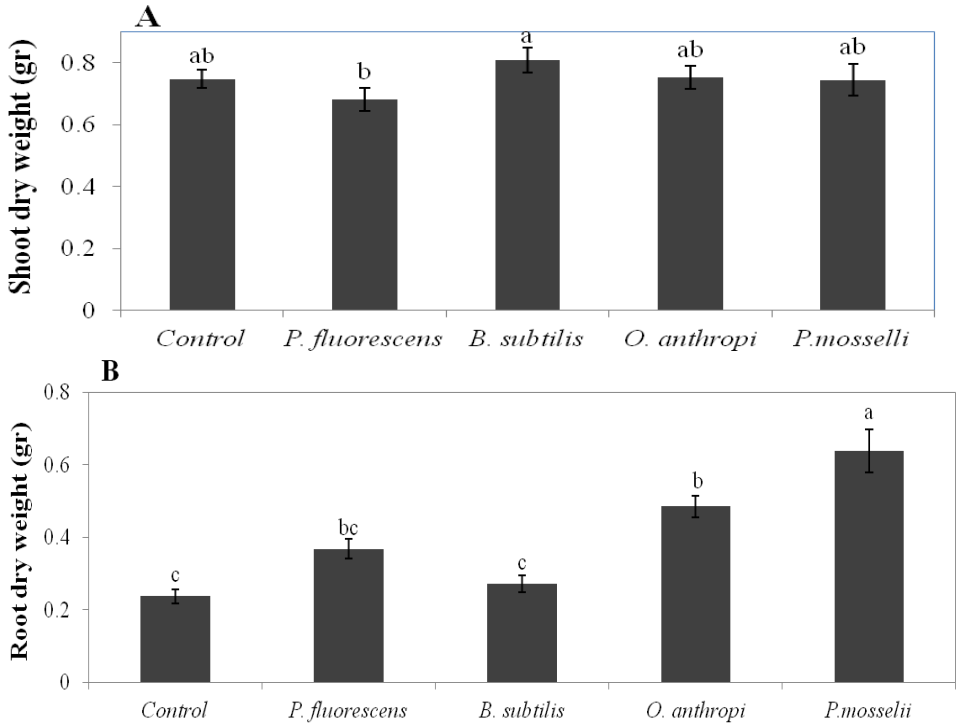
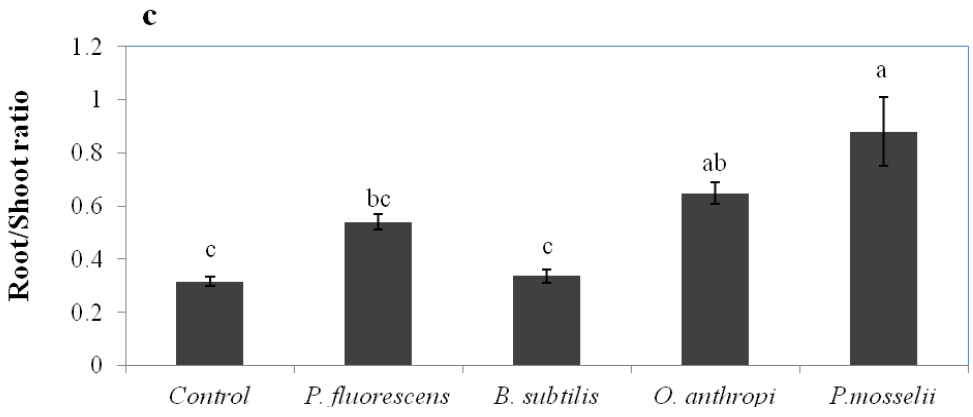


Figure 3. Effects of *P. fluorescens* UTSP50, *B. subtilis* UTSP40, *O. anthrupi* UTSP24 and *P. mosselii* UTSP6 on rice (*O. sativa* cv. Sariceltik) growth. **A,** Shoot dry weight; B, Root dry weight; C, root/shoot ratio. Bars represent LSD (least significant differences, P<0.05) for comparisons between treatments with ten replicates.



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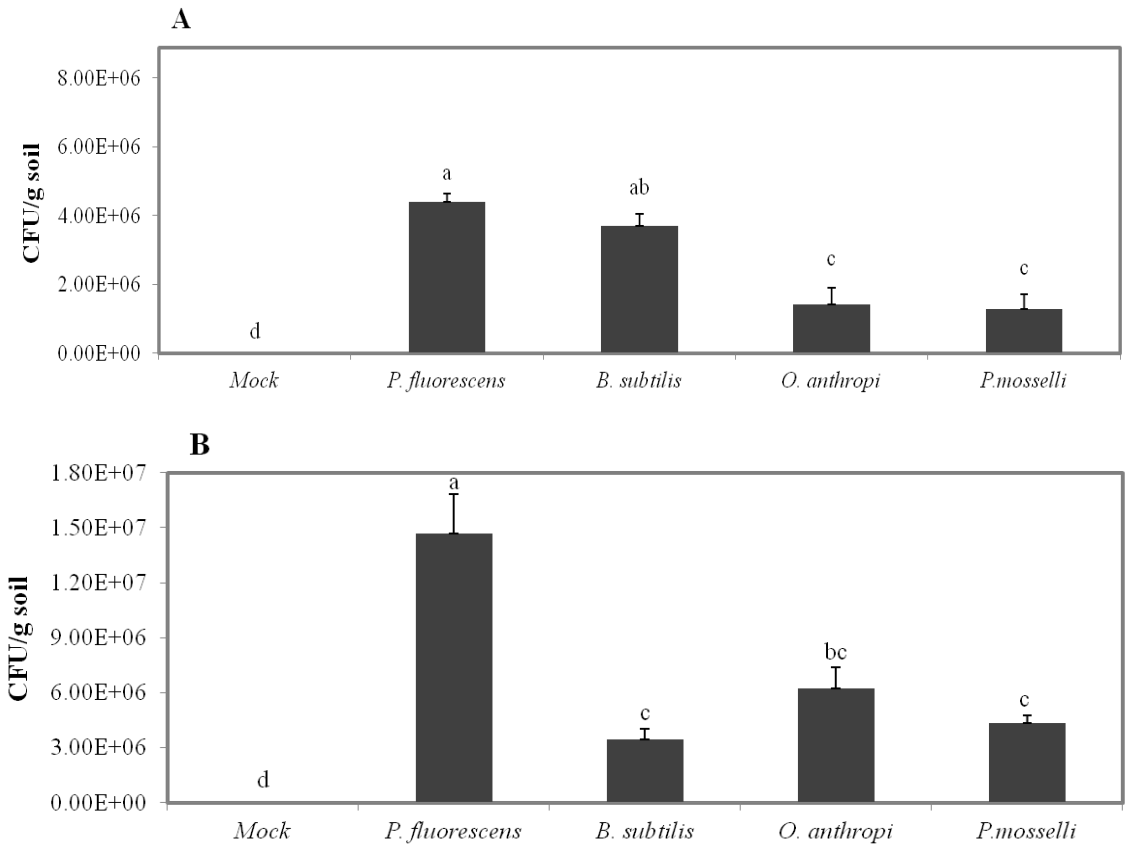

Figure 4. Population (colony forming unit or CFU/g soil), of *P. fluorescens* UTSP50, *B. subtilis* UTSP40, *O. anthropi* UTSP24 and *P. mosselii* UTSP6 in the rhizosphere of: **A,** *O. sativa* cv. Nipponbare; **B,** *O. sativa* cv. Sariceltik. Bars represent LSD (least significant differences, P<0.05) for comparisons between treatments with three replicates.

bacteria shows different performances on growth of various plant hosts, and it is explained that root exudates, varied in different plants, are substances that alter the condition of rhizosphere by changing pH level and mineral availability via desorption (Weston et al., 2012), which directly affects microbial combination in the rhizosphere and consequently plant productivity and health. Therefore, not only microorganisms, but also plants differ in the response to plant-microbe interaction in the rhizosphere, and based on the obtained results, it

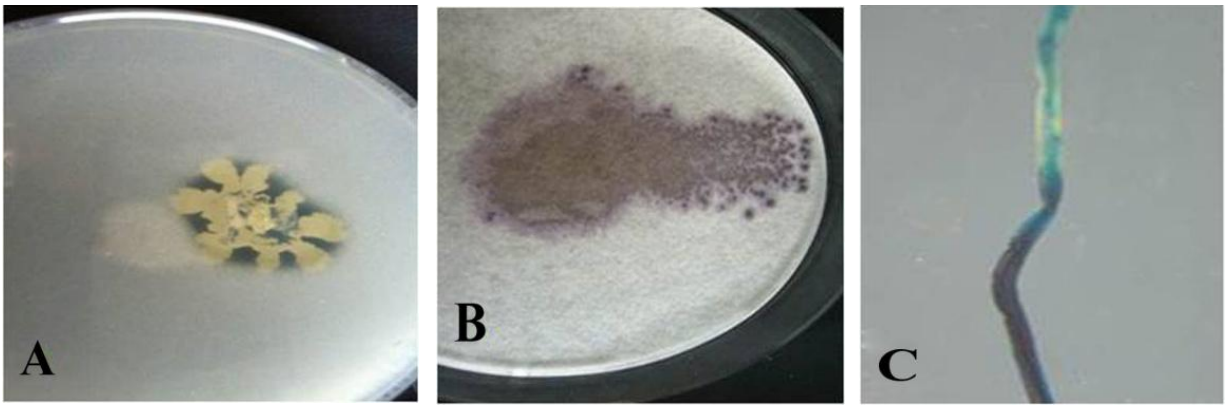

Figure 5. *Pseudomonas mosselii* UTSP6: **A,** A clear zone on Pikovskaya's agar medium in the Phosphate solubilization assay; **B,** production of IAA and red halo surrounding the bacterial colonies on the paper; **C,** expression of *DR5* promoter in large lateral roots of *DR5::GUS* transgenic rice plants treated with *P. mosselii* UTSP6.

is important to point out that root exudates play a crucial part in the establishment of bacteria unlike the population abundance in the soil. Hence, selection of PGPRs based on the host is important in a useful microbial performance on the plants. Furthermore, considerable root colonization by PGPRs is an essential factor to deliver the beneficial bacteria at the right place and time on the root, as poor root colonization results in decrease beneficial activity by PGPRs in host plant (Bais et al., 2004).

The phosphate solubilization ability of the bacteria was evaluated after three days, and *P. fluorescens* UTSP50 and *P. mosselii* UTSP6, by induction a clear zone, were considered as a phosphate solubilizing bacteria in Pikovskaya's agar medium (Figure 5A), extending the observations of Yulianti and Rakhmawati (2017) and Paul and Sinba (2017). Phosphorus is considered as an essential macronutrient for plants health and productivity (Koppelaar and Weikard, 2013). Phosphate solubilizing microorganisms (PSM) have been employed in agriculture and considered as important microbes due to their potential for soil improvement. Bacterial strains belonging to different genera such as *Pseudomonas* and *Bacillus* have the ability to solubilize inorganic phosphate compounds, including tricalcium phosphate and dicalcium phosphate, and reduce phosphate fertilizer application in the soil by 50% (Oteino et al., 2015). Fallah (2006) reported that the number of PSBs among total PSM, in the north of Iran, is around 88% and PSBs count from 0 to 10^7 CFU/g soil, with 3.98% population among total bacteria. It has been reported that higher concentrations of phosphate-solubilizing bacteria are commonly found in the rhizosphere soil in comparison with non-rhizospheric soil (Reyes and Valduz, 2006).

P. mosselii UTSP6 and *O. anthropi* UTSP24 produced auxin (IAA) *in vitro* and a red/pink halo appeared around bacterial colonies (Figure 5B), extending the results of Ji et al. (2014). After 4 weeks, GUS activity was detected in the roots of *DR5::GUS* transgenic rice plants treated with the bacteria, and GUS expression in root samples inoculated with *P. mosselii* UTSP6 and *O. anthropi* UTSP24 was much stronger than the other samples (Figure 5C), however, all the tested bacteria, in phyto chamber, were found to produce auxin and/or alter auxin maxima, differently. *DR5::GUS* gene is the synthetic auxin response reporter construct to localize regions of auxin responsiveness (Bai and De Mason, 2008). PGPRs affect root architecture by altering root auxin economy (Gladiano Junior et al., 2011; Iqbal and Hasnain, 2013). Auxin plays a critical role and an optimum level of auxin is required for several aspects of plant growth. IAA is the most common auxin in plants (Sreevidya et al., 2010), and IAA-producing bacteria are renowned to promote root elongation and plant growth (Malik and Sindhu 2011). The auxin produced by *P. fluorescens* strains improves plant productivity (Zamioudid et al., 2012; Iqbal and Hasnain, 2013). Importantly, IAA production by PGPRs varies among different species and strains, and is influenced by culture condition, growth stage and substrate availability (Mirza et al., 2001). In this study, results indicated that rhizosphere stimulates auxin production by beneficial bacteria or the presence of PGPRs just regulates transportation and gradient of auxin in plants.

Conclusion

There is a correlation between IAA production and phosphate solubilization in plants which plays a critical role in plant growth by regulating the expression of related genes (Kant et al., 2009). By further research on

the mechanisms of PGPRs, it can be relied on biological processes rather than agrochemicals to preserve soil fertility and plant health and productivity.

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