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Promotion of Upland Rice (*Oryza sativa* L.) Growth by Endophytic Bacteria Isolated from Nipa Palm (*Nypa fruticans*)

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Nitrogen and phosphorus have been identified as poorly available in the nipa palm ecosystems. Although it represents low nutrient environment, nipa can still grow efficiently and able to sustain high productivity. Possible reason is that symbiotic microbes perform mechanisms that involve conversion of atmospheric nitrogen to ammonium and nitrates that are usable by plants. In this study, five endophytic bacteria: NB_{or1}, *Enterobacter aerogenes*; NB_{or2}, *Raoultella planticola*; NQ_{b2}, *Pectobacterium cypripedii*; NA_{yr1}, *Serratia marcescens*; and NA_{ol2}, *Pantoea agglomerans* were evaluated for their potential in enhancing the growth of upland rice under screenhouse conditions at the Applied Biology Center for the Rice Environment, Philippine Rice Research Institute. These isolates were able to produce multiple plant growth-promoting compounds such as IAA, ACC-deaminase and phosphatase. Inoculation with isolates NB_{or1}, NB_{or2}, and NQ_{b2} significantly increased shoot fresh weight ranging from 73% to 89% relative to the uninoculated control. Similarly, inoculation with NB_{or1} and NQ_{b2} significantly increased root fresh weight by 238.34% and 204.36%, respectively. Highest shoot dry weight (8.91 g/pot) was obtained from NB_{or2} inoculated plants. Inoculation with NB_{or1} significantly increased root length by 30.21% relative to the uninoculated treatment. Moreover, inoculation with NB_{or2} and NA_{ol2} significantly increased root and shoot phosphorus uptake by 94.26% and 102.73%, respectively. The efficacy of the selected endophytic bacteria in enhancing the plant growth under screenhouse conditions suggests their potential as bio-fertilizing agent for upland rice. However, field assessment should be conducted to determine the effectiveness of endophytic nitrogen-fixing bacteria under the presence of biotic and abiotic stresses.

Keywords: Bio-fertilizing agent, endophytic, nipa palm, nitrogen-fixing bacteria, upland rice

Introduction

Nipa palm (*Nypafruticans*) is a mangrove palm that thrives naturally in river estuaries and brackish water environment in which salt and fresh water mingle (Nguyen, 2014). *N.fruticans* is the only species present in the genus

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Nypa. This palm is stem-less with tall erect fronds and underground rhizomatous stem possessing an extensive root system, well suited to resist swift running water (Hossain and Islam, 2015). Despite its usefulness as a large biomass-producing wild plant in mangrove areas, agricultural studies on this palm are still limited because of its uncommon living environment. Previous soil analysis in the habitat of nipa palm revealed that nutrient sources such as phosphorus and nitrogen, along with soil salinity, were poorly available (Sengupta and Chaudhuri, 1991). However, it was observed that the palm can grow efficiently and able to sustain high productivity even under unfavorable soil conditions. Hence, it has been speculated that symbiotic microbes perform unknown mechanisms that contribute in the fertility maintenance of nipa palm. Tang *et al*. (2010) was the first to report regarding endophytic nitrogen-fixing bacteria isolated from nipa palm. He observed that a group of *Burkholderia vietnamiensis* was highly active nitrogen-fixers of nipa palm and suggested that it can function as bio-fertilizing agent.

Hence, this study was conducted to evaluate the effectiveness of endophytic nitrogen-fixing bacteria isolated from nipa palm in enhancing the growth of crops such as rice.

Materials and methods

Five nitrogen-fixing bacteria previously proven to produce plant growth promoting compounds (Table 1) (Cruz *et al*., 2016) were tested under screenhouse conditions at the Applied Biology Center for the Rice Environment (ABCRE), Philippine Rice Research Institute (PhilRice) to determine their effects on shoot and root growth and nutrient uptake of upland rice variety, NSIC Rc192.

Table 1. Summary of ACC deaminase activity, IAA production, phosphate solubilization, N_2-fixation, siderophore production, and starch hydrolysis by the five isolates used in this study (Cruz *et al*., 2016).

Isolate	ACC deaminase	IAA production	Phosphate solubilization	Nitrogen fixation	Siderophore production	Starch hydrolysis
NB_{or1}	+	+	+	+	+	-
NB_{or2}	+	+	+	-	+	+
NQ_{b2}	+	+	+	+	-	-
NA_{yr1}	-	+	+	+	-	+
NA_{ol2}	+	+	+	+	+	-

Experimental design and treatments used

The pots were arranged in a randomized complete block design (RCBD) with 3 replications for each treatment. The experiment consisted of six inoculation treatments (NB_{or1}, $NB_{or2,}$ NQ_{b2}, NA_{yr1}, $NA_{ol2,}$ and uninoculated control).

Seed preparation and planting

Seeds were surface sterilized with concentrated sulfuric acid (H_2SO_4) for 30 seconds then washed with sterile distilled water seven times to rinse off H_2SO_4. Surface sterilized seeds were pre-soaked in a seven-day old culture broth for 30 minutes. After soaking, the seeds were directly planted into properly labeled pots.

Bacterial inoculation

Nitrogen-fixing bacteria was grown on culture broth for seven days. After incubation period, fifty ml of the cultured brothwas inoculated into the soil. Inoculation was done at the critical stages of rice (early growth stage, active tillering, and panicle initiation).

Plant sample collection and data gathered

Destructive sampling was done at 41, 76 and 106 days after sowing (DAS). Plant samples were taken from two pots per replication. Growth parameters such as shoot and root fresh weight, shoot dry weight and root length weremeasured.

Nutrient uptake analysis

The collected plant samples were oven-dried at 70 ºC for 24 hr. To determine the nutrient uptake, all gathered data on dry matter yields were used as inputs. Samples collected at 76 DAS (reproductive stage of rice) were used to determine the percentages of nutrient in plant tissues. Nutrient uptake was calculated by multiplying the percentage of each nutrient by the dry matter yield in gram.

Statistical analysis

All data gathered were subjected to analysis of variance (ANOVA) procedure using SAS 9.1.3 portable. Duncan's Multiple Range Test (DMRT) at $p<0.05$ was used to assess significant differences between treatments.

Results and Discussion

Effect of endophytic bacteria on shoot and root fresh weight and shoot dry weight of upland rice

Inoculation with endophytic bacteria caused significant effects on the shoot and root fresh weight and shoot dry weight of upland rice at 106 DAS (Table 2). Inoculation with isolates NB_{or1}, NB_{or2}, and NQ_{b2} significantly increased the shoot fresh weight by 73% to 89% relative to the uninoculated control. Likewise, inoculation with NB_{or1} and NQ_{b2} significantly increased root fresh weight by 238.34% and 204.36%, respectively. Highest shoot dry weight (8.91 g/pot) was obtained from NB_{or2} inoculated plants while lowest (3.64 g/pot) was obtained from uninoculated treatment. Moreover, inoculation with any of the selected isolates increased shoot dry weight by 40.11% to 119.78%. However, the effects of NA_{yr1} and NA_{ol2} on shoot and root fresh weight and shoot dry weight did not differ significantly from the control.

Table 2. Effects of endophytic bacteria inoculation on shoot and root fresh weight (SFW, RFW) and shoot dry weight (SDW) (g/pot) of upland rice at 106 days after sowing (DAS).

Inoculation	SFW	RFW	SDW
Uninoculated	24.96^{b}	4.59^{b}	3.64^{b}
NB_{or1}	43.19^{a}	15.53^{a}	5.10^{ab}
NB_{or2}	47.28^{a}	10.67^{ab}	8.91^{a}
NQ_{b2}	44.78^{a}	13.97^{a}	8.00^{a}
NA_{yr1}	26.36^{b}	4.41^{b}	6.70^{ab}
NA_{ol2}	33.09^{ab}	8.42^{ab}	6.61^{ab}

*Values with the same letter within a column are not significantly different at *P<0.05* according to DMRT.

An increase in the root surface area and the volume of soil foraged by the root, which leads to an enhanced biomass accumulation, is the most commonly proposed explanation for the beneficial effects of PGPB on plant growth. Also, they have a great impact on nitrogen nutrition by increasing nitrate (NO_3) uptake capacity, indirectly as a consequence of stimulated lateral root development and possibly directly by stimulating NO_3 transport systems (Mantelin and Touraine, 2004).

Recent study reported that endophytic bacteria isolated from pneumatophores of mangrove produces plant growth promoting substances and promotes plant growth. As well, it can solubilize phosphate molecules and fix atmospheric nitrogen (Janarthine and Eganathan, 2012). Similarly, Daivanai *et al*. (2014) reported that bacterial endophytes from mangrove increased the shoot and root length, fresh weight, and total chlorophyll content of rice.

Effect of endophytic bacteria on root length of upland rice

The effect of selected isolates in enhancing the root length of upland rice at 106 DAS are shown in Figure 1. Highest root length (43.10 cm) was exhibited by NB_{or1} inoculated plants. It significantly increased the root length by 30.21% relative to the uninoculated treatment. On the other hand, lowest root length (33.10 cm) was obtained from uninoculated plants. Although inoculation with either of the following endophytic bacteria: NB_{or2}, NQ_{b2}, NA_{yr1}, and NA_{ol2}, increased the root length of upland rice by 2.21-26.89%, statistical analysis revealed that only NB_{or1} inoculation was significantly different from the control.

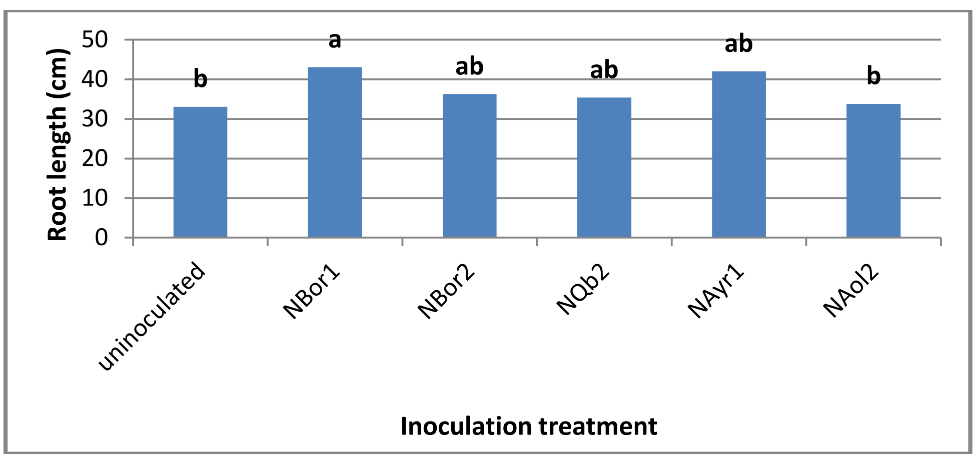

Fig. 1. Root length of upland rice as affected by endophytic bacteria inoculation at 106 DAS.

The positive effects of PGPB on plant growth are always correlated with remarkable changes in root morphology, such as increased lateral root length and root hair number. It is generally assumed that these developmental responses are triggered by phytohormones produced by the bacteria. Recently, it was shown that roots of plants release substances into soil, which in certain measure support colonization and nitrogen fixing activity of bacteria in the rhizosphere of plants. Aside from biologically fixed ammonia, it is known that these microorganisms are able to produce growth-promoting compounds such as IAA, ACC-deaminase and phosphatase that can influence plant growth effectively (Mantelin and Touraine, 2004).

It has long been known that rice can form natural associations with various nitrogen-fixing bacteria that are responsible for supplying the plants with fixed nitrogen (Gyaneshwar *et al*., 2001). In this study, the enhanced growth observed in rice may be attributed to the ability of the endophytic bacteria to fix atmospheric nitrogen as well as produce growth promoting compounds such as IAA and ACC-deaminase. Figure 2 shows the effect of endophytic bacterial inoculation on the growth of upland rice at 106 DAS. According to Mendes *et al*. (2007), endophytes are known to promote plant growth by producing IAA which increases root size and distribution, resulting in greater nutrient absorption from the soil (Li *et al*., 2008). In addition, they also produce ACC-deaminase which regulates the ethylene levels of plants and consequently contribute to a more extensive root system (Arshad *et al*., 2007). However, Glick *et al*. (2007) stated that it is likely that IAA and ACC-deaminase stimulate root growth in a coordinated manner.

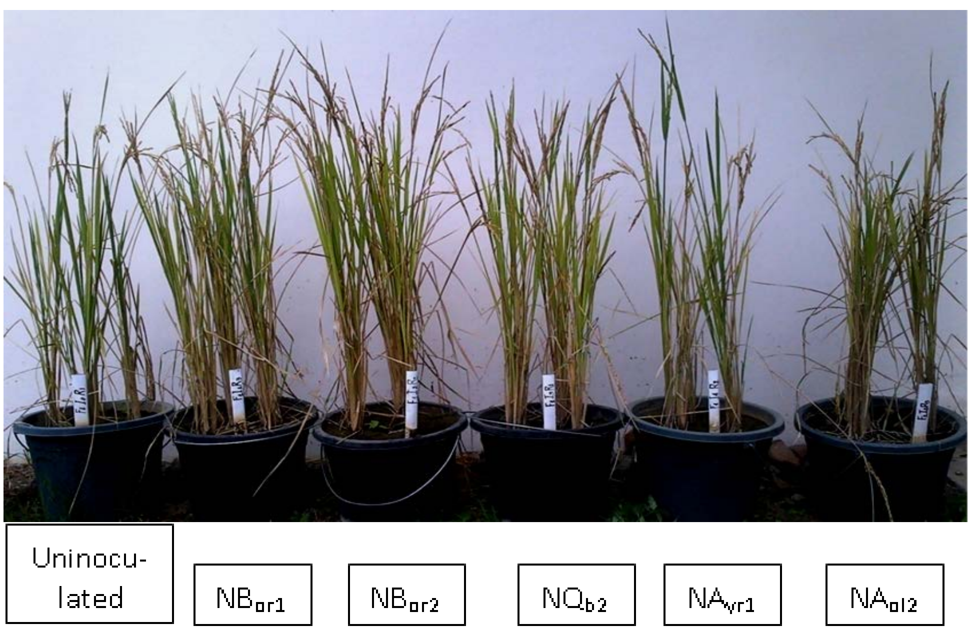

Fig.2. Upland rice as affected by inoculation with NB_{or1}, $NB_{or2,}$ NQ_{b2}, NA_{yr1}, and NA_{ol2} at 106 days after sowing (DAS).

Rashedul *et al*. (2009) demonstrated that the application of PGPB (by means of nitrogen fixation, phosphate solubilization or siderophore production) in greenhouse and field experiments showed a statistically significant increase in seed germination and plant growth, which means a better grain production efficiency.

Effect of endophytic bacteria on nutrient uptake of upland rice

Table 3 shows the effect of endophytic bacterial inoculation on nutrient uptake of upland rice at 76 DAS. Inoculation with NB_{or2} significantly increased root phosphorus uptake by 94.26% relative to the uninoculated treatment. Similarly, inoculation with NA_{ol2} significantly increased shoot P uptake by 102.73%.

Table 3. Effect of endophytic bacteria on nutrient uptake (mg/pot) of upland rice at 76 days after sowing (DAS).

Inoculation	**Root nutrient uptake**			**Shoot nutrient uptake**		
	N	**P**	**K**	**N**	**P**	**K**
Uninoculated	40.11 [a]	4.01 [b]	9.63 [a]	177.41 [a]	44.74 [b]	240.75 [a]
NB_{or1}	34.00 [a]	4.63[ab]	11.43 [a]	230.03 [a]	56.52 [ab]	324.77 [a]
NB_{or2}	67.95 [a]	7.79 [a]	18.05 [a]	271.23 [a]	72.71 [ab]	348.35 [a]
NQ_{b2}	38.00 [a]	4.30 [ab]	10.87 [a]	186.19 [a]	53.62 [ab]	297.84 [a]
NA_{yr1}	54.05 [a]	4.60 [ab]	15.81 [a]	203.71 [a]	53.62 [ab]	267.51 [a]
NA_{ol2}	46.45 [a]	3.95 [b]	15.63 [a]	252.13 [a]	90.70 [a]	372.84 [a]

*Values with the same letter within a column are not significantly different at $p<0.05$ according to DMRT.

In this study, all of the selected isolates are phosphate solubilizers (Table 1). According to Khan *et al*. (2007), phosphate solubilizing microorganisms (PSM) are able toconvert insoluble phosphatic compounds into soluble forms in soil and make them available for plant root uptake. The major aspect of P cycling and nutrient management is to increase the amount of free inorganic ions in soil. Mainly, these PSM facilitates P solubilization by organic acid production, extracellular enzyme production, chelation and exchange reactions (Sharma *et al*. 2013). According to Rodriguez *et al*. (1999), there have been number of reports on plant growth promotion by bacteria that have the ability to solubilize inorganic and/or organic P after their inoculationin soil. Stephen *et al*. (2015) demonstrated that inoculation with phosphate solubilizing bacteria positively increased the phosphorus content and uptake of plants which significantly reflected on the growth and yield of rice crops.

Highest root (67.95 mg/pot) and shoot (271.23 mg/pot) nitrogen uptake were obtained with NB_{or2} and NA_{ol2} inoculation, respectively. On the other hand, lowest root (34.00 mg/pot) and shoot (177.41 mg/pot) N uptake were observed in the NB_{or1} inoculated plants and uninoculated treatment, respectively. In terms of potassium uptake, inoculation with NB_{or2} increased the root K uptake by 87.44% relative to the uninoculated treatment. Likewise, NA_{ol2} inoculation increased the shoot K uptake by 54.87%.

The positive effects of bacterial inoculants on nutrient uptake may be related to the occurrence of PGP traits. Thus, the PGPB-based inoculation technology should be utilized along with appropriate levels of fertilization to achieve maximum benefits in terms of fertilizer savings, nutrient uptake, and plant growth (Souza *et al*., 2016).

Conclusion

The selected endophytic bacteria isolated from nipa palm were found as potential bio-fertilizing agent of rice as shown by their effects on shoot and root fresh weight, shoot dry weight, root length and nutrient uptake of upland rice. However, the effectiveness of the selected isolates under field conditions was not tested in this study. Hence, field assessment is recommended to determine the performance of the selected isolates along with the various factors present in the natural environment such as weather conditions, soil characteristics, and activity of the indigenous microbial flora of the soil.

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References

Arshad M., Saleem M. and Hussain S. (2007). Perspectives of bacterial ACC deaminase in phytoremediation. Trends in Biotechnology 25 (8): 356-62.

Cruz J.A. and Cadiente M.K.M. (2016). Assessment of Potential Plant Growth Promoting Compounds Produced *In Vitro* ByEndophytic Bacteria Associated with Nipa Palm (*Nypafruticans*). Philippine Journal of Crop Science (PJCS) 41 (2): 1-7.

Deivanai S., Bindusara A.S., Prabhakaran G. and Bhore S.J. (2014). Culturable bacterial endophytes isolated from Mangrove tree (Rhizophora apiculata Blume) enhance seedling growth in Rice. Journal of Natural Science, Biology and Medicine 5 (2): 437–444.

Glick B.R., Todorovic B., Czarny J., Cheng Z., Duan J. and McConkey B. (2007). Promotion of plant growth by bacterial ACC deaminase. Critical Reviews in Plant Sciences 26: 227-242.

Gyaneshwar P., James E.K., Mathan N., Reddy P.M., Reinhold-Hurek B. and Ladha J.K. (2001). Endophytic Colonization of Rice by a Diazotrophic Strain of Serratiamarcescens. Journal of Bacteriology 2634–2645.

Hossain M.F. and Islam M.A. (2015). Utilization of Mangrove Forest Plant: Nipa Palm (Nypa fruticans Wurmb.). American Journal of Agriculture and Forestry 3 (4): 156-160.

Janarthine S.R.O. and Eganathan P. (2012). Plant Growth Promoting of Endophytic *Sporosarcinaaquimarina* SjAM16103 Isolated from the Pneumatophores of *Avicennia marina* L. International Journal of Microbiology Article ID 532060, 10 p.

Khan M.S., Zaidi A. and Wani P.A. (2007). Role of phosphate-solubilizing microorganisms in sustainable agriculture - A review. Agronomy for Sustainable Development 27: 29-43.

Li J.H., Wang E.T., Chen W.F. and Chen W.X. (2008). Genetic diversity and potential for promotion of plant growth detected in nodule endophytic bacteria of soybean grown in Heilongjiang province of China. Soil Biology and Biochemistry 40: 238–246.

Mantelin S. and Touraine B. (2004). Plant growth-promoting bacteria and nitrate availability: impacts on root development and nitrate uptake. Journal of Experimental Botany 55 (394): 27-34.

Mendes R., Pizzirani-Kleiner A.A., Araujo W.L. and Raaijmakers J.M.. (2007). Diversity of cultivated endophytic bacteria from sugarcane: genetic and biochemical characterization of Burkholderia cepacia complex isolates. Applied Environmental Microbiology 73: 7259–7267.

Nguyen P.M. (2014). Factors influencing to nipa *Nypa fruticans* endosperm in canned food production. International Journal of Multidisciplinary Research and Development 1 (4): 126-131.

Rashedul I.M., Madhaiyan M., Deka Boruah H.P., Yim W., Lee G. and Saravanan V.S. (2009). Characterization of plant growth-promoting traits of free-living diazotrophic bacteria and their inoculation effects on growth and nitrogen uptake of crop plants. MicrobiolBiotechnol 19: 1213–1222.

Rodríguez H. and Fraga R. (1999). Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnology Advances 17: 319-339.

Sengupta A. and Chaudhuri S. (1991). Oecologia 87: 560-564.

Sharma B.S., Sayyed Z.R., Trivedi H.M. and Gobi A.T. (2013). Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. Springer Plus 2: 587.

Souza R.D., Schoenfeld R. and Passaglia L.M.P. (2016). Bacterial inoculants for rice: effects on nutrient uptake and growth promotion. Archives of Agronomy and Soil Science 62 (4): 561–569.

Stephen J., Shabanamol S., Rishad K.S. and Jisha M.S. (2015). Growth enhancement of rice (*Oryza sativa*) by phosphate solubilizing *Gluconacetobacter* sp. (MTCC 8368) and *Burkholderia* sp. (MTCC 8369) under greenhouse conditions. 3 Biotech 5: 831–837.

Tang S., Hara S., Melling L., Goh K. and Hashidoko Y. (2010). *Burkholderia vietnamiensis* Isolated from Root Tissues of Nipa Palm (*Nypa fruticans*) in Sarawak, Malaysia, Proved to be its Major Endophytic Nitrogen-Fixing Bacterium. Bioscience, Biotechnology and Biochemistry 74 (9): 1972-1975.

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