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Promotion of upland rice growth by actinomycetes under growth room condition

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A study was conducted to evaluate the effectiveness of actinomycetes on the growth of upland rice.Five actinomycete isolates were tested under growth room condition at the National Institute of Molecular Biology and Biotechnology (BIOTECH at UP Los Baños) to study their effects on rice root and shoot growth. Rhizosphere competence was also determined at 14 and at 30 days after sowing by comparing the cell population in the rhizosphere soil (R) with that in the non-rhizosphere soil (S) expressed as R: S ratio. In this study, actinomycetes increased root dry weight of upland rice by 24 to 71% at 14 days after sowing (DAS). Highest root dry weight (0.36g/magenta jar) was obtained with YB6y inoculation. On the other hand, inoculation did not significantly improve shoot and root fresh weight, and shoot dry weight at 14 DAS. All five isolates were rhizosphere competent. Rhizosphere competence was assessed by comparing the cell population in the rhizosphere soil (R) with that in the non-rhizosphere soil (S) expressed as R:S ratio. The actinomycete isolates colonized the roots of upland rice with population densities ranging from 5.9 x 10^5 to 1.2 x 10^7 CFU g^{-1} rhizosphere soil with R:S ratios of 0.8 to 1.1 at 14 days after sowing (DAS). The ability of actinomycetes to colonize the rhizosphere demonstrates their potential as plant growth-promoting inoculant. 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.

Keywords: actinomycetes, rhizosphere competence, R:S ratio

Introduction

At present, there is a low production of upland rice in the Philippines which is approximately 2 t/ha. Upland rice is seeded under dry conditions and depends on rainfall for moisture (De Datta, 1981). Many authorities recommend that upland areas that cannot be economically bunded, or that have sandy soil types, be converted to the growing crops such as maize, sorghum,

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soybeans, or sweet potatoes that have much more drought tolerance than rice (Chandler, 1979).

Weeds, decreased or excessive supply of nutrients, and moisture stress are some factors affecting upland rice production. Water application is the dominant factor affecting the growth and yield of rice as it was evidently clear in its effect on the agronomic responses at all stages of production. Field experiments conducted on upland rice (NERICA 2) relates water use pattern to its growth and yield. Total grain yield of 1.36 t ha^{-1} was attained in the treatment that received water most while the least grain yield of 0.16 t ha^{-1} was recorded in the treatment with least water application (Akinbile et al., 2007).

In recent years, it was recognized that activities in the plant root system and their associated physical and biological environment determine the productivity and quality of crops. The roots are populated by microorganisms which directly or indirectly affect plant growth. Biological processes associated with these microorganisms around the roots can be manipulated, thus, offering opportunity for optimizing crop productivity (Paterno, 2004).

The worldwide efforts in the search of natural products for plant growth promotion and crop protection have progressed significantly. Actinomycetes, especially those belonging to the genus *Streptomyces* sp., appear to be good candidates to find new approaches to control plant diseases (Behal, 2000). The agro-industry shows a marked interest for actinomycetes as a source of agro-active compounds and of biocontrol tools (Behal, 2000; Tanaka and Omura, 1993).

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, 1998). It is possible that certain rhizobacteria, including the actinomycetes, may act as plant growth enhancers.

The objective of the study is to evaluate the effectiveness of actinomycetes in enhancing the growth of upland rice under growth room condition.

Materials and methods

Growth Room Experiment

Five actinomycete isolates were tested under growth room condition at the National Institute of Molecular Biology and Biotechnology (BIOTECH at UP Los Baños) to study their effects on rice root and shoot growth. Rhizosphere competence was also determined at 14 and at 30 days after sowing by comparing the cell population in the rhizosphere soil (R) with that in the non-rhizosphere soil (S) expressed as R: S ratio.

Preparation of the Soil

Bulk sample of Lipa clay loam was collected, air-dried, pulverized, and passed through 2-mm sieve. Two hundred grams of soil was placed in magenta jars and the set-up was sterilized in an autoclave for 1hr. at 121°C for 3 consecutive days.

Seed Surface Sterilization

Rice seeds (cv NSIC Rc192) were soaked in concentrated H_2SO_4 for 30 seconds and washed with sterile distilled water seven times to remove H_2SO_4.

Planting

The surface sterilized seeds were planted in magenta jars containing sterilized soil. Sterilized SNAP (Simple Nutrient Addition Program) solution was used as nutrient source.

Inoculation

Selected isolates were the source of the inoculum. Surface sterilized seeds were pre-soaked in a seven day old culture broth for 30 minutes. A fifty mL inoculant was inoculated to the soil at sowing and at 14 days after sowing.

Measurement of Agronomic Parameters

Shoot and root fresh weight and dry weight were determined at 14 and 30 days after sowing (DAS).

Determination of Bacterial Population in the Rhizosphere and Non-rhizosphere soil

Duplicate samples were used to determine the bacterial population in the rhizosphere and in the non-rhizosphere soil at 14 DAS and at 30 DAS. Non-

rhizosphere soil samples were collected at 1-2 cm distance from the base of the plant, and at 1-2 cm depth. One gram of moist soil sample was diluted with 9 mL sterile water. After shaking the soil suspension, a series of five further 10-fold dilutions was made by transferring 1 mL soil suspension to 9 mL diluent. Aliquots of 0.1 mL of each of the six dilutions were spread on duplicate Arginine-Glycerol-Salt (AGS) agar plates. The colonies were observed after 7 days of incubation at room temperature. The number of bacterial cells per gram dry soil was determined after 7 days of incubation at room temperature.

The rhizosphere soil was collected from soil adhering to roots. Non-adhering soil was allowed to fall to the ground, and then the roots were placed in 9 mL diluent and shaken thoroughly. This soil solution was diluted to make a series of six 10-fold dilutions. Similarly, 0.1 mL of each of the six dilutions was spread on duplicate nutrient agar plates. The colonies were observed after 7 days of incubation at room temperature and expressed as number of colonies. The number of bacterial cells per gram dry soil was determined after 7 days of incubation at room temperature. To determine the oven dry weight, the roots were taken out from the 1st dilution vial, and the soil suspension was evaporated to dryness followed by overnight drying at 105°C.

Results and discussions

Effect of Actinomycete Inoculation on Upland

Rice under Growth Room Conditions

The potential of actinomycetes to enhance growth of upland rice was evaluated in a growth room experiment. Effects on root and shoot growth as well as their rhizosphere competence were studied.

Shoot and root growth:Inoculation with any of the selected isolates significantly increased root dry weight at 14 DAS ranging from 24% to 71% (Table 1). Highest root dry weight (0.36g/magenta jar) was obtained with YB6y inoculation. Figures 1 and 2 show the effect of inoculation on the rooting of upland rice.

Table 1. Effect of inoculation on upland rice growth under growth room condition

	14 DAYS AFTER SOWING				30 DAYS AFTER SOWING			
	Sfw	Rfw	Sodw	Rodw	Sfw	Rfw	Sodw	Rodw
Treatments								
1. Uninoculated	0.77a	2.43a	0.09a	0.21b	1.5a	3.70a	0.30a	0.97a
2. YB6y	0.7a	2.97a	0.09a	0.36a	1.83a	3.33a	0.33a	0.70a
3. AVermi3	0.77a	3.47a	0.09a	0.30a	1.87a	4.17a	0.33a	1.03a

4. AVermi7	0.8a	3.1a	0.10a	0.26a	1.5a	3.87a	0.30a	1.00a
5. NB_1	0.8a	3.1a	0.10a	0.30a	2.03a	3.77a	0.33a	0.83a
6. NB_3	0.8a	3.27a	0.10a	0.33a	2.23a	4.23a	0.37a	0.83a

*Values with the same letter for each growth measurement within a column are not significantly different.
*SFW- shoot fresh weight, RFW- root fresh weight, SODW- shoot oven dry weight, and RODW- root oven dry weight

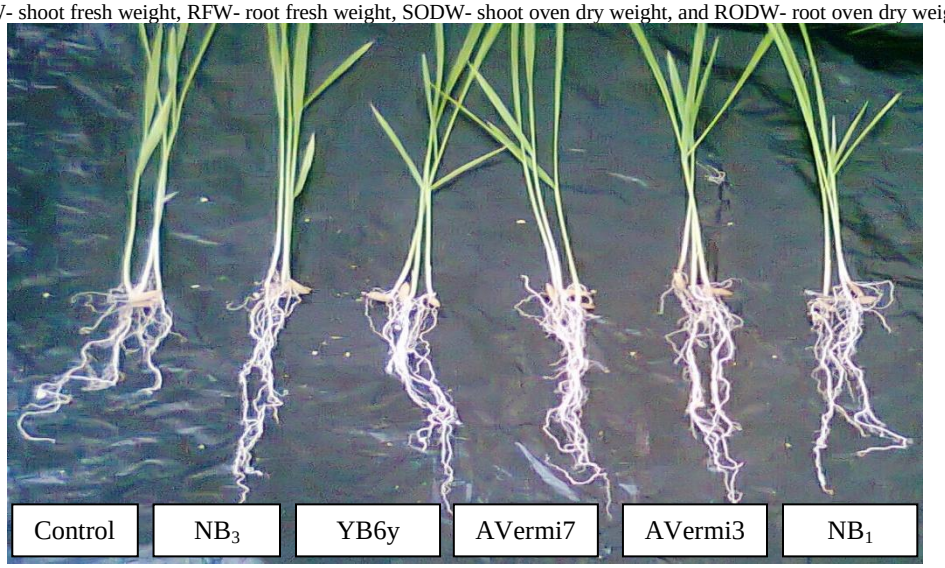

Fig. 1. Upland rice roots as affected by inoculation: control, NB_3, YB6y, AVermi7, AVermi3 and NB_1 at 14 days after sowing.

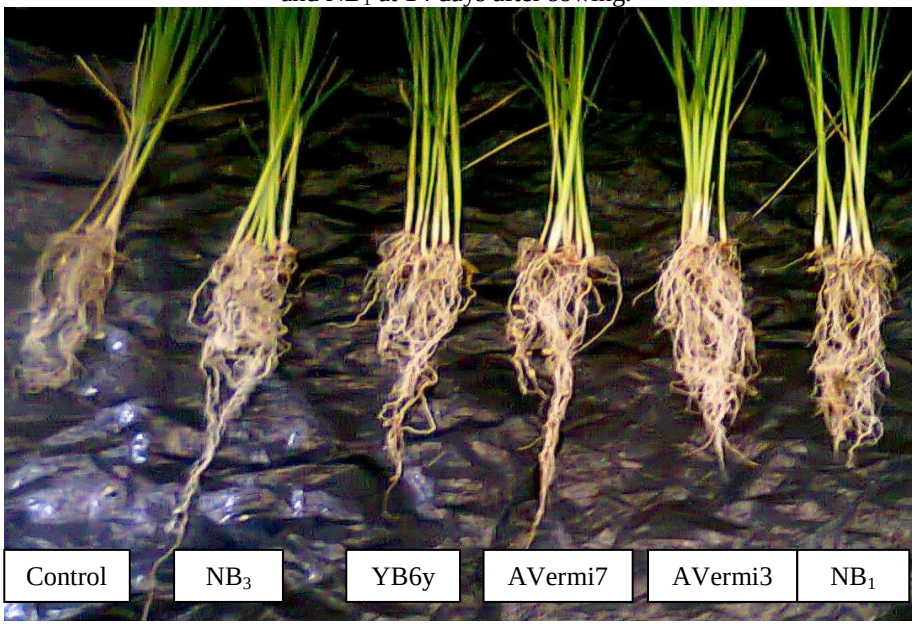

Fig. 2. Upland rice roots as affected by inoculation: control, NB_3, YB6y, AVermi7, AVermi3 and NB_1 at 30 days after sowing.

El-Tarabily (2008) observed that the application of *Streptomyces filipinensis no. 15* promoted growth of tomato. *S. filipinensis no. 15* produced both ACC deaminase and IAA. Itpromoted tomato root dry weight by 65% compared to the control treatment (El-Tarabily, 2008).

In this study, enhanced rooting can be attributed to the ability of the isolates to produce auxin and ACC deaminase. 1-Aminocyclopropane-1-carboxylate (ACC) is an immediate precursor of ethylene in higher plants. ACC deaminase-containing rhizobacteria can increase root growth by lowering endogenous ACC levels (Glick, 2005).

On the other hand, inoculation did not significantly improve shoot and root fresh weight, and shoot dry weight at 14 DAS, although there were 4% and 11% increases in shoot fresh weight and shoot dry weight, respectively, due to inoculation with any of the following: AVermi7, NB_1, and NB_3. Inoculation with any of the selected isolates increased root fresh weight at 14 DAS ranging from 22% to 43%.

Figures 3 and 4 show the growth of upland rice as affected by inoculation with YB6y, AVermi3, AVermi7, NB_1, and NB_3 at 14 and 30 days after sowing (DAS) under growth room conditions.

Rhizosphere competence of selected isolates:Rhizosphere competence was assessed by comparing cell population in the rhizosphere soil (R) with that in the non-rhizosphere soil (S) expressed as R:S ratio.

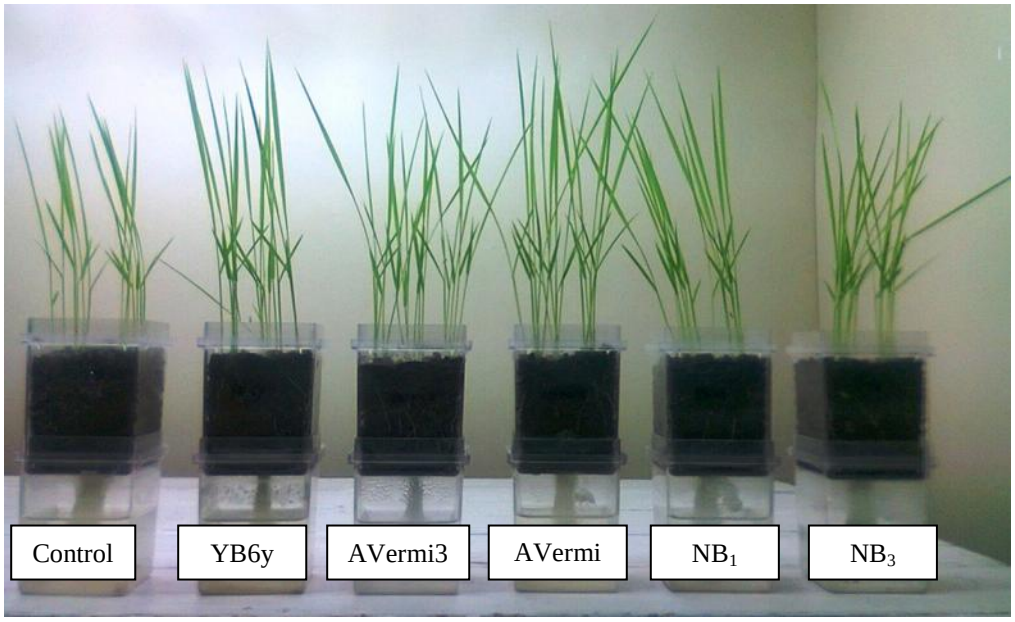

Fig. 3. Upland rice grown in Lipa clay loam as affected by inoculation with YB6y, AVermi3, AVermi7, NB_1, and NB_3 at 14 days after sowing (DAS).

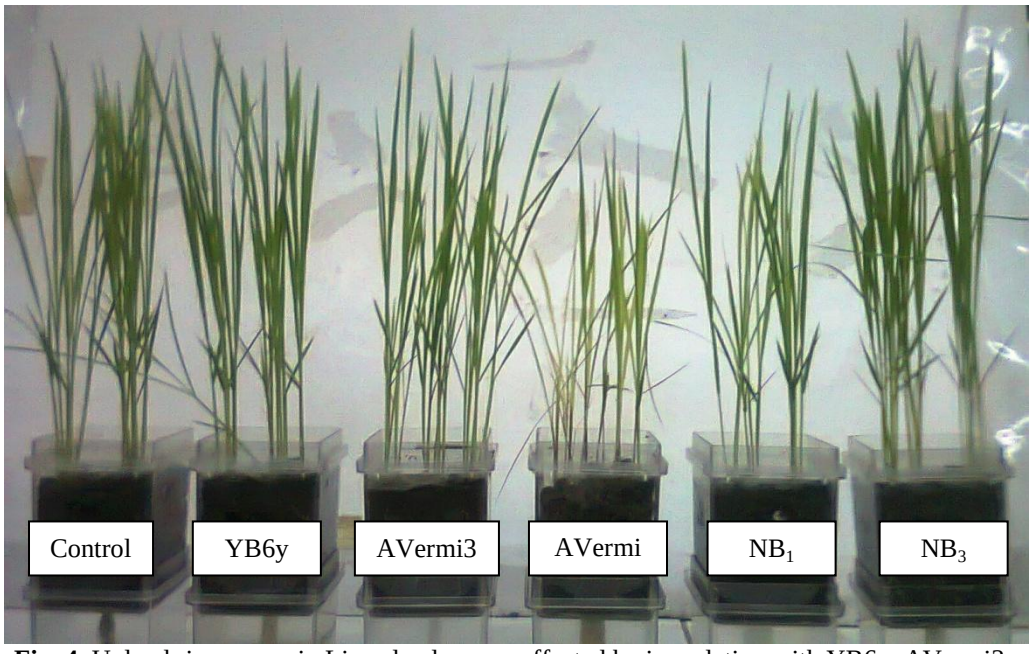

Fig. 4. Upland rice grown in Lipa clay loam as affected by inoculation with YB6y, AVermi3, AVermi7, NB_1, and NB_3 at 30 days after sowing (DAS).

All five isolates were rhizosphere competent. At 14 days after sowing (DAS), the actinomycetes colonized the roots of upland rice with population densities ranging from 5.9×10^5 to 1.2×10^7 CFU g^{-1} rhizosphere soil with R:S ratios of 0.8 to 1.1 at 14 DAS.

At 30 DAS, the resulting R:S ratio in YB6y is 0.96, 1.05 in AVermi3, 0.92 in AVermi7, 0.71 in NB_1, and 1.08 in NB_3 with population densities ranging from 8.1×10^4 to 2.1×10^6 CFU g^{-1} rhizosphere soil.

At 30 DAS, NB_3 population in the rhizosphere increased 100-fold with R:S ratio of 1.08 (Table 2). Bacterial population in the non-rhizosphere soil is lower compared with rhizosphere soil. Microbial population in the rhizosphere decreases as the distance from the root increases. This is due to the increase in nutrient levels in the rhizosphere soil (Thompson et al., 1992).

. The R:S ratio is helpful in determining the ability of PGPB to colonize the rhizosphere. Based on the results, the ability of the test isolates to survive in the rhizosphere and in the non-rhizosphere soil coupled with their ability to

colonize plant root established their persistent effects on plant growth. R: S ratio is directly correlated to the growth of plant (Alexander, 1977).

Rhizosphere competence is essential for enhanced plant growth promoting activity In this study, the application of selected isolates significantly improved root oven dry weight at 14 DAS. One possible reason is that, the selected isolates are rhizosphere competent. El-Tarabily (2008) observed that the application of rhizosphere-competent isolates was more effective in improving plant growth as compared with the non-rhizosphere competent isolate (El-Tarabily, 2008). Plant growth promoting bacteria need to colonize the rhizosphere and the rhizoplane, if they are to effectively influence plant growth, and the use of rhizosphere-competent isolates could ensure the targeted response (El-Tarabily and Sivasithamparam, 2006). Rhizosphere competence confers the microorganisms the required potency to be most effective at the plant root-soil interface where in addition to utilization of exuded compounds, roots can also absorb transformed/cleaved

Table 2. R: S Ratio of actinomycetes at 14 and 30 days after sowing (DAS).

Isolate	**14 DAS**			**30 DAS**		
	Rhizosphere	Non-rhizosphere		Rhizosphere	Non-rhizosphere	
	colony forming unit CFU g^{-1}	colony forming unit CFU g^{-1}	R:S ratio	colony forming unit CFU g^{-1}	colony forming unit CFU g^{-1}	R:S ratio
YB6y	5.9×10^5	6.0×10^6	0.86a	3.7×10^5	6.8×10^5	0.96a
AVermi3	2.1×10^6	3.4×10^5	1.10a	2.01×10^6	1×10^6	1.05a
AVermi7	1.2×10^7	2.8×10^6	1.10a	1.04×10^5	2.75×10^5	0.92a
NB_1	9.1×10^6	5×10^6	1.00a	8.1×10^4	8.57×10^6	0.71a
NB_3	6.8×10^5	1.9×10^7	0.80a	2.1×10^6	7.18×10^5	1.08a

*Meansfollowed by a common letter are not significantly different at 5% level by completely randomized design; R-rhizosphere soil, non-rhizosphere soil-S.Molecules readily. This ensures that activities occur as close to the root surface as possible (El-Tarabily, 2008).

Conclusion

Our findings show that actinomycete is a potential microbial inoculant as shown by their rhizosphere competence and drought-tolerant characteristics. 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.

Acknowledgements

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