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Enhancement of growth and yield of upland rice (*Oryza sativa* L.) var. NSIC Rc 192 by actinomycetes

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A study was conducted to evaluate the effectiveness of actinomycetes on the growth and yield of upland rice. Five previously screened actinomycetes for its growth promoting abilities: YB6y, AVermi3, AVermi7, NB_1, and NB_3 were tested under screenhouse condition at the Agricultual Systems Cluster, UPLB. They produced both indole-3-acetic acid (IAA) and 1-Aminocyclopropane-1-carboxylate (ACC) deaminase and were able to increased root dry weight of upland rice under growth room condition. In this study, inoculation with NB_1, AVermi7, YB6y and NB_3 in combination with full rate of fertilization significantly increased P uptake by 80 to 136% over the uninoculated control. At full rate of fertilization, Inoculation with NB_3 and AVermi7 significantly increased grain yield by 62% and 48% respectively, relative to uninoculated treatment. The significant increase in grain yield by NB_3 and AVermi7 demonstrate the potential of these actinomycetes as plant growth-promoting inoculant for upland rice. However, field assessment is recommended to determine the effect of biotic and abiotic stresses in the performance of promising actinomycetes.

Keywords: actinomycetes, plant growth, phosphorus uptake, grain yield, upland rice

Introduction

Majority of microbial populations present in soil are actinomycetes. Most actinomycete species are mesophilic and grow optimally at a pH near neutrality. They belong to an extensive and diverse group of gram-positive, aerobic, mycelia bacteria that play important ecological roles in soil nutrient cycling (Franco-Correa *et al*., 2010; El-Tarabily, 2006).

Actinomycetes are the most economically and biotechnologically valuable prokaryotes. They are responsible for the production of half of the

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discovered bioactive secondary metabolites. More than 50 genera have been used in human, veterinary medicine, agriculture, and industry. One of the genera of actinomycete is *Streptomyces. Streptomyces,* generally accounting for an abundant percentage of the soil microflora, is particularly effective colonizers of plant root systems and is able to endure unfavorable growth conditions by forming spores (Alexander, 1977).

According to Kanti (2005), actinomycetes produce some active cellulase enzymes which degrade cellulose. Actinomycetes can enhance plant growth by producing a promoter such as indole 3-acetic acid (IAA) and produce siderophores to help root.

Recent advances on the application of actinomycetes in cereal crop such as wheat was conducted. Aldesuquy *et al*. (1998) studied the effect of streptomycete culture filtrates on the growth of wheat plants. Shoot fresh mass, dry mass, length, and diameter, significantly increased with certain strains at varying sample times. *S. olivaceoviridis* had a pronounced effect on yield components (spikelet number, spike length, and fresh and dry mass of the developing grain) of wheat plants. The culture filtrates of all three strains appeared to enhance the growth and crop yield of wheat plants (Aldesuquy *et al*. 1998).

In greenhouse experiments showed that inoculation of rice with PGPB increased chlorophyll, leaf area, tiller number, plant height, root shoot biomass, and subsequently grain yield in rice. It was observed that PGPB contributed significant amount of nitrogen to increase rice plant biomass and improve nitrogen status of soil. It is possible that certain rhizobacteria, including the actinomycetes, may act as plant growth enhancers.

The general objective of the study is to evaluate the effectiveness of actinomycetes in enhancing the growth and yield of upland rice under screenhouse condition.

Materials and methods

Actinomycete isolates were tested under screenhouse condition at the Agricultural Systems Cluster (ASC), UPLB to study their effects on rice root and shoot growth, NPK uptake, and grain yield. NSIC Rc192 and Lipa clay loam was the variety and soil that was used, respectively. Bulk soil samples for the screen house experiment were collected from UPLB Experiment Station.

The study had 18 treatment combinations (six inoculation treatments x three fertilizer recommendations). The details of the variables are given below:
Rates of Fertilization (F) –mainplot

F_1 = no fertilizer addition

F_2 = ½ recommended rate of fertilizer

F_3 = recommended rate of fertilizer

Isolates (I) - subplot

I_0 = uninoculated
I_1 = YB6y
I_2 = AVermi3
I_3 = AVermi7
I_4 = NB_1
I_5 = NB_3

Selection of Isolates

Five isolates were selected on the basis of their growth promoting activities such as their IAA and ACC deaminase production. Growth rate was also considered as basis of selection. Isolates that were able to grow within 3 to 4 days were selected.

For the quantitative determination of IAA production, isolates were grown in Arginine-Glycerol-Salt (AGS) broth which consists of the following: Arginine Monohydrochloride, 1.0 g/l; Glycerol, 10 ml/l; K_2HPO_4, 1.0 g/l; NaCl, 1.0 g/l; AGS stock solution, 1.0 ml/l and $CaCO_3$, 1.0 g/l supplemented with tryptophan. After 7 days of incubation, the cultures were centrifuged at 5000 rpm for 15 minutes. One millilitre of the supernatant was mixed with 2 ml of Salkowski reagent and the appearance of a pink color indicated IAA production. The absorbance was measured at 530 nm and the quantity of IAA produced was estimated against the IAA standard.

Experimental Design

The pot experiment was laid out in a 6 x 3 factorial in split-split plot design with 4 replicates. Each replicate has 2 subsamples.

Chemical Characterization of Soils

Soils were analyzed for the following: pH, OM, total N, available P, and exchangeable K following Standard procedures (PCARRD, 1980).

Potting of Soils

Experiments were conducted in pots containing 5 kg air-dried soil. Each pot was labelled with the corresponding treatment.

Seed Surface Sterilization and Planting

Seeds were soaked in concentrated H_2SO_4 for 30 seconds and washed with sterile distilled water seven times to remove H_2SO_4. Surface sterilized seeds were pre-soaked in a seven day old culture broth for 30 minutes. Treated seeds were later planted into the pots by dibbling.

Inoculation

Actinomycete isolates were the source of the inoculum. Fifty mL of a seven day old broth culture was inoculated to the soil at the critical stages of rice (early growth stage, active tillering, and panicle initiation).

Fertilizer Application

N, P_2O_5, and K_2O were applied in a form of complete fertilizer (14-14-14), superphosphate (0-18-0), solophos (0-0-60), and urea (46-0-0) with the following rates: 0 rate, ½ rate (71.5 Kg/ha complete fertilizer, 55.5 Kg/ha superphosphate), and full rate (143 Kg/ha complete fertilizer, 111 Kg/ha superphosphate) on the first application (14 days after sowing) Second application was on 30 days after sowing with the following rates: 0 rate, ½ rate (21.75 Kg/ha urea, 16.7 Kg/ha solophos), and full rate (43.g Kg/ha urea, 33.33 Kg/ha solophos). Last application was on 45 days after sowing with the follwing rates: 0 rate, ½ rate (21.7 Kg/ha urea), and full rate (43.4 Kg/ha urea).

Collection of Plant Samples and Data Gathered

Plant samples were collected at 41, 76, and at 106 days after sowing (DAS). **S**hoot and root fresh and dry weights were measured in samples taken from two pots per replication. Grain yield was also determined.

Plant tissue analysis

The plant samples were oven dried at 70°C for 24 hours. All data on dry matter yields were used as inputs for NPK uptake determination. The percentages of nitrogen, phosphorus, and potassium in plant tissues were determined in samples collected at 76 DAS, during the reproductive stage of rice. The NPK uptake was calculated by multiplying the percentage of each nutrient (NPK) by the dry matter yield in gram.

Data analysis

All data gathered were organized into tables in accordance with the MS Excel Spreadsheet and then the data was exported into SAS software for Statistical Analysis. In assessing significant differences among treatments/treatment combinations, Duncan's Multiple Range Test (DMRT) was used.

Results and discussions

Chemical Characteristics of the Soil Used

The chemical characteristics of the soil used are the following: pH, 5.8; organic matter, 4.21%; phosphorus, 15 ppm, and potassium, 3.18 me/100g soil. Most soil actinomycetes grow in the pH range of 5.0-9.0 with an optimum close to neutrality. The abundance and activity of actinomycetes in the soil are affected by the availability of nutrients, nature and abundance of organic matter, salinity, relative moisture content, temperature, pH, and soil vegetation (El-Tarabily, 2006). High population of actinomycetes is also found in soils rich in organic matter.

NPK Uptake of Upland Rice

Table 6 shows the interaction of rates of fertilization and inoculation on phosphorus uptake of upland rice (shoots) at 76 DAS. Full rate of fertilization in combination with any of the following: YB6y, NB_1, NB_3, or AVermi7, significantly improved shoot phosphorus uptake. Within isolates, the addition of full fertilizer enhanced the performance of NB_3 and YB6y in terms of P uptake, whereas the application of half fertilizer rate and full fertilizer rate improved the performance of AVermi7. Highest shoot phosphorus uptake (1.32 g/pot) was obtained with NB_3 inoculation at full rate of fertilization. The lowest P uptake at full fertilization was observed in the uninoculated treatment. Inoculation with any of the selected isolates at full rate of fertilization increased shoot P uptake ranging from 45% to 136%.

Table 6. Interaction of rates of fertilization and inoculation on phosphorus uptake (g/pot) of upland rice (shoots) at 76 days after sowing (DAS)

	FERTILIZER		
	Zero	1/2 RRCF	Full RRCF
Uninoculated	0.79 A^{a}	0.84 AB^{a}	0.56 C^{a}
YB6y	0.45 A^{b}	0.80 AB^{ab}	1.25 AB^{a}
AVermi3	0.43 A^{a}	0.49 AB^{a}	0.81 BC^{a}
AVermi7	0.41 A^{b}	1.03 A^{a}	1.09 AB^{a}
NB_1	0.41 A^{b}	0.58 AB^{ab}	1.01 AB^{a}
NB_3	0.59 A^{b}	0.74 AB^{b}	1.32 A^{a}

*Capital letters represent comparison within a column while small letters represent mean comparison within a row. Means followed by a common letter are not significantly different at 5% level by DMRT; RRCF Recommended Rate of Complete Fertilizer.

In this study, both YB6y and NB_3 are phosphate solubilizers. Related studies show that phosphate solubilizing ability was widely exhibited by *Streptomyces* spp. Franco-Correa *et al*. (2010) observed that most of the actinomycete strains have the ability of solubilizing sparingly available inorganic P sources or mineralizing some P from the organic P sources in soil. Phosphate solubilizing ability of microorganisms is considered as one of the most important traits associated with plant P nutrition. Mechanism of mineral phosphate solubilization is assoociated with the release of low molecular weight organic acids, which through their hydroxyl and carboxyl groups chelate the cations bound to phosphate, thereby converting it into soluble forms (Kannapiran and Ramkumar, 2011). Phosphate-solubilizing bacteria often causes soil acidification, playing a key role in phosphorus solubilization. Therefore, they are considered the important solubilizers of insoluble inorganic phosphate (Zhu *et al*., 2011).

Adesemoye *et al*. (2008) observed that fertilizer treatment affect phosphorus uptake, but inoculant alone had no significant effect in corn tissue. Related studies in corn showed significant interaction effect of inoculants and fertilizer on N, P, and K uptake per gram of silage tissue. Application of inoculants have potential as inputs in integrated nutrient management systems to help reduce build up, leaching, or runoff of nutrients from fields. Inoculants promoted plant growth and yield.

Adesemoye *et al*. (2008) observed that there was a significant interaction between inoculation and fertilizer in growth and yield of corn. Height of plants on plots that received inoculants within plots of ammonium nitrate was greater than plants that received no inoculants. Analysis showed that fertilization and inoculation affected corn yields (including grain and silage) significantly. It is interesting that despite the drought, inoculants still produced better yield.

In terms of nitrogen and potassium uptake, there was no significant interaction between rate of fertilization and inoculation.

Grain Yield

Table 7 shows the mean effects of rates of fertilization and inoculation on grain yield of upland rice. Inoculation with NB_3 and AVermi7 significantly increased grain yield by 62% and 48% respectively, relative to uninoculated treatment. On the other hand, mean grain yield on the uninoculated treatment was not significantly different with the mean grain yield obtained in the YB6y and AVermi3 inoculation. Alizadeh *et al*. (2012) reported that in China, many studies have reported yield increased after bacterial inoculation for several crops; wheat (8.5-16%), rice (8.1-16%), maize (6-11%), beans (7-16%), sugar beet (15-20%), sorghum (5-10%), sweet potato (15-19%), linen (6-13%), oily turnip (16-18%), peanut (10-15%), and vegetables (13-35%). The application of rhizosphere competent, ACC deaminase-producing and IAA-producing actinomycetes also enhanced the growth and development of tomato under greenhouse condition (El-Tarabily, 2008). In white clover, Franco-Correa *et al*. (2010) observed increases in shoot biomass at four months and six months after planting due to ACC deaminase-producing actinomycetes.

Actinomycete isolates in this study produced both IAA and ACC deaminase. Inoculation with AVermi7 and NB_3 significantly improved mean grain yield of upland rice. This performance shows the advantage conferred to selected isolates due to their ability to produce both IAA and ACC deaminase. AVermi7 and NB_3 may increase plant growth, shoot phosphorus uptake, and yield of upland rice. Both YB6y and NB_3 are phosphate solubilizers.

Table 7. Effect of rates of fertilization and inoculation on grain yield (g/pot) of upland rice

	FERTILIZER			
	Zero	1/2 RRCF	Full RRCF	*Mean*
Uninoculated	2.14	4.49	3.90	*3.51 c*
YB6y	2.42	4.46	4.95	*3.94 c*
AVermi3	1.92	4.09	5.18	*3.73 c*
AVermi7	3.17	5.30	7.16	*5.21 ab*
NB_1	2.58	4.46	5.99	*4.34 bc*
NB_3	2.98	5.21	8.85	*5.68 a*
Mean	2.53 c	4.67 b	6.01 a	

* Means followed by a common letter are not significantly different at 5% level by DMRT; RRCF Recommended Rate of Complete Fertilizer.

Experiments conducted at BIOTECH showed that plants inoculated with PGPB increased the yield of ampalaya and lettuce by 90% and 40%, respectively. PGPB also caused *Dendrobium* to flower early, and *Mussaenda* and *Hibiscus* to grow heavier and longer roots. It was also found effective in the rooting of blackpepper cuttings and as a substitute for commercial rooting formulations (UPLB Horizon, 2006). The plant growth-promoting bacteria live in association with plants and improve plant growth by nitrogen fixation, growth hormone production.

Significant differences on grain yield were evident at different rates of fertilization. The application of full recommended fertilizer rate significantly improved the grain yield of upland rice regardless of inoculation. Full fertilizer rate application increased grain yield of upland rice by 138%, while only 85% increase in grain yield was attained with half fertilization rate.

Shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SODW), and root dry weight (RODW) were significantly correlated with the grain yield as shown in Table 8.

Table 8. Correlation analysis of shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SODW), and root dry weight (RODW) with grain yield at 41, 76, and 106 days after sowing (DAS)

	At 41 DAS	**At 76 DAS**	**At 106 DAS**
GRAIN YIELD	Sfw	Sfw	Sfw
	0.49687	0.65101	0.66265
	0.0001	*<.0001*	*<.0001*
	Rfw	Rfw	Rfw
	0.42517	0.59397	0.37602
	0.0014	*<.0001*	*0.0051*
	Sodw	Sodw	Sodw
	0.45661	0.57965	0.45411
	0.0005	*<.0001*	*0.0006*
	Rodw	Rodw	Rodw
	0.14613	0.43081	0.41496
	0.2917	*0.0011*	*0.0018*

Conclusion

Our findings show that actinomycete is a potential microbial inoculant as shown by their effects on plant growth, phosphorus uptake, and grain yield. However, field assessment of the promising actinomycetes is needed where

some factors affecting upland rice production such as weeds, decreased or excessive supply of nutrients, and moisture stress are present.

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