Journal of Plant Growth Regulation (2021) 40:494–508
https://doi.org/10.1007/s00344-020-10114-3

Growth and Yield Response of Upland Rice to Application of Plant Growth-Promoting Rhizobacteria

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Received: 18 January 2020 / Accepted: 6 April 2020 / Published online: 18 April 2020

Abstract

This study evaluated the effects of plant growth-promoting rhizobacteria (PGPR) isolates in enhancing upland rice growth and yield. Bacteria were isolated, screened for growth-promoting activities in vitro, biochemically identified, and tested under screenhouse conditions at the Philippine Rice Research Institute (PhilRice). Isolates exhibited growth-promoting activities, such as indole-3-acetic acid (IAA) production, tricalcium phosphate solubilization, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity, siderophore production, and starch hydrolysis. The screenhouse experiment was conducted with upland rice (PSB Rc23) as the test crop, sterilized and natural soils, and recommended rate of inorganic fertilizers (RRIF) as other source variables. Results showed that significantly heavier shoot and root fresh weights are evident in plants grown in sterilized soil. Plants treated with full RRIF exhibited superior growth in terms of plant height, shoot and root weights, and grain yield. Among inoculated and fertilized plants, comparable growth promotion was obtained with *Acidovorax delafieldii* combined with half inorganic fertilizer rate, in terms of root fresh weight, shoot and root dry weights, plant height, grain yield, and nitrogen, phosphorus, and potassium (NPK) uptake. Overall findings show that PGPR (*A. delafieldii*) in combination with 50% RRIF is as effective as full RRIF in enhancing growth and yield of PSB Rc23 rice, implying that dependence on chemical fertilizer can be reduced utilizing this PGPR. However, further evaluation of these bacterial isolates in actual field conditions is necessary to uncover their efficiency as potential biofertilizer.

Keywords Plant growth-promoting rhizobacteria · Inoculation · Upland rice · Plant growth and development · Growth and yield

Introduction

Rice (*Oryza sativa* L.) is the primary food grain of half of the world's population. This crop is the most common crop on the continents because of its high adaptability to different environments (Nascente et al. 2019). The Food and Agriculture Organization of the United Nations (2020) considers rice as a vital crop for food security throughout the world. Rice is cultivated in different ecosystems to about 150 million ha worldwide which are as follows: 75% irrigated, 17% rainfed lowland, and 4% upland condition (Nascente et al. 2019). In Asia, upland rice accounts to approximately 9% of the total rice area, while Latin America and West Africa share most of the upland rice acreage (Bernier et al. 2008; Kikuta et al. 2016). Upland rice can be grown under rainfed conditions and rarely require additional irrigation. Despite these benefits, this crop has low productivity due to inefficient acquisition of nutrients, especially of phosphorus (P), caused by unpredictable drought (Fageria et al. 1982). It is with this premise that numerous studies have been conducted integrating sustainable production system to increase its productivity (Adesemoye et al. 2008; Cruz et al. 2015; Guyasa et al. 2018; Santos and Cruz 2017).

Today, alternative systems are being sought to make agriculture more sustainable in application of limiting nutrients. Biofertilizers or microbial inoculants are the most attractive of these alternatives because of their positive impact on plant growth as well as the environment (Kantachote et al. 2016). Soil bacteria have been recognized as stimulating

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plant growth through the mobilization of nutrients in soils, numerous plant growth regulators, plant protection from pathogens, improvement of soil structure, and bioremediation of contaminated soils (Ahemad and Kibret 2014). Bacteria colonizing around the roots of or on plants are known as rhizobacteria, which are more versatile in nutrient transformation, mobilization, and solubilization than bulk soils (Hayat et al. 2010). Glick (2012) suggested that soil recycling and soil fertility are dominant deriving forces of rhizobacteria. PGPR are heterogeneous group of rhizobacteria associated with roots that can directly or indirectly improve the extent or quality of plant growth. Direct PGPR promotion involves providing the plant with plant growth-promoting substances like IAA, gibberellin, siderophores, ACC deaminase, and by mechanisms for various growth promoters such as phosphate solubilization and anti-fungal activity (Kloepper 1981).

Among the recently studied rhizobacteria on rice are the *Serratia* spp. (Nascente et al. 2019), *Bacillus* spp. (Guyasa et al. 2018; Rais et al. 2018), *Pseudomonas fluorescens* (Guyasa et al. 2018), *Serratia nematodiphila* (Chakraborty et al. 2013), and *Actinomycete* (Cruz et al. 2015). These studies have generally demonstrated that application of these microorganisms in either screenhouse of field experiments showed a significant increase in growth and yield parameters, like seedling viability and vigor, root dry weight, and grain yield, in several crops. In a study by Cruz et al. (2015), upland rice growth promotion was obtained with actinomycete inoculation through significant increase in P uptake and grain yield under screenhouse conditions. Nascente et al. (2019) studied the effectiveness of *Serratia* spp. on upland rice under field conditions and obtained significant increase in N uptake, shoot dry matter, and grain yield, among others. Although, these studies generally show the potential of PGPR to improve rice growth, there have been no studies about the effect of PGPR and its interaction with soil sterilization treatment and with varying rates of recommended inorganic fertilization.

The present study performed this gap through looking at the effect on nutrient content, growth parameters, and yield components. It is the primary goal of this study to investigate the effect of selected bacterial isolates on upland rice performance, with soil sterilization, fertilization, and inoculation as influencing factors.

Materials and Methods

Isolation of Bacteria

Plant and rhizosphere sample collection. Six upland rice plant samples were randomly collected at Sindon Bayabo, Ilagan City, Isabela, Philippines [17° 7′ 19.497″ N 122° 0′ 25.206″ E (DMS)]. Isolation of bacteria from the rhizosphere was performed based on the method of Cruz and Paterno (2014). The entire root system was removed out of the soil ground and gently tapped to remove soils adhering to the roots. Ten grams (10 g) of root samples from each plant was transferred into a 250-mL Erlenmeyer flask containing 100 mL sterile distilled H_2O and shaken for 24 h. The root–water mixture was diluted, and a series of four tenfold dilutions was made. Then, 0.1 mL of 10^{-3} and 10^{-4} dilutions was spread on duplicate Burks agar plates. The plates were incubated at room temperature, and morphologically different colonies appearing on the medium were isolated and sub-cultured.

Medium Used and Bacteria Purification

Burks medium (HiMedia) was used in the isolation and cultivation of bacteria from the rhizosphere samples. The medium was composed of (L^{-1}) 0.200 g magnesium sulfate, 0.800 g dipotassium phosphate, 0.200 g monopotassium phosphate, 0.130 g Calcium sulfate, 0.00145 g ferric chloride, 0.000253 g sodium molybdate, and 20 g sucrose. The medium was suspended in distilled water and was sterilized at 121 °C for 15 min. Color examination was done at every colony, and a loopful of each morphologically different colony was streaked on duplicate Burks agar plates. Pure cultures were assigned with codes were used for further experiments.

Screening for Growth-Promoting Activities

Bacterial isolates were screened and tested for the following plant growth-promoting activities: IAA production, phosphate solubilization, ACC deaminase, siderophore production, and starch hydrolysis. All media used in the assays were sterilized for 20 min at 121 °C.

IAA Production Assay

To measure the IAA production activity of bacteria, a loopful of each isolate was inoculated in nitrogen-free broth which consists of 10.65 g Burks medium and 500 mL distilled H_2O supplemented with 0.05 g L^{-1} tryptophan (Cruz and Paterno 2014; Shahab et al. 2009). After seven days of incubation, cultures were centrifuged for 10 min at 13,000 rpm in 4 °C. The IAA in the supernatant was detected colorimetrically by Salkowski's reagent (Reddy et al. 2010). The reagent used consisted of 3.0 mL 0.5 M $FeCl_3$, 90.0 mL H_2SO_4, and 150.0 mL distilled H_2O. One mL of the supernatant was reacted with 2.0 mL of the reagent and pink to red color transformation indicated positive reaction (Cruz et al. 2018).

Phosphate Solubilization Assay

To test phosphate solubilization activity, isolates were grown in modified Pikovskaya's medium (Bisen 2014) that contained the following ingredients: (L^{-1}) 5.0 g Ca3(PO_4), 0.2 g NaCl, 0.2 g KCl, 0.1 g $MgSO_4 \cdot 7H_2O$, 0.00025 g $MnSO_4 \cdot 7H_2O$, 0.00025 g $FeSO_4 \cdot 7H_2O$, 0.5 g $(NH_4)_2SO_4$, 0.5 g yeast extract, 10.0 g glucose, and 20.0 g agar. Bacterial isolates were spot inoculated onto the surface of the agar and incubated for 5 days. Clearing zone around the bacterial growth or colony indicated phosphate solubilization (Cruz and Paterno 2014; Shahab et al. 2009).

ACC Deaminase Activity Assay

To test ACC deaminase activity, isolates were grown in nitrogen-free Dworkin and Foster's minimal salts agar medium (DF-ACC agar) supplemented with 0.3 g L^{-1} ACC (Varma et al. 2007). Plates were incubated in the dark for 7 days, and growth of isolates on the media was taken as an indicator of its efficiency to utilize ACC and produce ACC deaminase (Cruz and Paterno 2014).

Siderophore Production Assay

To test siderophore production activity, the procedure of Schwyn and Neilands (1987) was followed. Chrome Azurol S (CAS) agar was prepared from four solutions, which were sterilized separately before mixing. The blue dye solution (solution 1) was prepared by mixing the 0.06 g CAS (in 50 mL distilled H_2O) and 0.0027 g $FeCl_3$ (in 10 mL 10mMHCl) with 40 mL of distilled H_2O containing 0.073 g of Hexadecyltrimethylammonium bromide (HDTMA). The resulting mixture was dark blue in color. Then, MM9/PIPES solution (solution 2) was prepared by dissolving 3.0 g KH_2PO_4, 5.0 g NaCl, and 10.0 g NH_4Cl in 100 mL distilled H_2O and diluting the solution in 750 mL distilled H_2O. The diluted solution was added with 32.24 g of piperazine-N,N'-bis[2-ethanesulfonic acid] (PIPES), and pH was adjusted to 6.8 through drop-by-drop addition of NaOH. Then, 15.0 g bacteriological agar was added, mixed, and set aside. Meanwhile, casamino acid solution (solution 3) was prepared by mixing 3.0 g of casamino acid (in 27 mL distilled H_2O) and 1.5 g 8-hydroxyquinoline (in 50 mL chloroform). Casamino acid (30 mL) was extracted using micropipette from the resulting two-layer solution. Lastly, 20% glucose stock (solution 4) was prepared by mixing 20.0 g of glucose in 100 mL distilled H_2O. All four solutions were sterilized at 121 °C for 20 min and CAS agar (blue color) was prepared aseptically. Bacterial isolates (four isolates per plate) were spot inoculated on the agar's surface. Microbial siderophore production was determined by the formation of orange halos or clearing zone around the bacterial colony after 1 week of incubation at room temperature (Varma and Chincholkar 2007).

Starch Hydrolysis Assay

To test starch hydrolysis activity, isolates were streaked on the starch agar consisting (L^{-1}) of 3.0 g beef extract, 10.0 g soluble starch, and 15.0 g bacteriological agar. After 24 h of incubation, the plate's surface was submerged with iodine solution to detect the presence or absence of starch. Microbial starch hydrolysis was revealed as clearing zone surrounding the bacterial growth (Cappuccino and Sherman 2007).

Selection and Identification of Isolates

Four isolates were selected based on their growth-promoting traits. Three of these isolates ($IBBw_{1a}$, $IBBy_1$, $IBBy_{2d}$) outclass among others—each of which was positive for IAA production, phosphate solubilization, and starch hydrolysis, respectively. Meanwhile, the other isolate ($IBBw_{2e}$) holds multiple growth-promoting activities—IAA production, phosphate solubilization, ACC deaminase activity, and siderophore production. Selected isolates were identified biochemically using the Biolog GEN III Microbial Identification System analysis. Identification process was performed according to manufacturer's instruction (Wozniak et al. 2019). All readings and interpretation of results were performed by Biolog's Microbial Identification System Software at the Natural Sciences Research Institute (NSRI), University of the Philippines (UP) Diliman, Quezon City, Philippines.

The Biolog GEN III Microbial ID System revealed the identity of four selected isolates (Table 1). However, only two of them passed the similarity value criteria. These are the *Ralstonia pickettii* (IBB_{y1}) and *Acidovorax delafieldii* ($IBBy_{2d}$) with similarity values of 0.668 and 0.652, respectively. In essence, isolate $IBBw_1$ was identified as *Rhizobium rhizogenes* while $IBBw_{2e}$ as *Burkholderia pyrrocinia* but with low similarity values (0.291 and 0.348), these identifications cannot be considered. Wozniak et al. (2019) defined similarity as the "degree of similarity of physiological profile of the test strain with the strain deposited in the Biolog GEN III database." Wong et al. (2015) reported that acceptable similarity values are those that are > 0.5, and values lower than 0.5 should be recorded as "NO ID."

Screenhouse Experiment

Soil Sampling

Maligaya clay soils were used and analyzed at the Agricultural Systems Institute (ASI), College of Agriculture (CA),

Table 1 Selected isolates, their growth promoting activities, and identification results

Selected isolate	IAA production	Phosphate solubilization	ACC deaminase activity	Siderophore production	Starch hydrolysis	Identification result and similarity value
$IBBw_{1a}$	+	−	−	−	−	*Rhizobium rhizogenes* (0.291)*
$IBBy_{1}$	−	+	+	+	−	*Ralstonia pickettii* (0.668)
$IBBw_{2e}$	+	+	+	+	−	*Burkholderia pyrrocinia* (0.348)*
$IBBy_{2d}$	−	−	+	+	+	*Acidovorax delafieldii* (0.652)

+ positive; − negative

*Considered as NO ID and the original isolate code was retained

Table 2 Chemical properties of Maligaya clay soil used in this screenhouse experiment

Property	Value
pH (1:2.5 soil to water diluent)	6.30
OM (%)	0.61
Total N (%)	0.05
Extractable P (mg kg^{-1} soil)*	Low*
Extractable K (meq 100 g^{-1} soil)	0.17
$CEC_{Effective}$ (meq 100 g^{-1} soil)	22.61

*Qualitative value was presented since the quantitative value of *P* exceeds the normal *P* values for Maligaya clay soil series. Determined using soil test kit

University of the Philippines Los Baños (UPLB) (Table 1). In soil sampling, the protocol set by Tan (2005) was followed where 10 points were selected within a 1 ha-area field. Using a hand hoe and a shovel, surface litter was initially removed, and soils were collected from a depth of 0–20 cm around each selected point with 2-m radius. Collected soils were placed in clean polypropylene sacks with 60 kg-capacity. Samples were transported in the laboratory using a hauling vehicle by the Philippine Rice Research Institute (PhilRice). Soil samples were bulked and evenly mixed together in clean plastic sheets, air dried, and composited. An approximate total of 1200 kg soil samples (20 sacks) were obtained.

Soil Chemical Properties

Chemical properties of collected soils are shown in Table 2. Soils used had near neutral pH, low OM content, low N, low K, and high CEC. Soil pH was measured potentiometrically. Organic matter content was measured using modified Walkley and Black Method, while total nitrogen (N) was determined using modified Kjeldahl Method. Available phosphorus (P) was determined using modified Olsen method, while exchangeable potassium (K) was analyzed using NH_4OA_c extraction-flame photometer method. Additionally, cation exchange capacity (CEC) was determined using sodium acetate method. All analyses were done following the methods described in a handbook on "Standard Methods of Analysis for Soil, Plant Tissue, Water and Fertilizer" prepared by PCAARRD (1980).

Treatments and Design

The screenhouse experiment was conducted at the Philippine Rice Research Institute (PhilRice), Maligaya, Science City of Muñoz, Nueva Ecija, Philippines. Sterilized and natural soils, recommended rate of inorganic fertilizer (RRIF), and selected bacterial isolates were used as the source variables. The experiment was laid out in factorial arrangement in randomized complete block design (RCBD) in four replicates. Details of the above variables are shown below:

Factor A—soil treatment (S)

S_1 = Sterilized soil S_2 = Natural soil

Factor B—inoculation and fertilization treatment (IF)

IF_1 = uninoculated, 0% RRIF
IF_2 = $IBBw_{1a}$
IF_3 = *R. pickettii*
IF_4 = $IBBw_{2e}$
IF_5 = *A. delafieldii*
IF_6 = $IBBw_{1a}$ + 50% RRIF
IF_7 = *R. pickettii* + 50% RRIF
IF_8 = $IBBw_{2e}$ + 50% RRIF
IF_9 = *A. delafieldii* + 50% RRIF
IF_{10} = uninoculated, 100% RRIF

Potting Material

Plastic pails were used as pots containing 8-kg air-dried and pulverized soils, composited and sieved using a 2-mm wire mesh. Sterilization of soils was done in an autoclave at 121 °C for 1 h for 3 consecutive days (Cruz et al. 2014). Each pot was labeled with the corresponding treatment. Meanwhile, PSB Rc23 rice seeds, a 108-day duration upland rice variety, were used in this experiment. Initially, seeds were washed with sterile distilled water, soaked subsequently in 2.5% Sodium hypochlorite for 20 min, and then in 70% ethanol for 30 s. Seeds were washed afterward with sterile distilled water for three times (Shahzad et al. 2017).

Table 3 Population of four selected rhizobacterial isolates used in inoculation

Selected isolate	Population of bacteria (CFU 0.1 mL^{-1})			
	Seed bacterization	First application	Second application	Third application
$IBBw_{1a}$	$>3.0\times10^9$	$>3.0\times10^9$	$>3.0\times10^9$	5.7×10^8
R. pickettii	$>3.0\times10^9$	$>3.0\times10^9$	$>3.0\times10^9$	$>3.0\times10^9$
$IBBw_{2e}$	$>3.0\times10^9$	1.9×10^9	1.7×10^9	1.8×10^9
A. delafieldii	1.6×10^9	8.5×10^8	7.8×10^8	5.6×10^8

Bacteria Preparation and Inoculation

Prior to sowing, selected isolates were grown in Burks medium agar and were incubated for 5 days at room temperature. Bacterial suspension (10^6 cell mL^{-1}) was prepared in sterile distilled water (Ma et al. 2011). Surface-sterilized seeds were soaked in bacterial suspension for 2 h. Inoculated seeds (five per pot, then thinned into three) were planted into the soil by dibbling. Then, 50 mL of bacterial suspension was inoculated into the soil at the following critical stages: early growth [10 days after sowing (DAS)], active tillering (24 DAS), and panicle formation (38 DAS) (Cruz et al. 2014). Population count of four bacterial isolates were determined and expressed as colony-forming units (CFUs) to confirm the presence of viable cells in each of the bacterial suspension applied to the plants (Cruz and Cadiente 2015). These were done during seed bacterization stage and three field applications (Table 3). Such population is fair, and within the acceptable count of bacterial population in a suspension (10,000 CFU mL^{-1} or more) according to Malarkey and McMorrow (2011). This supports the viability of the bacterial isolates applied to plants.

Fertilizer Application

Recommended rate of inorganic fertilizer (RRIF), 100-60-60 kg N, P_2O_5, and K_2O, was determined based on the results of the quantitative soil analysis, except for P. Fertilizer materials (FM) used were applied in exact proportion in the following splits:

1st Application (10 DAS) : ¼ N, ½ $P_2O_5$and ½ K_2O of RRIF
2nd Application (24 DAS) : ½ N, ½ $P_2O_5$and ½ K_2O of RRIF
3rd Application (38 DAS) : ¼ N of RRIF

The first application consisted of 54.38 kg of ha^{-1} urea, 166.67 kg of ha^{-1} solophos, and 50 kg ha^{-1}muriate of potash. In the second application, 108.70 kg ha^{-1}, 166.67 kg ha^{-1} solophos, and 50 kg ha^{-1} muriate of potash have been applied. In the third application, only 54.35 kg ha^{-1} urea was used. Given below is the amount of each fertilizer material applied per 8-kg soil pot at full recommended rates. Half of these values were used for treatments with 50% RRIF. The formula used in the calculation is also presented:

1st Application : 0.2174 g urea, 0.6667 g solophos and 0.20 g muriate of potash
2nd Application : 0.4348 g urea, 0.6667 g solophos and 0.20 g muriate of potash
3rd Application : 0.2174 g urea only

$$\text{FM (kg per ha)} = \frac{\text{Weight of nutrient}}{\%\ \text{nutrient}},$$

$$\text{FM (g per 8 kg)} = \frac{\text{FM (kg per ha)} \times 8\ \text{kg soil}}{2 \times 10^6 \text{kg soil}} \times 1000$$

Water Management

After sowing, watering was done uniformly everyday among all plants to maintain soil moisture at about field capacity during the entire growth cycle. The pots were subjected to wetting and drying cycles similar to the methods performed by Fageria et al.'s (2014) experiment on upland rice.

Plant Tissue Analysis

All plants per pot were collected at maturity, oven-dried at 70 °C for 24 h (Cruz et al. 2014), and sieved using a 0.45-mm wire mesh. Ground composite samples from every treatment were sent to Visayas State University (VSU) Central Analytical Services Laboratory (CASL) for analysis of N, P, and K in plant tissues following standard procedures by PCAARRD (1980). Nutrient uptake determination was done using collected data on shoot dry matter yield, excluding grains. Calculation of N, P, and K uptake of crop was done by multiplying percentage of each nutrient (N, P, and K) by the shoot dry matter yield expressed in grams per plant.

Agronomic Parameters

Plant Height, Root Length, and Dry Matter

Plant height and root length were measured at physiological maturity using a meterstick. Height was measured from the base of plant to the tip of the tallest tiller of the plant. For root length, the root portion was removed manually from the soil. Any loose soils were washed off and the length was measured from the crown roots to the tip of the primary root. Dry matter was measured as the oven-dry weight of shoot and root. It is the average weight of three plants in every treatment. A digital weighing scale (LCD display type) was used with a precision of 0.01 g.

Grain Yield and Its Components

Harvesting was done manually after physiological maturity. Grain yield and its following components were determined: number of spikelets per panicle, number of productive and unproductive tillers, and 100-grain weight. Grain yield was determined by weighing the total grain yield per plant per pot and then expressed in average. Moisture content (MC) of seeds was adjusted to 14%. Yield expressed in grams per pot was computed following the formula presented below. Tiller count was determined by counting both grain-bearing and non-grain-bearing tillers. The spikelet count per panicle was determined by counting both filled and unfilled ones and expressed in percentage. Weight per 100 grains was determined by taking 100 grains from each treatment combination.

$$\text{Grain yield (at 14\% MC)} = \text{grain yield (g)} \times \frac{100 - \text{MC}}{86}$$

Data Analysis

Screenhouse experiment data were analyzed by analysis of variance (ANOVA) using SAS Software, and treatment means were compared relative to the control (uninoculated) following Tukey's Studentized Range (HSD) tests. Unless indicated otherwise, differences were only considered when significant at $P < 0.05$.

Results

Plant Growth Promoting Activities

A total of 25 bacterial isolates were obtained from the upland rice rhizosphere using Burk's N-free medium. These were purified and preserved on agar plates.

IAA Production

Isolates were initially screened for their ability to produce IAA. It was observed that only 12 out of 25 isolates were able to synthesize IAA as confirmed by the pink to red color transformation after the reaction of the tryptophan-supplemented culture broths with the Salkowski's reagent (Fig. 1a). Interestingly, isolates $IBBw_{1a}$ and $IBBw_{1b}$ were noted to produce a distinct red color among others indicating higher IAA-producing potential of these rhizospheric bacterial isolates (Cruz and Paterno 2014).

Phosphate Solubilization

The 25 bacterial isolates were tested using Pikovskaya's modified agar containing tricalcium phosphate to measure their solubilizing efficiency (Bisen 2014). There were seven out of 25 isolates ($IBBy_1$, $IBBy_{2a}$, $IBBy_{3b}$, $IBBy_{2c}$, $IBBy_{3c}$, $IBBw_{2e}$, and $IBBw_{1b}$) that showed halo zone around the bacterial colony indicative of the solubilization activity in its vicinity (Fig. 1b).

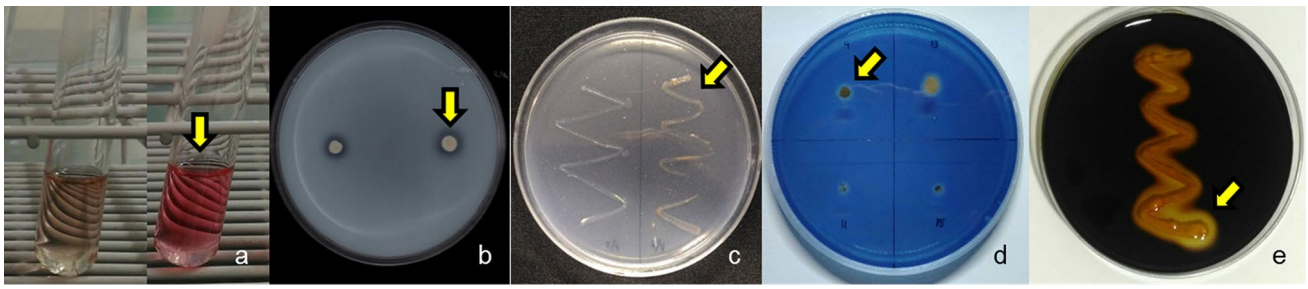

Fig. 1 Positive indicators of the five assays: **a** IAA production—pink to red color, **b** phosphate solubilization—halo zone around the colony, **c** ACC deaminase activity—growth of bacteria, **d** siderophore production—orange halos around the colony, and **e** starch hydrolysis—clearing zone around bacterial growth (Color figure online)

ACC Deaminase Activity

Twenty out of 25 isolates showed ACC deaminase activity showing growth on Dworkin and Foster's minimal salts agar medium amended with ACC (DF-ACC agar) (Fig. 1c).

Siderophore Production

Siderophore production by the bacterial isolates was indicated by the formation of orange halos in CAS agar by the bacteria after 1 week of incubation at room temperature (Fig. 1d). Results showed that 12 out of 25 tested isolates had the ability to produce siderophores.

Starch Hydrolysis Assay

Only one out of the 25 isolates screened was positive for starch hydrolysis activity. A clearing zone around bacterial growth was observed on this isolate indicative of its capacity to hydrolyze starch through production of various exoenzymes (Fig. 1e).

Nutrient Content

NPK Uptake

The combined bacterial inoculation and fertilization showed significant effects in N uptake. Half fertilization rate + *A. delafieldii* recorded the highest shoot N uptake (61.46 mg plant^{-1}), which is significantly (138%) higher than the untreated plants. Closer look at data showed that combined fertilization of 50% RRIF and inoculation with the other isolate also yielded comparable shoot N uptake with plants in 100% RRIF (Table 4). In terms of P uptake, lower values than N were observed across all treated plants which ranged from 0.033 to 0.124 mg plant^{-1}. Potassium uptake data revealed significantly higher values (by 62%) in plants grown in sterilized soil. Application of full RRIF significantly increased shoot K uptake over the untreated plants. Substituting 50% of RRIF with inoculation of *R. pickettii*, *B. pyrrocinia,* and *A. delafieldii* resulted to comparable shoot K uptake values relative to full RRIF-treated plants.

Morpho-agronomic Traits

Plant height was significantly influenced by inoculation–fertilization and soil treatments. Inoculation of any of the selected isolates alone did not significantly enhance plant height over full RRIF-treated plants. However, substituting half of RRIF with inoculation resulted to comparable plant height with full RRIF-treated plants. Meanwhile, significant effect was also evident in two different soil treatments (Fig. 2). Significant increase of about 11% was observed in plants grown in sterilized soil conditions regardless of inoculation and fertilization (Table 5). In terms of root length, the effect of inoculation–fertilization and soil treatments showed no significant differences among inoculated and fertilized plants. However, significant difference was observed between two soil treatments as there was 37% increase in plants grown in sterilized soil relative to natural soil.

For dry matter, comparison among means revealed that bacterial inoculation alone did not significantly enhance shoot and root oven-dry weights relative to the uninoculated plants. Likewise, inoculation of any of selected isolates combined with 50% RRIF did not significantly improve shoot and root oven-dry weights relative to full RRIF-treated plants. However, *A. delafieldii* inoculation + 50% RRIF gave statistically comparable result with full fertilization rate, and this was significantly higher than the uninoculated plants (Table 6). Meanwhile, significant increase in oven-dry weights (29% and 55%, respectively) was observed in sterilized soil relative to natural soil. Figure 3 shows the roots of upland rice in sterilized and natural soil conditions as affected by inoculation and fertilization treatments.

For grain yield, generally, inoculation with any of the selected isolates alone did not significantly enhance yield relative to full recommended rate. The heaviest grain yield per plant was still obtained with full RRIF treatment (Table 7). Half fertilization treatment in combination with *A. delafieldii* produced comparable grain yield relative to full RRIF-treated plants, but not significant with inoculation of IBBW$_{1a}$, *R. pickettii* and *B. pyrrocinia,* and half RRIF. Significant increase (34%) in grain yield per plant was observed in plants grown in sterilized soil conditions relative to yield obtained in natural soil.

For productive tillers, the highest tiller count was obtained with full fertilization, while inoculated plants had the least count ranging from 1 to 2 only. An average of 3 productive tillers were observed among inoculated and fertilized plants. Results further showed significant effects on productive tiller count among inoculated and fertilized plants but not on soil-treated plants and on their interactions. Meanwhile, unproductive tillers among inoculated–fertilized plants only ranged from 0 to 1 and showed no significance in both factor treatments (Table 7).

The effect of inoculation–fertilization and soil treatments showed no significant differences in percent filled spikelets per panicle among all treated plants (data not shown). Meanwhile, an average number of filled spikelets were observed to be significantly higher by 26% in plants grown in sterilized soil than in natural soil, but no

Table 4 Effect of inoculation and fertilization on shoot nitrogen, phosphorus, and potassium uptake (mg plant^{-1}) of upland rice in sterilized and natural soil conditions

Inoculation and fertilization treatment	Nitrogen		Mean	Phosphorus		Mean	Potassium		Mean
	Soil treatment			Soil treatment			Soil treatment		
	Sterilized	Natural		Sterilized	Natural		Sterilized	Natural	
1 Uninoculated, 0% RRIF	16.12	34.90	25.51^{bc}	0.052	0.058	0.055^{ab}	48.71	49.45	49.08^{bc}
2 IBBw$_{1a}$	20.29	23.52	21.91^{c}	0.027	0.048	0.038^{ab}	33.81	26.96	30.39^{c}
3 *R. pickettii*	31.72	10.55	21.13^{c}	0.058	0.022	0.040^{ab}	56.16	13.48	34.82^{c}
4 IBBw$_{2e}$	24.30	16.43	20.37^{c}	0.032	0.034	0.033^{b}	65.19	25.27	45.23^{c}
5 *A. delafieldii*	50.16	21.12	35.64^{abc}	0.199	0.049	0.124^{a}	82.65	27.42	55.04^{bc}
6 IBBw$_{1a}$ + 50% RRIF	41.96	34.17	38.06^{abc}	0.080	0.052	0.066^{ab}	52.48	49.71	51.09^{bc}
7 *R. pickettii* + 50% RRIF	43.65	28.83	36.24^{abc}	0.085	0.053	0.069^{ab}	107.68	43.92	75.80^{abc}
8 IBBw$_{2e}$ + 50% RRIF	44.61	34.95	39.78^{abc}	0.063	0.050	0.057^{ab}	79.94	56.07	68.00^{abc}
9 *A. delafieldii* + 50% RRIF	59.78	63.21	61.46^{a}	0.132	0.050	0.091^{ab}	95.90	85.86	90.88^{ab}
10 Uninoculated, 100% RRIF	65.75	47.98	56.86^{ab}	0.122	0.101	0.112^{ab}	126.92	85.87	106.40^{a}
MEAN	39.83	31.57		0.085^{a}	0.052^{b}		74.94^{a}	46.40^{b}	

Means followed by a common letter are not significantly different at 5% level, HSD

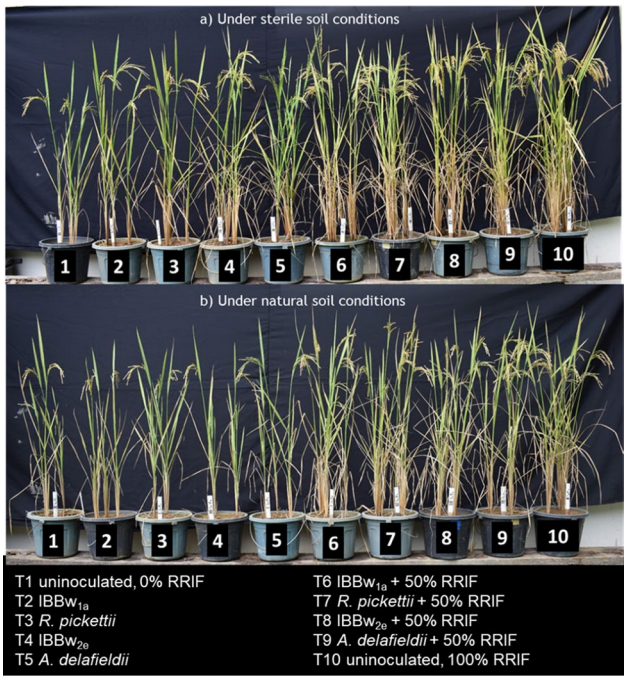

Fig. 2 Growth of upland rice (PSB Rc23) in **a** sterilized and **b** natural soil conditions as affected by inoculation and fertilization treatments

significant effects among inoculated and fertilized plants. In terms of 100-grain weight, the effect of inoculation–fertilization and soil treatments showed no significant differences observed among all treated plants. Likewise, no interaction effect was observed between two factor treatments (Table 8).

Discussion

Plant Growth Promoting Activities

The soil rhizosphere is a complex zone surrounding a plant root where multiple bacterial species live, giving direct and indirect beneficial effects to the host plants (Reddy et al. 2010). Root-colonizing bacteria or most known as rhizobacteria play important roles in plant growth and development and can also protect host plants against soil-borne pathogens. Several genus and species of rhizobacteria have recently been isolated from the rice plant, including *Serratia* sp. (Nascente et al. 2019), *Streptomyces mutabilis* (Cruz and Paterno 2014), *Pseudomonas fluorescens* (Guyasa et al. 2018), and *Burkholderia pyrrocinia* (Rego et al. 2018) which were mostly reported to show plant growth-promoting activities in both screenhouse and field conditions. In the present study, a total of 25 bacterial isolates were obtained from the rhizosphere of upland rice which showed growth-promoting potential based on a series of assays.

Prominent among the mechanisms used by PGPR strains is the bacterial synthesis of phytohormone indole-3-acetic acid (IAA) (Sayyed et al. 2019). This plant-derived PGPR compound is the most common auxin hormone (Zažímalová et al. 2014) and is naturally occurring in plants (Vidhyasekaran 2014). In the current study, 12 isolates were positive for IAA production. IAA is one of the most important plant hormones which have profound effects in growth and development of crops. Previous studies reported growth promotion and stimulation using IAA-synthesizing bacteria, such as *Bacillus* spp. and *Pseudomonas fluorescens* (Guyasa et al. 2018), *Lysinibacillus sphaericus* (Shabanamol et al.

Table 5 Effect of inoculation and fertilization on plant height and root length (cm) at maturity of upland rice (PSB Rc23) in sterilized and natural soil conditions

Inoculation and fertilization treatment	Plant height			Root length		
	Soil treatment		Mean	Soil treatment		Mean
	Sterilized	Natural		Sterilized	Natural	
1 Uninoculated, 0% RRIF	100.45	90.08	95.26^{ab}	43.8	33.7	38.8
2 IBBw$_{1a}$	94.10	85.08	89.59^{b}	46.5	32.0	39.2
3 *R. pickettii*	99.65	82.80	91.23^{b}	45.7	30.1	37.9
4 IBBw$_{2e}$	101.98	86.75	94.36^{ab}	45.4	29.2	37.3
5 *A. delafieldii*	93.48	84.48	88.98^{b}	46.3	33.0	39.7
6 IBBw$_{1a}$ + 50% RRIF	99.33	98.05	98.69^{ab}	44.7	38.1	41.4
7 *R. pickettii* + 50% RRIF	103.48	90.15	96.81^{ab}	49.5	32.8	41.1
8 IBBw$_{2e}$ + 50% RRIF	98.43	95.08	96.75^{ab}	39.7	33.9	36.8
9 *A. delafieldii* + 50% RRIF	107.73	100.10	103.91^{a}	45.6	36.7	41.2
10 Uninoculated, 100% RRIF	113.40	96.55	104.98^{a}	45.8	31.5	38.6
MEAN	101.20^{a}	90.91^{b}		45.3^{a}	33.1^{b}	

Means followed by a common letter are not significantly different at 5% level, HSD

Table 6 Effect of inoculation and fertilization on shoot and root oven-dry weight (g plant^{-1}) of upland rice (PSB Rc23) in sterilized and natural soil conditions

Inoculation and fertilization treatment	Shoot oven-dry weight			Root oven-dry weight		
	Soil treatment		Mean	Soil treatment		Mean
	Sterilized	Natural		Sterilized	Natural	
1 Uninoculated, 0% RRIF	3.69	3.88	3.78^{cd}	1.82	0.88	1.35^{c}
2 IBBw$_{1a}$	2.60	2.79	2.69^{d}	1.13	0.81	0.97^{c}
3 *R. pickettii*	4.11	2.13	3.12^{cd}	1.42	0.63	1.03^{c}
4 IBBw$_{2e}$	4.90	2.22	3.56^{cd}	1.92	0.57	1.24^{c}
5 *A. delafieldii*	6.69	2.66	4.67^{cd}	2.85	0.98	1.92^{bc}
6 IBBw$_{1a}$ + 50% RRIF	6.57	5.94	6.25^{bcd}	3.71	2.28	2.99^{bc}
7 *R. pickettii* + 50% RRIF	7.91	5.57	6.74^{bcd}	4.65	2.62	3.64^{bc}
8 IBBw$_{2e}$ + 50% RRIF	6.28	7.61	6.94^{abc}	2.87	3.64	3.26^{bc}
9 *A. delafieldii* + 50% RRIF	9.42	8.63	9.02^{ab}	5.11	4.35	4.73^{ab}
10 Uninoculated, 100% RRIF	12.85	8.88	10.86^{a}	8.35	5.04	6.69^{a}
Mean	6.50^{a}	5.03^{b}		3.38^{a}	2.18^{b}	

Means followed by a common letter are not significantly different at 5% level, HSD

2018), and *Enterobacter* sp. (Saengsanga 2018), all of which were isolated from upland rice rhizosphere. Wahyudi et al. (2011) revealed that the IAA-synthesizing property of bacteria is an effective tool for screening beneficial microorganisms. Inoculation of IAA-producing bacteria can promote lateral roots and root hair formation (Mohite 2013) and can improve plant's tolerance to salinity and stress (Kang et al. 2019).

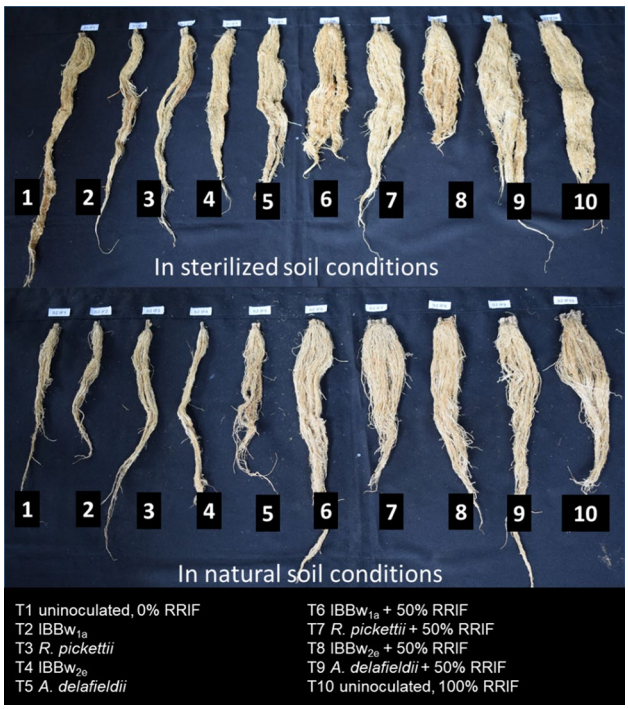

Fig. 3 Roots of upland rice (PSB Rc23) in sterilized and natural soil conditions as affected by inoculation and fertilization treatments

Phosphorus is one of the essential plant nutrients, which plays an important role in growth and development of crops (Selim 2018). Frequently, this mineral nutrient is present in relatively insoluble forms, hence considered as a limiting nutrient on plant growth (Bünemann et al. 2010). In this study, seven bacterial isolates have been found to have the capacity to solubilize tricalcium phosphate and can be considered phosphate solubilizing bacteria (PSB). Similar studies by Cruz and Paterno (2014) and Tripti and Anshumali (2012) showed the same results signifying PSB suitability as plant growth stimulant particularly in phosphorus-deficient soils. These PSB are known to have the ability synthesize organic acids as verified by previous studies (Purwaningsih et al. 2019; Rasul et al. 2019; Sarker et al. 2014). The results further indicate the commercial prospect of these isolates to be developed as biofertilizer for improving the P nutrition of the crop (Khan et al. 2014). The use of PSB inoculants reported to have simultaneously increased phosphorus uptake and yield of crops. Strains of the genera *Pseudomonas*, *Bacillus,* and *Rhizobium* are among the most powerful phosphate solubilizers (Rodriguez and Fraga 1999).

Plant growth promoting rhizobacteria have also been documented to have the ability to produce ACC deaminase. Production of this enzyme has an added benefit in host plants by reducing the level of stress-inducing ethylene (Karthikeyan et al. 2012). The results of the present study have found out that upland rice rhizosphere is a good source of ACC deaminase-producing bacteria as shown by most of the isolates that tested positive of the assay. The compound ACC is a precursor to plant ethylene levels, and ACC deaminase enzyme can limit ACC's availability by hydrolyzing ACC into ammonia and α-ketobutyrate (Glick 2005). This mechanism has been reported in several studies involving rhizobacteria, such as *Streptomyces venezuelae* (Yoolong et al.

Table 7 Effect of inoculation and fertilization on grain yield (g plant^{-1}), and number of productive and unproductive tiller of upland rice in sterilized and natural soil conditions

Inoculation and fertilization treatment	Grain yield		Mean	Productive tiller		Mean	Unproductive tiller		Mean
	Soil treatment			Soil treatment			Soil treatment		
	Sterilized	Natural		Sterilized	Natural		Sterilized	Natural	
1 Uninoculated, 0% RRIF	3.29	2.64	2.97^{bcde}	2	2	1.9^{bcde}	0	0	0.1
2 IBBw$_{1a}$	1.60	1.32	1.46^{e}	1	1	1.0^{e}	0	1	0.5
3 *R. pickettii*	2.33	1.39	1.86^{de}	1	1	1.3^{de}	1	0	0.3
4 IBBw$_{2e}$	3.57	1.43	2.50^{bcde}	2	1	1.5^{de}	0	0	0.3
5 *A. delafieldii*	2.84	1.62	2.23^{cde}	2	1	1.8^{cde}	1	1	0.6
6 IBBw$_{1a}$ + 50% RRIF	4.43	4.47	4.45^{bc}	3	2	2.5^{bcd}	0	1	0.5
7 *R. pickettii* + 50% RRIF	5.14	3.27	4.21^{bcd}	3	3	3.0^{bc}	0	0	0.3
8 IBBw$_{2e}$ + 50% RRIF	3.70	4.57	4.14^{bcd}	2	4	3.0^{bc}	1	1	0.9
9 *A. delafieldii* + 50% RRIF	5.07	4.62	4.84^{ab}	4	3	3.3^{ab}	1	1	0.6
10 Uninoculated, 100% RRIF	9.19	5.50	7.35^{a}	5	4	4.5^{a}	1	0	0.4
Mean	4.12^{a}	3.08^{b}		2.4	2.3		0.5	0.4	

Means followed by a common letter are not significantly different at 5% level, HSD

Table 8 Effect of inoculation and fertilization on number of filled spikelets and 100-grain weight of upland rice in sterilized and natural soil conditions

Inoculation and fertilization treatment	Number of filled spikelets		Mean	100-Grain weight		Mean
	Soil treatment			Soil treatment		
	Sterilized	Natural		Sterilized	Natural	
1 Uninoculated, 0% RRIF	70	45	57	2.84	2.92	2.88
2 $IBBw_{1a}$	47	36	42	2.49	2.58	2.54
3 *R. pickettii*	58	45	51	3.08	3.06	3.07
4 $IBBw_{2e}$	74	43	58	2.87	2.27	2.57
5 *A. delafieldii*	55	40	47	3.04	2.12	2.58
6 $IBBw_{1a}$ + 50% RRIF	59	64	61	2.92	2.70	2.81
7 *R. pickettii* + 50% RRIF	53	42	47	2.77	3.37	3.01
8 $IBBw_{2e}$ + 50% RRIF	45	40	42	2.48	2.80	2.64
9 *A. delafieldii* + 50% RRIF	48	53	50	2.73	3.86	3.29
10 Uninoculated, 100% RRIF	69	51	60	2.69	2.83	2.70
Mean	58[a]	46[b]		2.79	2.82	

Means followed by a common letter are not significantly different at 5% level, HSD

2019), *Streptomyces manipurensis* (Tamreiha et al. 2019), and *Burkholderia* sp. (Sarkar et al. 2018). The production of ACC deaminase hydrolytic enzyme can be a useful tool to mitigate plant stress caused by adverse environmental conditions (Chandra et al. 2018). Plant growth is often subjected to ethylene-producing stress; thus, by lowering the level of ACC in the affected plant, the amount of ethylene synthesis is limited as well as its damage (Soleimani et al. 2018).

The production of siderophores is also one of the important characteristics of rhizobacteria. Siderophore-producing bacteria help plant to compete against other microorganisms under iron-limited conditions, giving greater root colonization in plant tissues (Varma and Chincholkar 2007). The current study revealed the siderophore-producing capacity of 12 out of 25 tested isolates. The role of siderophore in plant tissues is that they bind to available form of iron in the rhizosphere (Fe^{3+}) making it unavailable to plant pathogens and thereby protecting crop health (Barton and Hemming 2012). This has been recently documented in the studies of Wozniak et al. (2019) and Tamreiha et al. (2019). Aside from plant protection, siderophores also have role in plant growth promotion. Linu et al. (2019) reported that inoculation of siderophore-producing bacteria *Pseudomonas aeruginosa* can significantly enhance chili growth under greenhouse conditions. Priyanka et al. (2017) also proved the crop specific growth-promoting trait of siderophore *Pseudomonas* spp. on wheat, chickpea and bottlegourd, and rice.

Rhizobacteria are also reported to have the capacity to hydrolyze starch. Starch hydrolysis is mainly due to the catalytic activity of both acids and enzymes secreted by the bacteria (Tate 2000). The results of this study have interestingly found out only one positive for starch hydrolysis among 25 isolates. The appearance of a halo zone around the bacterial growth has confirmed this activity. This isolate is a potential plant growth stimulant. It is known that starch molecules are too large to enter the bacterial cell membrane, so starch-hydrolyzing bacteria excrete exoenzymes, such as α-amylase and oligo-1,6-glucosidase, which split starch into smaller fragments that are of metabolic value to bacteria (Kharwar et al. 2014) and this breaks the glycosidic linkages between sugar subunits. Evangelista et al. (2017) likewise found out only few starch-hydrolyzing bacteria (five out of 55 isolates) in rice root samples. The effects of starch-hydrolyzing bacteria on crops were also studied by Gusain et al. (2015) and Pandey et al. (2013), showing beneficial effects on plant biomass and nutrient uptake. Sudan et al. (2018) also argued that starch-hydrolyzing bacteria have the potential for industrial applications in various crops, such as rice, wheat, and corn.

Inoculation Effects on Upland Rice

PGPR can influence rice growth under screenhouse conditions (Cruz et al. 2015; Cruz and Cadiente 2016; Santos and Cruz 2017). However, until now, no information was available about the effects on crop growth and productivity, considering the influence of soil sterilization and varying recommended fertilizer rates. As research today are aimed toward improving upland rice productivity using environment-friendly approaches, the results of this study contribute to this growing body of literature by exploring on this gap. This study brings promising data on PGPR isolates which showed comparable growth performance. Despite not at all statistically significant, this can be a better fertilizer-saving technology specially for upland rice areas which are constrained with nutrient acquisition.

The general observation that superior results are obtained with sterilized soil relative to natural soil is an indication

that isolates introduced in the soil have survived in the absence of competing indigenous microorganisms, like inherent protozoans, which could possibly ingest soil bacteria (Trevors 1996). Although no quantification was done as to bacterial survival, it is evident that soil sterilization process was effective in promoting positive host–PGPR interactions. Selected bacterial isolates in this case had greater capacity of expression of its potential as growth promoter since possible antagonistic activity against plant pathogens was inhibited (Pérez-Montaño et al. 2014). Additionally, the use of sterilization may have caused the elimination of denitrifying bacteria causing lesser loss of nitrogen. Sterilized soil gave superior results due to control of denitrifying microbes, leading to greater nitrogen available for rice than being liberated into the atmosphere (Rosenblueth et al. 2018). On the other hand, reduction of growths in natural soil is probably a result of certain microflora working as root pathogens which competed at some point with the inoculant microorganisms and other indigenous soil community (Gamliel and van Bruggen 2016). This can be seen in terms of height and oven-dry weight of both shoots and roots, P and K uptake, number of filled spikelets, and grain yield.

There were no significant effects observed with bacterial inoculation and fertilization. However, comparable growth in relation to 100% RRIF as a result of combined bacterial inoculation and fertilization (*A. delafieldii* + 50% RRIF) was obtained in most parameters, particularly in nutrient content, dry matter weights, and grain yield. This effect suggests a consistent evidence for synergistic effect of such combined application. This means that *A. delafieldii* is of great potential in reducing farmers' dependence on chemical fertilizers by 50%. This can be partly attributed to phytohormone production by bacteria. Pérez-Montaño et al. (2014) reported that PGPR in rice were able to produce plant hormones and other abilities (i.e., N_2 fixation and P solubilization). These characteristics help enhance nutrient uptake by host plants, biosynthesis of various metabolites, and resistance to stressful conditions, among others. This further revealed the beneficial effect of inoculation as function of improved root development. Indole-3-acetic acid production by PGPR stimulates root tissue development by increasing capacity of root system to provide nutrients and water required for above-ground biomass function (Tate 2000). In screenhouse experiments, Cruz et al. (2014) showed enhanced root fresh weight in upland rice with bacterial inoculation.

In summary, growth under sterilized soil showed superior results relative to natural soil as antagonistic activities against plant pathogens and denitrifying microbes were inhibited. The results further suggest that there is a synergistic relationship between *A. delafieldii* and half RRIF. Such characteristics are common among plant growth-promoting rhizobacteria. It is known that *A. delafieldii* can produce ACC deaminase in vitro. The role of ACC deaminase in decreasing ethylene levels by enzymatic hydrolysis of ACC into α-ketobutyrate and ammonia has been documented as one of the major mechanisms of PGPR in promoting root and plant growth. ACC deaminase-producing bacteria can protect stressed plants from some of deleterious effects of stress ethylene (Glick 2005). In addition, the application of inorganic fertilizer increased the fertility of soil and influenced its productivity. The use of *A. delafieldii* in combination with half RRIF can promote comparable growth and productivity while, at the same time, saving inorganic fertilizer. This has advantages in reducing crop production costs and environmental pollution, as well.

Conclusion

The present study has found out that upland rice-associated rhizobacteria produce growth-promoting compounds that may stimulate and enhance plant growth. The screenhouse experiment revealed that *A. delafieldii* was relatively efficient in enhancing upland rice growth under screenhouse conditions. This isolate when combined with 50% RRIF can obtain comparable growth promotion relative to full RRIF in terms of root fresh weight, shoot and root oven-dry weights, plant height, productive tiller count, grain yield, and NPK uptake. This suggests that it is possible to obtain comparable yields of upland rice with the complimentary inoculation of *A. delafieldii* and half fertilizer recommendation rate. Hence, farmers' dependence on chemical fertilizer can be reduced. However, it is recommended to further assess the selected bacterial isolates by confirming their effectiveness as microbial inoculants in actual field conditions.

Acknowledgements The authors would like to acknowledge the Department of Agriculture—Philippine Rice Research Institute (DA-PhilRice), Central Luzon State University (CLSU), and the Department of Science and Technology (DOST), Accelerated Science and Technology Human Resource Development Program—National Science Consortium (ASTHRDP-NSC) for the materials and facilities utilized in this work. The King Mongkut's Institute of Technology Ladkrabang (KMITL) is also acknowledged for its access to scholarly articles that are reviewed in this study.

Compliance with Ethical Standards

Conflict of interest There is no conflict of interest.

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