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# Effectiveness of Various *Pseudomonas* spp. and *Burkholderia caryophylli* Containing ACC-Deaminase for Improving Growth and Yield of Wheat (*Triticum aestivum* L.)

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**Abstract** This study assessed the possible role of different traits in selected plant growth-promoting rhizobacteria (PGPR) for improving wheat growth and yield under natural conditions. Rhizobacteria exhibiting 1-aminocyclopropane-1-carboxylate (ACC)-deaminase activity were isolated and screened for their growth-promoting activity in wheat under axenic conditions. Five isolates belonging to *Pseudomonas* and one *Burkholderia caryophylli* isolate that showed promising performances under axenic conditions were selected and characterized for *in vitro* ACC-deaminase activity, chitinase activity, auxin production, P solubilization, and root colonization. These isolates were then used as inocula for wheat cultivated under natural conditions in pot and/or field trials. Significant increases in root elongation, root weight, tillers per pot, 1,000-grain weight, and grain and straw yields were observed in response to inoculation with PGPR in the pot trials. Inoculation with these PGPR was also effective under field conditions and increased the wheat growth and yield significantly. However, the efficacy of the strains was inconsistent under the axenic, pot, and field conditions. *Pseudomonas fluorescens* ($ACC_{50}$), which exhibited a relatively high *in vitro* ACC-deaminase activity, chitinase activity, auxin production, and P solubilization and more intensive root colonization, was the most efficient isolate under the field conditions. Therefore, these results demonstrated that ACC-deaminase activity is an efficient parameter for the selection of promising PGPR under axenic conditions. However, additional traits of PGPR, including auxin production, chitinase activity, P solubilization, and root colonization, are also important for selecting PGPR as biofertilizers.

**Keywords:** Ethylene, ACC-deaminase, plant growth-promoting traits of rhizobacteria, wheat, field conditions

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The direct use of microorganisms to promote plant growth continues to be an area of rapidly expanding research. As such, the ability of specific root-colonizing bacteria (rhizobacteria) to increase the growth and yield of crop plants is currently attracting considerable attention among microbiologists. Beneficial free-living rhizobacteria, which have been shown to improve plant health or increase yield, are usually referred to as plant growth-promoting rhizobacteria (PGPR) [19] or yield-increasing bacteria (YIB) [35].

There are several mechanisms by which PGPR affect plant growth. The beneficial effects of these bacteria have been attributed to their ability to produce various compounds (such as phytohormones, vitamins, and siderophores), fixation of atmospheric nitrogen, phosphate solubilization, and/or production of antibiotics and chitinase that suppress deleterious rhizobacteria [5, 16, 27, 30]. However, some other unidentified mechanisms may also be involved, and one of the most plausible action mechanisms to improve plant growth could be the changes in the endogenous levels of plant growth-regulating substances caused by PGPR [12–15, 18].

Ethylene is a plant hormone that is involved in the regulation of many physiological responses [1, 3, 28]. Although low levels of ethylene appear to enhance root initiation and growth, higher levels of ethylene, produced by fast-growing roots, can inhibit root elongation [21]. Thus, any factor/stimulus that causes a change in the endogenous level of ethylene in a plant results in modified growth and development [3, 4, 18].

The synthesis of ethylene in plants is directly related with the concentration of 1-aminocyclopropane-1-carboxylic acid (ACC) [22]. It has been discovered that certain microorganisms contain an enzyme, ACC-deaminase, that hydrolyzes ACC into ammonia and α-ketobutyrate [12, 14, 23, 32, 33], thereby acting as a sink for ACC. Decreased

levels of ACC result in lower levels of endogenous ethylene, which eliminate the potential inhibitory effects of higher ethylene concentrations [2, 14]. Consequently, these bacteria are very useful in promoting the growth of plants exposed to biotic or abiotic stress(es) by eliminating the negative effects of stress-induced ethylene [2, 36]. It has also been observed that strains without ACC-deaminase activity could not promote the growth of inoculated plants [31].

During the last decade, there have been several reports on the effect of inoculation with PGPR containing ACC-deaminase activity on the root growth of various plants under axenic conditions [11, 13, 15, 20, 26, 32, 33]. However, there are relatively few reports on the effect of inoculation with these PGPR under field conditions. Accordingly, this study assessed selected PGPR containing ACC-deaminase as regards promoting the growth and yield of wheat under natural conditions (pot and field trials). Other traits of these PGPR, including their auxin (Indol acetic acid, IAA) production, chitinase activity, P solubilization, and root colonizing ability, were also studied *in vitro* to gain a better understanding of the contribution of such traits in improving wheat growth and yield under field conditions.

## MATERIALS AND METHODS

### Isolation and Screening Under Axenic Conditions

Rhizosphere soils were collected from wheat fields, and 97 rhizobacteria were isolated using a dilution plate technique with a salt minimal medium [10] containing ACC as the sole nitrogen source (enrichment technique). The collected rhizobacteria were purified by streaking on fresh plates five times, stored in 20% glycerol at -20°C, and then screened for growth-promoting activity in wheat under axenic conditions. Six strains showing more promising results in different trials were selected for further testing under wire house (pot trials) and field conditions.

### Characterization and Identification of Strains

The ACC-deaminase activity was determined by monitoring the amount of ammonia generated on the hydrolysis of ACC by the rhizobacterial isolates containing ACC-deaminase, as described previously [32], whereas the chitinase activity of the selected rhizobacteria was determined as described by Chernin *et al.* [9]. The *in vitro* auxin production by the selected isolates was determined as indole acetic acid (IAA) equivalents in the presence and absence of L-tryptophan (an auxin precursor) using the protocol described by Khalid *et al.* [17], the phosphorus solubilizing activity determined according to the qualitative method described by Mehta and Nautiyal [24], and the root colonization ability of the different strains in maize studied under axenic conditions, as described by Simons *et al.* [34]. In the latter case, samples were obtained from each pot three weeks after sowing, where 0.2 g of the root tips was removed and shaken vigorously in 5 ml of sterilized water in an orbital shaking incubator at 100 rev/min for 15 min. The bacterial suspension was diluted from $10^{-1}$ to $10^{-5}$, and a sterilized DF minimal salt medium containing ACC as the sole nitrogen source poured into petri plates along with 1 ml of each dilution. The petri dishes were then incubated at 28±1°C for bacterial growth, the colonies counted using a colony counter, and the CFU/ml calculated. The rhizobacteria exhibiting the highest growth-promoting activity under axenic conditions were identified using a Biolog identification system (Microlog System Release 4.2, Hayward, CA, U.S.A.). For identification with the Biolog system, the rhizobacteria were grown on a Biolog universal growth medium. The cells were removed from the agar plate using a sterile swab to avoid any transfer of nutrients from the agar medium into the suspension. As such, the swab was twirled and pressed against the inside surface of the glass tube above the fluid line to break up any clumps and release the cells. The swab was then moved up and down the wall of the tube until the rhizobacteria mixed with the fluid and became a homogenous clump-free suspension. The turbidity was adjusted to an appropriate value for each rhizobacterium, and the suspension poured into a multichannel pipette reservoir. Eight sterile tips were fastened securely onto an 8-channel repeating pipetter, the tips primed, and 150 µl of the inoculum poured into each well. The microplate was then covered with a lid and incubated at 30°C for 6 h. Thereafter, the microplate was loaded onto a microplate reader and identified putatively using Microlog software (Microlog System Release 4.2, Hayward, CA, U.S.A.).

### Preparation of Inocula

The inocula were prepared by growing the selected rhizobacterial strains ($ACC_3$, $ACC_7$, $ACC_9$, $ACC_{14}$, $ACC_{50}$, $ACC_{73}$) in 250-ml flasks containing the DF minimal salt medium [10]. The medium was incubated at 28±1°C for 48 h in an orbital shaking incubator at 100 rev/min. The optical density of each inoculum was measured and a uniform population of rhizobacteria ($10^8$–$10^9$ CFU/ml) maintained at the time of inoculation. Peat was ground to pass through a 2-mm 40-mesh and autoclaved at 121°C for 20 min. A 100-ml inoculum of the selected rhizobacteria was mixed with 1 kg of peat and incubated for 24 h at 28±1°C before being used for seed coating, with a seed to peat ratio of 1:1 (w/w).

### Pot Trials

The wheat seeds (cv. TJ-83) were surface sterilized by dipping in a 95% ethanol solution for 5 min and 0.2% $HgCl_2$ solution for 3 min, and then washed thoroughly with

sterilized water [17]. For the inoculation, the seeds were dressed using the inoculated peat mixed with 25 ml of a 10% sugar solution. For the uninoculated control, the seeds were coated with the sterilized (autoclaved) peat treated with sterilized broth and 25 ml of a 10% sugar solution. Ten inoculated wheat seeds were sown in each pot containing 12 kg/pot sandy clay loam soil (pH 7.8; electrical conductivity, 3.5 dS/m; organic matter, 0.78%; total nitrogen, 0.05%; available phosphorus, 7.8 mg/kg, and extractable potassium, 127 mg/kg soil). After germination, thinning was carried out to leave four uniform seedlings in each pot. Six replications were performed for each treatment. The pots were arranged in a wire house with ambient light and temperature according to a completely randomized design. The fertilizers NPK at 150-120-70 kg/ha were applied in the form of urea, single super phosphate, and muriate of potash, respectively. All the PK and half the N were mixed with the soil at the time of sowing, while the remaining N was applied in a solution form at a tillering stage. The pots were irrigated with good quality canal water [electrical conductivity, 0.03 dS/m; sodium adsorption ratio, 0.26 $(mmol/l)^{1/2}$, and residual sodium carbonates, 0] meeting the irrigation quality criteria for crops in the area [6]. Data regarding the root elongation, root weight, number of tillers, 1,000-grain weight, plus straw and grain yields were collected at harvesting (153 days after seeding). Nitrogen, phosphorus, and potassium contents in grain and straw were determined as described by Ryan *et al.* [29].

### Field Trials

A field experiment was also conducted during 2004–2005 to study the ability of the selected rhizobacteria to increase the growth and yield of wheat under natural conditions. The seeds were inoculated as described above. After drying overnight, inoculated and uninoculated (treated with sterilized peat) seeds were sown in a field (at a rate of 60 kg/ha). The soil was clay loam (32% clay), EC of 0.36 dS/m, pH (8.02), organic matter (0.75%), Olson P (6.105 mg/kg), and $NH_4AC$ K (169.75 mg/kg). The wheat seeds were sown using a drill, keeping a row to row and plant to plant distance of 25 cm with a plot size of 2.5 m× 5 m. The treatments were replicated four times using a randomized complete block design.

The fertilizers P and K at 120 and 70 kg/ha were applied as single super phosphate and potassium sulfate, respectively, with a basal dose to all the plots at the time of sowing. Additionally, nitrogen at 150 kg/ha in the form of urea was applied in two stages (half the dose at sowing and the remaining half at tillering). Canal water was used for irrigation. At maturity (153 days after seeding), 10 plants per treatment were harvested and data regarding the plant height, tillers/m, number of grains/spike, 1,000-grain weight, plus straw and grain yields recorded. The grain and straw samples were oven dried at 70°C and ground in a Wiley grinding mill. The nitrogen, phosphorus, and potassium contents in the grain and straw were determined as described by Ryan *et al.* [29].

### Statistical Analysis

The data collected were statistically analyzed using a completely randomized design in the case of the pot trials and a randomized complete block design for the field trials. The means were compared using a least significant difference test. The correlation coefficients between a pair of trait means were determined and the significance of the correlation determined using Student's t-test. All the statistical tests were performed at $p$=0.05, using the software MSTATC.

## RESULTS

In a series of trials, 97 isolates of rhizobacteria were screened for their root growth-promoting activity under axenic conditions, and six promising isolates ($ACC_3$, $ACC_7$, $ACC_9$, $ACC_{14}$, $ACC_{50}$, $ACC_{73}$) were selected (data not shown). The selected strains of rhizobacteria were identified and characterized for ACC-deaminase activity, chitinase activity, auxin production, phosphorus solubilization, and root colonization under gnotobiotic conditions. Among the six selected strains, four were identified as fluorescent pseudomonads ($ACC_3$, $ACC_{14}$, $ACC_{50}$, and $ACC_{73}$) and the remaining two as *Pseudomonas putida* biotype A ($ACC_9$)

**Table 1.** Identification and plant growth-promoting attributes of selected strains of rhizobacteria (average of three replicates).

| Isolate | Species | ACC-deaminase activity (nmol $NH_3$/g biomass/h) | Chitinase activity | IAA production (mg/l) | | Phosphorus solubilizing activity | Root colonization (CFU/g root) |
|---|---|---|---|---|---|---|---|
| | | | | Without L-tryptophan | With L-tryptophan | | |
| $ACC_3$ | *Pseudomonas fluorescens* biotype G | 207±13 | Positive | 4.1±0.5 | 9.0±0.7 | Positive | $5.0\times10^6$ |
| $ACC_7$ | *Burkholderia caryophylli* | 342±3 | Negative | 3.0±1 | 7.7±0.9 | Negative | $2.0\times10^8$ |
| $ACC_9$ | *Ps. putida* biotype A | 278±11 | Negative | 0 | 3.1±0.6 | Negative | $8.5\times10^5$ |
| $ACC_{14}$ | *Ps. fluorescens* | 103±10 | Negative | 0 | 3.0±1.3 | Negative | $4.0\times10^6$ |
| $ACC_{50}$ | *Ps. fluorescens* | 302±5 | Positive | 8.5±1.3 | 15.3±0.8 | Positive | $7.0\times10^6$ |
| $ACC_{73}$ | *Ps. fluorescens* biotype F | 393±7 | Positive | 0 | 8.1±0.4 | Positive | $1.0\times10^6$ |

**Table 2.** Effect of inoculation with rhizobacteria containing ACC-deaminase on root elongation, root weight, tillers/plant, 1,000-grain weight, grain yield, and straw yield of wheat in pot trials (average of six replicate pots with four plants per pot).

| Treatment | Root elongation (cm) | Root weight (g/plant) | Tillers/plant | 1,000-grain weight (g) | Grain yield (g/plant) | Straw yield (g/plant) |
|---|---|---|---|---|---|---|
| Uninoculated control | 18.5±1.1d[a] | 0.60±0.04 d | 4.1±0.10 c | 28.8±0.7 d | 3.32±0.3 c | 5.26±0.6 c |
| *Ps. fluorescens* biotype G ($ACC_3$) | 21.5±0.6 c | 0.93±0.02 b | 4.9±0.29 ab | 34.5±1.5 ab | 4.08±0.15 b | 6.77±0.3 b |
| *Burkholderia caryophylli* ($ACC_7$) | 24.3±0.4 a | 1.10±0.08 a | 5.5±0.32 a | 33.5±0.9 bc | 4.74±0.25 a | 7.56±0.39 a |
| *Ps. putida* biotype A ($ACC_9$) | 21.4±0.8 c | 0.83±0.02 c | 5.1±0.20 ab | 35.8±0.8 a | 3.99±0.18 b | 6.62±0.12 b |
| *Ps. fluorescens* ($ACC_{14}$) | 21.1±0.9 c | 0.83±0.03 c | 4.7±0.10 b | 32.2±1.0 c | 3.51±0.21 c | 5.69±0.23 c |
| *Ps. fluorescens* ($ACC_{50}$) | 21.8±1.2 bc | 0.93±0.04 b | 5.1±0.21 ab | 34.2±1.1 ab | 3.72±0.19 b | 6.42±0.29 b |
| *Ps. fluorescens* biotype F ($ACC_{73}$) | 23±0.3 b | 0.90±0.06 bc | 5.1±0.15 ab | 33.7±0.5 bc | 4.11±0.20 b | 6.65±0.17 b |

[a]Means sharing the same letter(s) in a column do not differ significantly according to least significant difference test ($P<0.05$).

and *Burkholderia caryophylli* ($ACC_7$). The ACC-deaminase activity of the PGPR strains varied from 103 to 393 nmol $NH_3$/g biomass/h (Table 1). Three strains, *Ps. fluorescens* biotype G ($ACC_3$), *Ps. fluorescens* ($ACC_{50}$), and *Ps. fluorescens* biotype F ($ACC_{73}$), were positive for chitinase activity. All the strains tested produced IAA in the presence of the auxin precursor L-tryptophan, yet only three strains ($ACC_3$, $ACC_7$, and $ACC_{50}$) were able to produce IAA in the absence of L-tryptophan. *Ps. fluorescens* ($ACC_{50}$) was the most effective for producing IAA both in the presence and absence of L-tryptophan. Three rhizobacterial isolates ($ACC_3$, $ACC_{50}$, and $ACC_{73}$) were able to solubilize tricalcium phosphate. All the strains varied in their ability to colonize roots under gnotobiotic conditions. *B. caryophylli* ($ACC_7$) was identified as the most efficient wheat root colonizer ($2.0\times10^8$ CFU/g root), followed by *Ps. fluorescens* ($ACC_{50}$), which colonized up to $7.0\times10^6$ CFU/g root.

Table 2 summarizes the data on the effect of inoculation with PGPR containing ACC-deaminase on root elongation, root weight, tillers/pot, 1,000-grain weight, and grain and straw yields in the pot trials. The results revealed that all the test strains significantly increased the root elongation when compared with the uninoculated control, where *B. caryophylli* ($ACC_7$) was the best with a 31% increase, followed by *Ps. fluorescens* biotype F ($ACC_{73}$) with a 24% increase over the uninoculated control. The effect of inoculation with rhizobacteria containing ACC-deaminase was more obvious on the root weight, as all the PGPR caused significant increases (up to 83%) in root weight compared with the uninoculated control (Table 2). Again, *B. caryophylli* ($ACC_7$) was the most effective in increasing the root weight compared with the other strains. Similarly, the number of tillers/pot increased significantly after inoculation, ranging from 16 to 35% above that for the uninoculated control. Inoculation with the PGPR also significantly increased the 1,000-grain weight (up to 24% higher than the uninoculated control) and, except for $ACC_{14}$, significantly increased the grain yield by 6 to 43% compared with the uninoculated control. Once again, inoculation with *B. caryophylli* ($ACC_7$) resulted in the maximum increase in grain yield compared with the other strains. A similar result was also observed for the straw yield, where inoculation with *B. caryophylli* ($ACC_7$) resulted in the maximum increase (44% higher than the uninoculated control). The effect of inoculation with PGPR on the nutrient uptake in the pot trials is summarized in Table 3. The inoculation with PGPR (except $ACC_{14}$) significantly increased the wheat nitrogen uptake, where *B. caryophylli* ($ACC_7$) inoculation produced the maximum increase of 39% compared with the uninoculated control, followed by

**Table 3.** Effect of inoculation with rhizobacteria containing ACC-deaminase on total nitrogen, phosphorus, and potassium uptake of wheat in pot trials (average of six replicate pots with four plants per pot).

| Treatment | Total uptake (mg/pot) | | |
|---|---|---|---|
| | Nitrogen | Phosphorus | Potassium |
| Uninoculated control | 429±19 d[a] | 131±14 c | 458±15 c |
| *Ps. fluorescens* biotype G ($ACC_3$) | 508±16 b | 152±6 b | 568±25 a |
| *Burkholderia caryophylli* ($ACC_7$) | 598±31 a | 174±15 a | 588±13 a |
| *Ps. putida* biotype A ($ACC_9$) | 504±17 b | 162±12 ab | 537±21 b |
| *Ps. fluorescens* ($ACC_{14}$) | 433±29 cd | 142±13 bc | 464±19 c |
| *Ps. fluorescens* ($ACC_{50}$) | 462±13 c | 144±10 bc | 518±23 b |
| *Ps. fluorescens* biotype F ($ACC_{73}$) | 532±15 b | 162±10 ab | 521±20 b |

[a]Means sharing the same letter(s) in a column do not differ significantly according to least significant difference test ($P<0.05$).

**Table 4.** Correlation between PGPR/plant traits and wheat growth/yield parameters in pot trials.

| PGPR/plant traits | r value | | | | | |
|---|---|---|---|---|---|---|
| | Root elongation | Root weight | Tiller/plant | 1,000-grain weight | Grain yield | Straw yield |
| Root colonization | 0.84* | 0.9* | 0.86* | $-0.2^{NS}$ | 0.83* | 0.76* |
| ACC-deaminase activity | 0.72* | $0.48^{NS}$ | 0.79* | $0.35^{NS}$ | $0.62^{NS}$ | 0.67* |
| Root weight | 0.95** | - | 0.96** | $0.65^{NS}$ | 0.92** | 0.94** |

*Significant correlation at $p$=0.05.
**Highly significant correlation at $p$=0.05.
$^{NS}$Nonsignificant correlation at $p$=0.05.

*Ps. fluorescens* biotype F ($ACC_{73}$) with a 24% increase. Among the six isolates, only four significantly increased the phosphorus uptake, where *B. caryophylli* ($ACC_7$) caused the maximum increase of 33% compared with the uninoculated control. The data also revealed that inoculation with all the PGPR (except $ACC_{14}$) significantly increased the potassium uptake, ranging from 1 to 28% above the uninoculated control. Again, inoculation with *B. caryophylli* ($ACC_7$) increased the potassium uptake the most. A significant correlation was observed between the PGPR traits (ACC-deaminase activity and root colonization) and the wheat growth/yield parameters (Table 4).

All the strains studied in the pot trials were also tested in the field as regards their efficacy to increase the growth and yield of wheat, and the results revealed that all the strains significantly increased the plant height compared with the uninoculated control (Table 5), with the maximum increase of 16% produced by *Ps. fluorescens* biotype F ($ACC_{73}$) inoculation. Inoculation with all six PGPR isolates also significantly increased the number of tillers/$m^2$, where *Ps. fluorescens* biotype F ($ACC_3$) was the most promising with 9% more tillers than the uninoculated control (Table 5). The data regarding the number of grains/spike revealed that only two strains, *Ps. fluorescens* biotype F ($ACC_3$) and *Ps. fluorescens* ($ACC_{50}$), significantly increased the number of grains/spike (11.7% and 10% more than the uninoculated control, respectively). Inoculation with all the rhizobacterial isolates, except *Ps. fluorescens* ($ACC_{14}$), significantly increased the 1,000-grain weight compared with the uninoculated control (Table 5), where the maximum increase was recorded with *Ps. fluorescens* ($ACC_{50}$) at 34% higher than the uninoculated control. Inoculation with all PGPR strains, apart from $ACC_{14}$, significantly increased the grain yield when compared with the uninoculated control (Table 5), where the most effective isolate was *Ps. fluorescens* ($ACC_{50}$) with a 39% increase compared with the uninoculated control, followed by *Ps. fluorescens* biotype F ($ACC_3$) with a 22.7% increase. Similarly, inoculation with all the strains, except *Ps. fluorescens* ($ACC_{14}$), significantly increased the straw yield compared with the uninoculated control. Again, the maximum increase in straw yield resulted from inoculation with *Ps. fluorescens* ($ACC_{50}$) and was 14% higher compared with the uninoculated control. As shown by the results (Table 6), inoculation with all the strains, except $ACC_{14}$, significantly increased the nitrogen uptake compared with the uninoculated control, where *Ps. fluorescens* ($ACC_{50}$) was the most effective, causing a 32% increase compared with the uninoculated control. Inoculation with four of the strains ($ACC_3$, $ACC_9$, $ACC_{50}$, $ACC_{73}$) significantly increased the wheat phosphorus uptake, and the maximum increase was recorded with *Ps. fluorescens* ($ACC_{50}$) at 40% higher than the uninoculated control. Only three strains ($ACC_3$, $ACC_9$, $ACC_{50}$) significantly increased the potassium uptake compared with the uninoculated control, where *Ps. fluorescens* ($ACC_{50}$) again produced the best result (24%) (Table 6).

**Table 5.** Effect of inoculation with rhizobacteria containing ACC-deaminase on plant height, tillers/$m^2$, number of grains/spike, 1,000-grain weight, grain yield, and straw yield of wheat in field trials (average of four replicates).

| Treatment | Height (cm) | Tillers/$m^2$ | No. of grains/ spike | 1,000-grain weight (g) | Grain yield (kg/ha) | Straw yield (kg/ha) |
|---|---|---|---|---|---|---|
| Uninoculated control | 63.7±2.1 c[a] | 336.0±5.3 c | 39.7±1.7 b | 25.0±1.0 c | 2599±98 d | 4611±157 b |
| *Ps. fluorescens* biotype G ($ACC_3$) | 72.0±0.9 ab | 366.0±4.9 a | 44.3±1.5 a | 31.2±0.8 b | 3183±121 b | 5104±151 a |
| *Burkholderia caryophylli* ($ACC_7$) | 71.0±1.3 ab | 365.7±5.2 a | 42.5±1.9 ab | 31.5±0.9 ab | 2895±86 c | 4621±114 b |
| *Ps. putida* biotype A ($ACC_9$) | 72.2±1.2 a | 349.2±3.8 b | 43.0±1.2 ab | 31.2±1.0 b | 2895±141 c | 4821±98 b |
| *Ps. fluorescens* ($ACC_{14}$) | 69.0±1.5 b | 346.5±4.8 b | 42.0±1.5 ab | 26.2±1.5 c | 2507±96 d | 4686±108 b |
| *Ps. fluorescens* ($ACC_{50}$) | 72.7±1.3 a | 361.7±4.2 a | 43.8±1.0 a | 33.5±1.7 a | 3624±121 a | 5254±146 a |
| *Ps. fluorescens* biotype F ($ACC_{73}$) | 73.7±2.1 a | 350.2±4.0 b | 42.8±1.4 ab | 31.5±1.3 ab | 3058±130 bc | 4662±161 b |

[a]Means sharing the same letter(s) in a column do not differ significantly according to least significant difference test ($P$<0.05).

**Table 6.** Effect of inoculation with rhizobacteria containing ACC-deaminase on total nitrogen, phosphorus, and potassium uptake of wheat in field trials (average of four replicates).

| Treatment | Total uptake (kg/ha) | | |
|---|---|---|---|
| | Nitrogen | Phosphorus | Potassium |
| Uninoculated control | 117±4.3 c[a] | 22±1.2 d | 140±5.1 d |
| *Ps. fluorescens* biotype G ($ACC_3$) | 137±2.1 b | 27±1.5 b | 160±4.5 ab |
| *Burkholderia caryophylli* ($ACC_7$) | 124±1.7 b | 24±0.9 cd | 147±6.8 bcd |
| *Ps. putida* biotype A ($ACC_9$) | 124 b±1.5 | 25±1.1 bc | 155±4.1 bc |
| *Ps. fluorescens* ($ACC_{14}$) | 108±6.8 c | 22±1.6 d | 143±3.8 cd |
| *Ps. fluorescens* ($ACC_{50}$) | 155±3.9 a | 31±1.9 a | 173±8.6 a |
| *Ps. fluorescens* biotype F ($ACC_{73}$) | 130±2.8 b | 26±1.3 bc | 148±3.6 bcd |

[a]Means sharing the same letter(s) in a column do not differ significantly according to least significant difference test ($P<0.05$).

## DISCUSSION

All the rhizobacterial strains isolated on the basis of ACC-deaminase activity had a notably positive effect on the wheat root growth in the pot trials, and significantly increased the root elongation and root weight compared with the uninoculated control. This effect may have been due to a decrease in ethylene synthesis in the inoculated roots, which is supported by the coefficient of the correlation between the *in vitro* ACC-deaminase activity and the root elongation (r=0.72*) in the pot trials (Table 4). It was also found that the bacterial strain without ACC-deaminase did not promote the growth of the inoculated plants [31], confirming that the ACC-deaminase trait of the PGPR was primarily responsible for the growth promotion. Many researchers have already reported that, under gnotobiotic conditions, seed and/or root inoculation with rhizobacteria promotes root growth through ACC-deaminase activity [7, 11, 13, 14, 32, 36]. The PGPR strains that colonized the wheat roots the most were found to increase the wheat growth parameters, as seen by the positive correlation between the root colonization and the different plant growth and yield parameters (Table 4). Thus, it is highly likely that the strains that intensively colonized the wheat roots hydrolyzed more ACC, owing to a higher number of PGPR per gram root weight. Overall, the best performing strains were found to have a good combination of many plant growth-promoting attributes.

In the case of the field trials, inoculation with PGPR was also found to be effective in increasing the wheat growth and yield, although the efficacy of the strains differed from that in the pot trials. *Ps. fluorescens* ($ACC_{50}$) was identified as the most effective for increasing the wheat yield and yield-contributing parameters, and it is highly probable that the greater effectiveness of this strain may be related to its high auxin production and P solubilization, and relatively high root colonization ability and chitinase activity, in addition to its ACC-deaminase activity (Table 1). *B. caryophylli* ($ACC_7$), which was identified as the most effective in the pot trials, also significantly increased the wheat growth and yield, yet was less effective than *Ps. fluorescens* ($ACC_{50}$) under the field conditions. The superiority of *Ps. fluorescens* ($ACC_{50}$) over *B. caryophylli* ($ACC_7$) may have been related to the chitinase activity of $ACC_{50}$, which made it more competitive under the field conditions. This premise is supported by the observation that three out of the four fluorescent pseudomonads tested possessed chitinase activity and showed a relatively better performance under the field conditions than in the pot trials. Other factors not determined in this study may also have been important in determining the ability of the strains to survive and increase the wheat growth under the field conditions compared with the pot trials, such as the ability of the strains to inhibit pathogens, *etc.* The production of antifungal metabolites by fluorescent pseudomonads has also been previously reported to suppress soilborne fungal pathogens [8, 25]. *Pseudomonas putida* biotype A ($ACC_9$) and *Pseudomonas fluorescens* ($ACC_{14}$) did not prove as effective as the other strains, in both the pot and field trials, possibly because of their relatively poor root colonization ability, lower ACC-deaminase activity, no IAA production without L-TRP, absence of chitinase activity, and no P solubilization. This suggests that ACC-deaminase activity is a good tool for the efficient selection of promising PGPR; nonetheless, other growth-promoting attributes of PGPR should also be considered for the selection/screening of effective PGPR strains. Thus, strains with a good combination of these traits (ACC-deaminase activity, auxin production, root colonization, P solubilization, chitinase activity, siderophore production, antibiotic production) are likely to perform better as inocula for biofertilizer formulations. Such studies could also lead to the development of statistical models of the contribution of these plant growth-promoting traits of PGPR to increase crop growth and yield.

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