

# Increasing of Rice Yield by Using Growth Promoting Endophytic Bacteria from Swamp Land

Siti Nurul Aidil Fitri and Nuni Gofar<sup>1</sup>

Received 4 December 2009 / accepted 11 August 2010

## ABSTRACT

**Increasing of Rice Yield by Using Growth Promoting Endophytic Bacteria from Swamp Land (SNA Fitri and N Gofar):** Swamp land has can be used as a paddy field that will be potential as a rice source However, this land has some limiting factors such as low fertility. On the other hand, continuous used of inorganic fertilizer to improve soil fertility will also have some disadvantages. Therefore, an alternative method as fertilizers complement is needed. Biofertilizer is potential to be developed. Previous research had succeeded to explore and selected some bacteria from rice tissues grown on swamp land. That research had found two bacteria Consortium were named as Growth Promoting Endophytic Bacterial Consortium (GPEBC). The aims of this research were (1) to evaluate the effect of a GPEBC population density and a level of N fertilizer on plant N absorption, and rice yield in the swamp soil, and (2) to find out the optimal population density of GPEBC and optimal dosage of N fertilizer on plant N absorption and rice yields in the swamp soil. The research used a factorial completely randomized design with 3 factors and 3 replicates. The first factor was a kind of GPEBC which consisted of Consortium A and consortium B. The second factors was population density of GPEBC which consisted of 0 CFU mL<sup>-1</sup>, 10<sup>7</sup> cfu mL<sup>-1</sup>, 10<sup>9</sup> cfu mL<sup>-1</sup>, and 10<sup>11</sup> cfu mL<sup>-1</sup>. The third factor was N-fertilizer dosages which consisted of 50% of plant nitrogen necessity (equivalent to 57.50 kg N ha<sup>-1</sup>), 75% of plant nitrogen necessity (equivalent to 86.25 kg N ha<sup>-1</sup>), and 100 % of plant N necessity (equivalent to 115 kg N ha<sup>-1</sup>). The research showed that GPEBC of the Consortium B had a better effect on rice yields than Consortium A. The population density of 10<sup>7</sup> cfu mL<sup>-1</sup> of GPEBC increased the growth and the yield of rice grown on swamp soil. Treatment combination of 75% of plant N necessity, and 10<sup>7</sup> cfu mL<sup>-1</sup> of population density produced the best production of Consortium B (GPEBC) for rice grown on swamp soil.

---

**Keywords:** Bacterial consortium, nitrogen fertilizers, rice, swamp land

## INTRODUCTION

Swamp land (“lebak”) has a high potential as a rice resource according to land availability. There is about 1.1 million hectares swamp land in South Sumatra, and from this area only 288.637 hectares are used for rice cultivation (BPS 2005). However this land has some limiting factors such as low of a biological, chemical and physical fertility. Lebak is a flatting topography area where located in both side of rivers. This area is flooded during rainy season and the flooding is not influenced by water sea level.

Paddy is very important crop in the world. It is a staple food for 70% of world population. Paddy

demand increases because of the increasing of world population (Britto and Kronzucker 2004). Increasing of rice production in marginal area should be developed by applicable technology such as application of indigenous bacteria especially rhizosphere microbe that live in plant root systems. Those bacteria have some disadvantages such as a low ability to adapt in a new environment (Kimura *et al.* 1992). Therefore, an endophytic bacteria is developed. Those bacteria live in plant tissue but it is not parasitic to host plant even it is useful for host plant (Sturz and Nowak 2000). The endophytic bacteria can infect host plant not only by root plant but also by flowers, stems, and cotyledon (Zinniel *et*

---

<sup>1</sup>Faculty of Agriculture, Sriwijaya University, Jalan Palembang- Prabumulih Km 32 Indralaya – 30662, South Sumatera, Indonesia. Email: gandiswijaya@yahoo.co.id, phone: 081377971010

al. 2002). Using endophytic bacteria as a growth promoter all at once as a nitrogen ( $N_2$ ) fixation can be an alternative technology that more friendly to environment.

Thakuria *et al.* (2004) reported that application of bacteria as biofertilizer for growth promoting has some advantages such as nutrients solubilizers, growth hormone production, nitrogen fixation, and activation of disease resistant mechanism. Tan and Zou (2001) reported that every high plant has some endophytic bacteria which can produce organic compounds or secondary metabolic. This phenomenon is caused by co-evolution or transfer of genetic secondary metabolic from plant host to endophytic bacteria.

Nitrogen fertilizer is an absolutely agriculture input that has to be applied to achieve plant high yield in marginal land. However, the efficiency of N fertilizer is low (Hossain *et al.* 2005), for example in rice cultivation N that could be used by rice plant was only 60% from N application (Cassman *et al.* 1998).

Boddey *et al.* (1995) reported that in Brazil, using  $N_2$  fixation bacteria which was isolated from sugar cane with certain cultivars decrease half of nitrogen that was required by plant. Those bacteria fixed about 150 kg N ha<sup>-1</sup> per year. Setiawati (2004) reported that dry rice plant that was inoculated by growth promoting endophytic bacteria Consortium (GPEBC) could decrease the N fertilizer application. The optimal dosage of GPEBC should be studied because the endophytic bacteria need to adapt to a new environment. Moreover Setiawati (2004) argued that the highest nitrogenase activity was produced by 10<sup>-11</sup> cfu mL<sup>-1</sup> dosage of GPEBC in Ultisol. Gofar *et al.* (2007) evaluated and collected some GPEBC from lebak in South Sumatra. They reported that Consortium A (consisting of *Pseudomonas fluorescens*, *Klebsiella pneumoniae* and *Enterobacter aerogenes*) and Consortium B (consisting of *Pseudomonas aeruginosa*, *P. diminuta*, *Klebsiella pneumonia*, and *Bulkkholderia cepacia*) bacteria Consortium had a high potential as GPEBC.

The present study was undertaken to: (1) evaluate the effect of population density of GBEP and N fertilizer to the rice yield that cultivated in the swamp soil, (2) find out the optimal population density of GBEP and optimal dosage of N fertilizer for the highest rice yield.

## MATERIALS AND METHODS

### Preparation of Soils and Plant

The rice (Ciherang Variety) was cultivated on pots. Soil as medium were collected from swamp area located 5 km from Palembang, South Sumatera. The soil was prepared by drying and filtering, each pot was filled with 10 kg of swamp soil.

### Experimental Setup

The research used a randomized completely design with 3 factors and 3 replicates. Every experimental unit was set duplo, so this experiment totally used 144 pots (2 x 4 x 3 x 3 x 2). The first factor was a kind of GPEBC which consisted of Consortium A and Consortium B that was collected by Gofar *et al.* (2007), the second factor was population density of GPEBC which consisted of 0 cfu mL<sup>-1</sup>, 10<sup>7</sup> cfu mL<sup>-1</sup>, 10<sup>9</sup> cfu mL<sup>-1</sup>, and 10<sup>11</sup> cfu mL<sup>-1</sup>, the third factor was N-fertilizer dosage in which consisted of 50% of plant N need (equivalent to 57.50 kg N ha<sup>-1</sup>), 75% of plant nitrogen necessity (equivalent to 86.25 kg N ha<sup>-1</sup>), and 100% of plant nitrogen necessity (equivalent to 115 kg N ha<sup>-1</sup>).

The observed parameters were: (1) soil properties such as pH (H<sub>2</sub>O and KCl), C-organic, N-total, P-Bray, K, Na, Ca, Mg, Cation Capacity Exchange, Al, H and Al, (2) 100 seeds weight, and (3) yield and percentage of empty seed.

### Data Analysis

Data were analyzed by analysis of variance (ANOVA) for significance difference ( $P < 0.05$ ) and least significant differences (LSD) test at  $P < 0.05$  were used to separate treatment means for all properties.

## RESULT AND DISCUSSION

### Soil Fertility

In general, soil which was used in this experiment was categorized as a low to medium fertility soil. This criterion had been shown by high soil acidity, low of K-dd and Ca, low of Mg. While, C-organic content was categorized as medium, total N and available P content were categorized as medium level, Cation Exchange Capacity was categorized as medium, Na was categorized as medium. This result was parallel

Table 1. Effect of inoculum type, inoculum density and N dosage on the rice yield

Treatments	Rice yield (g plant <sup>-1</sup> )
Kind of Inoculum	
Consortium A	60.37 a
Consortium B	64.56 b
Inoculum density (cfu mL <sup>-1</sup> )	
0	54.68 a
10 <sup>7</sup>	62.57 a
10 <sup>9</sup>	67.01 b
10 <sup>11</sup>	65.61 b
N dosage (% plant need)	
50	56.63 a
75	68.87 b
100	61.92 a

Note: Values with different letters are significantly different according to LSD test ( $P \leq 0.05$ ).

to result of Subagyo (2006). He reported that lebak soil has pH 4.0 to 5.5, macro element content was categorized as low and medium.

**Rice Yield**

Each bacterial consortium, their density and dosage of N fertilizer affected significantly all rice yield component, except for the percentage of empty seeds and there was no interaction among them (Table 1). Yield of rice which was inoculated with consortium A produced less yield comparing to yield of rice which was inoculated with consortium B. Rice yield which was produced by control treatment (no bacteria consortium inoculation) was the lowest, while the treatment with consortium produced significantly higher yield than in a control, except for 10<sup>7</sup> cfu mL<sup>-1</sup> density of bacteria. Isaac (1992) reported that soaking seed in the highest density of inoculants would increase infection probability of bacteria to seeds. Because there is osmotic differences between seeds and high density of bacterial consortium suspension, as a consequence the bacteria is pushed into the seeds via pedicel. Setiawati (2004) also reported that 10<sup>11</sup> cfu mL<sup>-1</sup> density of bacteria produced the best effect on growth and yield of dry rice because of this density gave the highest nitrogenase activity. Our result showed that the higher density of consortium, the higher the yield of rice. Inoculation of 0 cfu mL<sup>-1</sup>, 10<sup>7</sup> cfu mL<sup>-1</sup>, and 10<sup>9</sup> cfu mL<sup>-1</sup> indicated bacterial consortium inoculums has mutualism symbiosis with the rice plant.

According to Morris (2001) and Gofar (2004) plant tissue was an optimal habitat for pathogen and also non pathogen microbe. Benefit effect from interaction between non pathogen microbe and host plant is growth promoting for host plant because the microbe can produce phytohormone. Furthermore, Susilowati *et al.* (2004) reported that a number of bacterial endophytic could stimulate rice and maize growth via their capability to produce indole-3-acetic acid (IAA) phytohormone and nitrogen fixation. It is showed that plant N absorption with microbe inoculation treatment produced a higher plant N absorption (Table 2) than control.

**Nitrogen Absorption**

The type of bacterial consortium were not affected plant N absorption, but the density and N dosage significantly affected plant N absorption although without interaction among them (Tabel 2). De Datta (1981) argued that vegetative growth of rice plant depended on soil nitrogen availability whereas the vegetative growth has a high correlation to the plant yield. It is relevance to the report of Wallenstein *et al.* (2003) that application of 150 kg N ha<sup>-1</sup> caused decreasing of microbial as much as 68% comparing to control. Arteca (1995) explained that IAA phytohormone included auxin hormone where this hormone stimulates cell development and cell

Tabel 2. Effect of inoculum type, inoculum density and N dosage on the N absorption by plant.

Treatments	N absorption (g plant <sup>-1</sup> )
Kind of Inoculum	
Consortium A	1.35
Consortium B	1.41
Inoculum density (cfu mL <sup>-1</sup> )	
0	0.91 a
10 <sup>7</sup>	1.49 b
10 <sup>9</sup>	1.47 b
10 <sup>11</sup>	1.63 c
N dosage (% plant need)	
50	1.27 a
75	1.52 b
100	1.34 a

Note: Values with different letters are significantly different according to LSD test ( $P \leq 0.05$ ).

Tabel 3. Effect of inoculum type, inoculum density and N dosage on the tillering.

Treatments	Number of Tillering (Tiller plant <sup>-1</sup> )
Kind of Inoculum	
Consortium A	24.94 a
Consortium B	29.11 b
Inoculum density (cfu mL <sup>-1</sup> )	
0	25.56 a
10 <sup>7</sup>	26.56ab
10 <sup>9</sup>	27.83 b
10 <sup>11</sup>	28.17 b
N dosage (% plant need)	
50	25.33 a
75	29.71 b
100	26.04 a

Note: Values with different letters are significantly different according to LSD test (P ≤ 0.05).

enlargement. Because of that the plant that was applied by auxin hormone would growth vigorous comparing to control plant.

**Number of Tillering**

The type of bacterial consortium, their density and N dosage significantly affected the number of tillering and there was no interaction among them (Tabel 3).

Increasing of paddy nitrogen content which was inoculated by endophytic bacteria was likely to be caused nitrogen supply from bacteria. Moreover, the bacteria also produced phytohormone. The phytohormone stimulates hairy root production that can increase nutrient absorption. Hubbel and Kidder (2001) argued that endophytic bacteria could increase nitrogen content of plant host. Pa'dua *et al.* (2001) had proved that endofitic bacteria of IAA phytohormone production which is inoculated to paddy seedling can increase plant nitrogen content. Our result showed that the inoculants consortium microbe (10<sup>7</sup> cfu mL<sup>-1</sup>, 10<sup>9</sup> cfu mL<sup>-1</sup>, and 10<sup>11</sup> cfu mL<sup>-1</sup>) treatment produced higher biomass than control treatment especially on number of tiller (Table 3).

**Seed Weight**

The type of bacterial consortium, their density and N dosage significantly affected the percentage of 100 seeds wigth, although no interaction among them (Tabel 4).

Tabel 4. Effect of inoculum type, inoculum density and N dosage on the weight of 100 seeds.

Treatments	Weight of 100 seeds (g)
Kind of Inoculum	
Consortium A	2.73 a
Consortium B	2.84 b
Inoculum density (cfu mL <sup>-1</sup> )	
0	2.61 a
10 <sup>7</sup>	2.75 b
10 <sup>9</sup>	2.92 c
10 <sup>11</sup>	2.88 bc
N dosage (% plant need)	
50	2.79 a
75	2.91 b
100	2.65 a

Note: Values with different letters are significantly different according to LSD test (P ≤ 0.05).

This result was similar to research result of Setiawati *et al.* (2004). They reported that seeds which were soaking in highest density of bacteria consortium solution would be infected by endofitic bacteria where the bacteria would support part of N plant necessity. It was predicted that this treatment also produced the highest 100 seeds weight (Table 4) and the lowest percentage of empty seeds (Table 5).

Tabel 3. Effect of inoculum type, inoculum density and N dosage on the percentage of empty seeds.

Treatments	Percentage of empty seed (%)
Kind of Inoculum	
Consortium A	5.28
Consortium B	5.48
Inoculum density (cfu mL <sup>-1</sup> )	
0	7.39 b
10 <sup>7</sup>	4.52 a
10 <sup>9</sup>	5.06 a
10 <sup>11</sup>	4.45 a
N dosage (% plant need)	
50	2.85 a
75	4.99 b
100	8.31 c

Note: Values with different letters are significantly different according to LSD test (P ≤ 0.05).

## Percentage of Empty Seed

A nitrogen fertilizer application significantly affected the plant yield (Table 1). The application of nitrogen as much as 57.50 kg N ha<sup>-1</sup> (50% plant need) produced the lowest yield whereas the application of 86.25 kg N ha<sup>-1</sup> (75% plant need) produced the highest yield. Even though application N fertilizers as much as 115 kg N ha<sup>-1</sup> (100% plant need) had no different yield with application of 57.5 kg N ha<sup>-1</sup> (50% plant need). It was predicted that N dosage of 115 kg N ha<sup>-1</sup> caused high concentration N in the soil. This fact can be proved by the percentage of empty seeds where the 115 kg N ha<sup>-1</sup> treatment produced the highest a percentage of empty seeds (Table 5). De Datta (1981) reported that over supply of nitrogen in soil cause a high of empty seed of rice plant.

## CONCLUSIONS

The results showed clearly that Growth Promoting Endophytic Bacterial Consortium (GPEBC) A had a better effect on rice production than Consortium B. The population density of 10<sup>7</sup> cfu mL<sup>-1</sup> of GPEBC could increase the growth and production of rice grown in the swamp soil. The combination treatment of 75% of plant nitrogen needed and 10<sup>7</sup> cfu mL<sup>-1</sup> of population density produced the best production of Consortium B GPEBC rice grown in the swamp soil.

## REFERENCES

- [BPS] Badan Pusat Statistik. 2005. Sumatera Selatan dalam Angka. Badan Pusat Statistik Propinsi Sumatera Selatan (in Indonesian).
- Arteca RN 1995. Plant Growth Substances Principles and Applications. Chapman and Hall New York, 352 p.
- Boddey RM, DC de Oliveira, S Uguiaga, VM Reis, FL de Olivares, VLD Baldani and J Dobreiner. 1995. Biological nitrogen fixation associated with sugar cane and rice, Contributions and prospect for improvement. *Plant Soil* 174: 195-209.
- Britto DT and HJ Kronzucker. 2004. Bioengineering Nitrogen Acquisition in Rice: Can Novel Initiatives in Rice Genomics and Physiology Contribute to Global Food Security. *BioEssays* 26 (6): 683-692.
- Cassman KG, S Peng, DC Olk, JK Ladha, W Reichardt, A Doberman and U Singh. 1998. Opportunities for increased nitrogen-use efficiency from improved resource management in irrigated rice systems. *Field Crops Res* 56: 7-39.
- De Datta SK. 1981. Advances in Soil Fertility Research and Nitrogen Fertilizer Management for Lowland Rice. Proceeding of the meeting of the International Network on Soil Fertility and Fertilizer Evaluation for Rice. Griffith, New South Wales, Australia 16-20 April 1985.
- Gofar N, A Napoleon and MU Harun. 2007. Eksplorasi Bakteri Endofitik Pemacu Tumbuh Asal Jaringan Tanaman Padi Rawa dan Pasang Surut Sumatera Selatan. Laporan Penelitian Hibah Bersaing. Universitas Sriwijaya, Indralaya (in Indonesian).
- Gofar N. 2004. Pertumbuhan tanaman jagung (*Zea mays* L.) pada tanah ultisol yang diinokulasi dengan konsorsium mikroba daun pemacu tumbuh. *Tan Trop* 7 (1):13-22.
- Hossain MF, SK White, SF Elahi, N Sultana, MHK Choudhury, QK Alam, JARother and JL Gaunt. 2005. The efficiency of nitrogen fertiliser for rice in Bangladeshi farmers' fields. *Field Crops Res* 93: 94-107.
- Hubbell DH and G Kidder. 2001. Biological Nitrogen Fixation. University of Florida. <http://edis.ifas.ufl.edu/BODYSS180>.
- Isaac S. 1992. Fungal-Plant Interaction: Endophytic Symbiosis. Chapman and Hall. London, pp. 316-327.
- Kimura R, M Nishio and K Katoh. 1992. Utilization of phosphorus by plant after solubilization by phosphate solubilizing microorganism in soil. National Grassland Research Institute. Nasu, Japan and National Agriculture Research Centre, Tsukuba, Japan.
- Morris C. 2001. The Infact Biofilms on the Ecology and Control of Epiphytic Bacteria. Interdisciplinary Plan Biology Seminar Speaker, January 29, 2001. Plan Pathology Station, INRA, France.
- Pa'dua VLM, HP Masuda, HM Alves, KD Schwarcz, VLD Baldani, PCG Ferreira and AS Hemerly. 2001. Effect of endophytic bacterial indole-acetic acid (IAA) on rice development. Dept Bioquimica Medica, Rio de Janeiro
- Setiawati MR. 2004. Peran Konsorsium Bakteri Endofitik Penambat N<sub>2</sub> Asal Tumbuhan Ekosistem Air Hitam Kalimantan Tengah Dalam Meningkatkan Hasil Padi Gogo pada Humic Hapludult. Disertasi pada Universitas Padjajaran (tidak dipublikasikan), Bandung.
- Sturz AV and J Nowak. 2000. Endophytic communities of rhizobacteria and strategies required to create yield-enhancing associations with crops. *Appl Soil Ecol* 15: 183-190.
- Subagyo H. 2006. Lahan Rawa Lebak In: DA Suriadikarta, U Kurnia, HS Mamat, W Hartatik and D Setyorini (eds). Karakteristik dan Pengelolaan Lahan Rawa. Balai Besar Penelitian dan Pengembangan Sumber Daya Lahan Pertanian. Bogor, pp. 99-116 (in Indonesian).

***SNA Fitri and N Gofar: Growth Promoting Endophytic Bacteria from Swamp Land***

- Susilowati DN, R Saraswati, Wlasanti, and E Yuniarti. 2004. Isolasi dan seleksi mikroba diazotrof endofitik dan penghasil zat pemacu tumbuh pada tanaman padi dan jagung. In: M Machmud, Harnoto, TS Silitonga, K Mulya, IS Dewi, M Yunus, and IN Orbani (eds). Prosiding Seminar Hasil Penelitian Rintisan dan Bioteknologi Tanaman, Balai Penelitian Bioteknologi dan Sumber Daya Genetik Pertanian, pp. 128-143.
- Tan RX and WX Zou. 2001. Endophytes: a rich source of functional metabolites. *Nat Prod Rep* 18: 448-459.
- Thakuria D, NC Talukdar, C Goswami, S Hazarika, RC Boro and MR Khan. 2004. Characterization and screening of bacteria from rhizosphere of rice grown in acidic soils of assam. *Curr sci* 86: 978-985.
- Wallenstein M.D., W.H. Sholesinger, S.K. Rhee, and J. Zhou. 2003. Effect of Nitrogen Fertilization on Soil Microbial Communities. *Geograp Res Abs, Eur Geophys Soc* 5: 13087.
- Zinniel DK, P Lambrecht, NB Harris, Z Feng, D Kuczmarski, P Higley, CA Ishimaru, A Arunajumari, RG Barletta and AK Vidaver. 2002. Isolation and characterization of endophytic colonizing bacteria from agronomic crops and prairie plants. *Appl Environ Microbol* 68 (5): 2198-2208.