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Effectiveness of *Enterobacter cloacae* to Promote the Growth and Increase the Yield of Rice

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Abstract
Rice productivity in Indonesia is relatively lower than the rice productivities in other rice growing countries. In order to increase the rice productivity, it is necessary to find agents that can improve and promote the rice growth. This study was done to determine potential use of *Enterobacter cloacae* isolated from rhizospheres of *Imperata cylindrica* to promote the growth and increase the yield of rice under green house experiment. Five isolates of *E. cloacae* namely Al2N, Al3Bt, Al9Sa, Al6G, Al7Kla were tested for their effectiveness to promote the growth and increase the yield of rice. Treatments with isolates of *E. cloacae* significantly improved the growth of rice seedlings, in which the plant height, root length and number of root significantly ($p<0.05$) higher on treated plants when compared to control. These treatments were also significantly increased the number of tillers, content of chlorophyll in the leaf, content of macro nutrients in the leaf, the dry weight of shoot and the dry weight of root, but did not significantly ($p>0.05$) affect the plant height. Number of panicles per hill and percentage of filled grain per hill of treated plants were significantly higher than that of control, but the weight of 1000 grains did not significantly different. Rizhobacteria treatment increased the rice yield ranged from 22.53% to 26.13% when compared to control. No significant different were observed on all parameters among treatments with isolates of *E. cloacae* suggested that all isolates resulted in similar level of effect to the growth and yield of rice. These isolates may be further developed as plant growth promoting agents to increase the yield of rice in Indonesia.
Keywords : *Enterobacter cloacae*, plant growth promoting rhizobacteria, rice yield.

1. Introduction

Rice is still consumed as main staple food by most of Indonesian people with the average consumption rate of 139 kg per capita per year. The total rice production in Indonesia in 2010 was 66.47 million tons and decreased to 65.9 million tons in 2011 (Suswono, 2012). The rice productivity was about 4.56 ton/ha, which is relatively lower than other rice growing countries such Australia 8.22 ton/ha, Japan 5.85/ton/ha and China 6.06 ton/ha (USDA, 2004). In 2011 the rice productivity in Indonesia was 5.14 ton/ha (BPS, 2012). This data indicated that there is a possibility to increase the rice productivity and production in Indonesia through the improvement of rice growth by using indigenous plant growth promoting rhizobacteria.

Rhizobacteria are bacteria that colonize the rhizospheres of diverse plants. There are several rhizobacteria that have been known as PGPR such as Pseudomonas, *Enterobacter*, *Azospirilum, Azotobacter*, *Burkholderia*, *Bacillus* and *Serratia* (Alam *et al*., 2001; Vidyasekaran et al., 1997; Khalimi *et al*., 2012). Several reports available on the role of rhizobacteria in promoting the plant growth and increase the plant yield. Seed treatment with *Pseudomonas fluorescens* strain Pf1 significantly increased the rice yield (Vidhyasekaran *et al*., 1997). Biswas *et al*. (2000) reported that the rice inoculated with *Rhizobium* sp. IRBG74 could increase the rice yield by 11,6%. Alam *et al*. (2001) proved that treatment by soaking the root of rice seedling for 24 h with the suspensions of *Azotobacter nigricans*, *A.armeniacus*, *Bacillus sphaericus*, *B. megaterium*, *Enterobacter* and *Xhantobacter* could increased the rice yield by 15,03%. Study done by Khalimi and Suprapta (2012) showed that the treatment with *Pantoea agglomerans* isolates PaJ and BS2a isolated from the rhizosphere of groundnut could increase the yield of rice cultivar Cicih Medang Selem by 154.17% when compared to control. This study was done in order to test the effectiveness of *Entrobacter cloacae* isolates, isolated from the rhizospheres of *Imperata cylindrica* to promote the growth and increase the yield of rice.

2. Materials and Methods

2.1 Isolates of Enterobacter cloacae

Five isolates of *E. cloacae* were used in this study namely Al3Bt, Al6G, Al7Kla, Al9Sa and Al12N. All of these isolates were isolated from the rhizospeheres of *Imperata cylindrica* grown in Bali Island Indonesia and provided by the Laboratory of Biopesticide Faculty of Agriculture Udayana University.

2.2 Test for PGPR Activity

All five isolates of *E. cloacae* were tested for their plant-growth promoting activity on rice seedlings. The rice seeds cultivar Ciherang were soaked in sterile distilled water for 24 h, and drain up and placed on a

Petri dish with wet Whatman No.2 filter paper for germination. The germinated seeds were soaked in rhizobacterial suspension (10^7 CFU/ml) and air dried for an hour in the Laminar Flow Cabinet. The seedlings that soaked only with distilled water were used as control. The seedlings were then grown in glass cultural bottles containing 0.01% KNO_3 and distilled water. Five bottles were prepared for each isolate. The cultures were maintained in cultural cabinet with 12 h lightening of flourescens lamps for 10 days at temperature 28 $\pm$ 2°C. The growth of shoots and roots were determined and compared among treatments and control.

2.3 Test for PGPR Activity under Green House Condition

Five isolates of *E. cloacae* namely Al3Bt, Al6G, Al7Kla, Al9Sa and Al12N were tested for their effectiveness to promote the growth and increase the yield of rice cultivar Ciherang under green house condition. This experiment was designed according to randomized block design (RBD) consisting of 6 (six) treatments *i.e.* isolates Al3Bt, Al6G, Al7Kla, Al9Sa, Al12N and control. Each treatment was replicated 4 (four) times, thus there were 24 experimental units in this experiment. Each experimental unit consisting of 10 plastic pots of 30 cm diam. Cultural medium used in this experiment was soil taken from rice field at 20 cm depth mixed with compost (3:1, w/w). Application of *E. cloacae* was done through seed treatment, where the germinated seeds were soaked in *E. cloacae* suspension (10^7CFU/ml) for 2 h before sowing on a tray (30 cm x 40 cm in size). Treatment with rhizobacteria was also done at transplanting by incorporating 10 ml *E. cloacae* suspension (10^7CFU/ml) per pot.

Several parameters were observed during this experiment, such as plant height, number of tillers, content of chlorophyll using Chlorophyll Meter SPAD-502 (Konika, Minolta, Japan). The content of macro elements such as N, P and K was measured at 30 days after transplanting. Content of N was determined with Kjeldhal method, while the content of P was determined with Spectrophotometer after mixing the sample with Barton solution (Nadeem *et al*., 2006). Content of K was determined with *Gallenkamp Flame Photometer* at wave length of 767 nm (Oyewale, 2005). The dry weights of root and shoot were measured at 60 days after transplanting. Number of productive tillers, number of grain per panicle, percentage of filled grains, weight of 1000 grain and weight of grain per hill were measured at harvest time.

2.4 Statistical Analysis

All data were subjected to the analysis of variance (ANOVA) and continued by Duncan, s multiple range test (DMRT) at 5% level.

3. Results and Discussion

3.1 Plant Growth Promoting Activity

All five isolates of *E. cloacae* significantly ($p<0.05$) increased the plant height, root length and number of root when compared to control as presented in Table 1. The plant height increased by 26.71% to 30.62%; the root length increased by 47.95% to 52.39%, and number of root increased by 38.25% to 47.82%. These data indicated that *E. clocae* promoted both the growth of shoot and root; however the improvement of root system was better than the shoot. The root system of a plant determines the capacity of plant to acquire nutrients and water. Auxin is one of plant growth hormones that have a close connection with the root development particularly on primary root length, number of lateral roots and number of root hairs (Overvoorde *et al*., 2010). Murphy *et al*. (2000) reported that treatment of tomato plant with rhizobacteria resulted in better and bigger growth. The growth of plant was increased because rhizobacteria produced growth hormones such as auxin, ethylene, cytokinin and gibberellins which are necessary for plant growth (Husen, 2009).

There are several species of rhizobacteria have been reported as PGPR to rice plant, such as *Pantoea agglomerans* (Khalimi *et al*., 2012); *Bradyrhizobium* sp. IRBG271 (Biswas *et al*., 2000); *Burkholderia caryophylli* ACC7 (Shaharoona *et al*., 2007), *Rhizobium leguminosarum* 128C56C (Han and Lee, 2005); *Azospirillum brasilense* (Kannan and Ponmurugan, 2010). Other study showed that two isolates of rhizobacteria, PaJ and BS2a isolated from the rhizospeheres of groundnut have been proven to promote the growth of soybean, corn, rice and tomato on green house experiments (Khalimi *et al*., 2012).

Table 1: Plant growth promoting activities of five isolates of *E. cloacae* on rice seedlings of Cultivar Ciherang

No.	Treatment	Plant height (mm)	Root length (mm)	Number of root
1	Control	51.63 b*	14.64 b	13.07 b
2	Al2N	66.82 a (29.42%)**	21.66 a (47.95%)	18.76 a (43.53%)
3	Al3Bt	67.41 a (30.36%)	22.17 a (51.43%)	18.07 a (38.25%)
4	Al9Sa	65.63 a (27.12%)	22.31 a (52.39%)	18.34 a (40.32%)
5	Al6G	65.42 a (26.71%)	21.89 a (49.52%)	19.32 a (47.82%)
6	Al7Kla	67.44 a (30.62%)	22.23 a (51.84%)	19.32 a (47.82%)

* Values followed by the same letters in the same column are not significantly different ($p>0.05$) according to Duncan's *Multitle Range Test* at 5% level.

**Values in the parenthesis indicated the percentage of increase when compared to control.

3.2 Effect of E. clocae to the Growth of Rice

Based on analysis on variance to the growth parameters of rice showed that treatment with isolates of *E. cloacae* significantly ($p< 0.05$) increased the number of tillers, chlorophyll content in the leaf, dry weight of shoot and dry weight of root, but did not significantly increase the plant height.

3.3 Plant height and Number of Tillers

The maximum plant height was obtained at 56 days after transplanting (DAT). Treatment with *E. clocae* did not significantly increase the plant height ($p>0.05$), but significantly ($p<0.05$) increased the number of tillers as presented in Table 2. The growth of plant on control and the plant treated with *E. cloacae* isolate Al7Kla is shown in Fig.1. No significant different ($p>0.05$) were found among treatments with *E. colacae* isolates on the number of tiller. This result suggested that all four isolates have similar potential as promoter of tiller formation of rice. Other study showed that inoculation of strains *Agrobacterium, Burkholderia, Enterobacter,* and *Pseudomonas* could promote the growth of rice (Souza, 2012). Application of *Serratia marcescens* NBRI1213 significantly increased the plant height, the dry wight of shoot, root length and dry weight of root of *Piper betle* L. by 81%, 68%, 152% and 290% respectively (Lavania, 2006). Murphy *et al.* (2000) reported that treatment of tomato plant with rhizobacteria resulted in better and bigger growth. The growth of plant was increased because rhizobacteria produced growth hormones such as auxin, ethylene, cytokinin and gibberellins which are necessary for plant growth (Husen, 2009**).** Treatment with *Pantoea agglomerans* could increase the number of maximum tiller of rice cultivar Cicih Medang Selem by 93.27% to 120.19% when compared to control (Khalimi *et al*., 2012). Other study done by Hussain *et al*. (2009) showed that treatment of the root of rice with *Mesorhizobium ciceri* strain CRI-32 increased the number of maximum tillers by 25,34%.

Table 2. Maximum plant height and number of tillers

Treatment	Maximum plant height (cm)	Number of tillers
Control	95.25 a*	46.17 b
Al2N	96.02 a	57.08 a (23.63%)**
Al3Bt	96.25 a	57.67 a (24.91%)
Al9Sa	95.50 a	56.33 a (22.01%)
Al6G	95.58 a	56.33 a (22.01%)
Al7Kla	96.50 a	57.83 a (25.25%)

* Values in the same column followed by the same letters are not significantly different ($p>0.05$) according to the Duncan's Multiple Range Test at 5% level.
** Values in the parenthesis indicated the percentage of increase when compared to control.

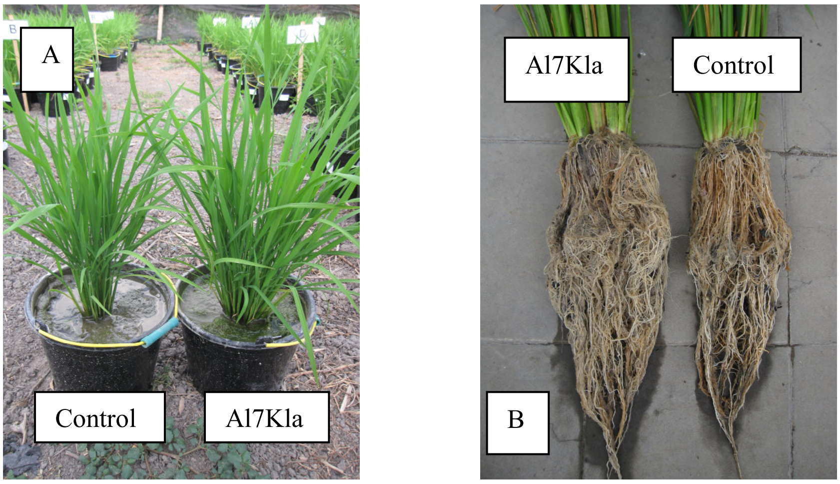

Figure 1. Rice plants of cultivar Ciherang of control (A, left) and treated with *E. cloacae* isolate Al7Kla (A, right), the roots of rice plant treated with *E. cloacae* isolate Al7Kla (B, left) and control (B, right).

3.4 Content of macro nutrients and chlorophyll in the leaf

Analysis of the content of macro nutrients in the leaf 30 days after transplanting showed that treatment with *E. cloacae* increased the content of N by 9.20% to 12.93% when compared to control. This treatment increased the content of P by 15.09% to 17.30% and increased the content of K by 20.63% to 23.32% (Table 3).

This result suggested that the intake of macro nutrients by the plants is higher on rice plants treated with *E. clocae*. This is in line with the content of chlorophyll in the leaf as presented in Table 4. Chlorophyll content of plants with *E. cloacae* treatments significantly ($p<0.05$) higher than that of control at 30, 45, and 60 days after planting.

Khalimi *et al*. (2012) proved that treatment with *Pantoea agglomerans* significantly increased the content of macro nutrients such as N, P and K and increased the content of chlorophyll. Treatment with *P. agglomerans* increased N, P and K content by 59.09% to 72.72%; 57.14% to 78.57% and 33.33% to 59.09% respectively when compared to control. Biswas *et al.* (2000) reported that rice plant treated with *Bradyrhizobium* sp. IRBG271 increase the intake of N, P and K by 27,87%; 19,82% and 10,97%. Similar result was reported by Shaharoona *et al*. (2007) in which treatment of wheat seeds with the suspension of *Burkholderia caryophylli* ACC7 could increase the intake of N, P and K by 39.39%; 32.82% and 28.38%.

Table 3. Content of macro nutrients in the leaf at 30 days after planting

Treatment	N (%)	P (ppm)	K (%)
Control	1.47 b*	319.55 b	2.23 b
Al2N	1.64 a (11.56%)**	369.49 a (15.63%)	2.69 a (20.63%)
Al3Bt	1.58 a (9.20%)	367.76 a (15.09%)	2.74 a (22.87%)
Al9Sa	1.63 a (10.88%)	374.66 a (17.25%)	2.75 a (23.32%)
Al6G	1.62 a (10.20%)	373.96 a (17.03%)	2.70 a (21.08%)
Al7Kla	1.66 a (12.93%)	374.83 a (17.30%)	2.72 a (21.97%)

* Values in the same column followed by the same letters are not significantly different ($p>0.05$) according to the Duncan's Multiple Range Test at 5% level.
** Values in the parenthesis indicated the percentage of increase when compared to control.

Table 4: Chlorophyll content in the leaf

Treatment	30 DAP*	45 DAP	60 DAP
Control	36.15 b**	37.45 b	37.24 b
Al2N	38.49 a	39.87 a	40.35 a
Al3Bt	38.57 a	39.99 a	40.10 a
Al9Sa	38.54 a	39.79 a	39.98 a
Al6G	38.38 a	39.86 a	40.51 a
Al7Kla	38.78 a	40.17 a	40.94 a

* Days after planting
** Values in the same column followed by the same letters are not significantly different ($p>0.05$) according to the Duncan's Multiple Range Test at 5% level.

3.5 Dry weight of shoot and root

Dry weight of shoots and roots of plants treated with rhizobacteria significantly ($p<0.05$) higher than that of control (Table 7). The dry weight of shoots increased by 27.42% to 30.28% while the dry weight of shoot increased by 36.94% to 40.41% when compared to control. No significant different were found on the dry weight of shoot and dry weight of root among treatments with *E. clocae* isolates.

These results are similar to the results of other study done by Kumar (2011) where application of *Enterobacter cloacae* PfR8 to the chilli pepper could increase plant height and root length 38.4% and 50.0% respectively. Treatment with *Enterobacter* sp. C1D significantly increased the root length and plant height of green bean (Subrahmanyam and Archana, 2011). Inoculation of *Serratia* sp. SY5 on corn, significantly increase the weight of root which indicated that *Serratia* sp. SY5 act as bio-stimulant that could promote the plant growth (Koo and Kyung-Suk, 2009). Treatment with two isolates of *Pantoea agglomerans i.e.* Paj and BS2a could increase the dry weight of shoot of rice by 377% to 511% (Khalimi *et al*., 2012). Kannan and Ponmurugan (2010) reported that treatment of *Azospirillum brasilense* on rice cultivar CO43 increased the dry weight of root by 41.95%. *Pantoea agglomerans* was reported to produce indole-3-acetic acid (IAA) that can suppress the growth of main roots, but promote the growth of lateral roots and increased the number of root hairs (Bucio *et al*., 2007).

Table 5: Dry weight of shoot and root at 60 days after planting

Treatment	Dry weight of shoot (g)	Dry weight of root (g)
Control	62.28 b*	50.21 b
Al2N	80.59 a (29.40%)**	68.76 a (36.94%)
Al3Bt	79.46 a (27.59%)	70.26 a (39.93%)
Al9Sa	79.36 a (27.42%)	69.61 a (38.64%)
Al6G	80.55 a (29.34%)	69.57 a (38.56%)
Al7Kla	81.14 a (30.28%)	70.50 a (40.41%)

* Values in the same column followed by the same letters are not significantly different ($p>0.05$) according to the Duncan's Multiple Range Test at 5% level.

** Values in the parenthesis indicated the percentage of increase when compared to control.

3.6 Effects of E. cloacae *to the Yield of Rice*

Results of statistical analysis showed that treatment with five isolates of *E. cloacae* significantly increased the number of panicles per hill. The plants of control resulted in 31.58 panicles per hill, which was significantly lower than that of plants treated with *E. cloacae*. However no significant different ($p>0.05$) was found among the plants treated with *E. cloacae* (Table 6). No significant different occurred on the weight of 1000 grains among all treatments.

The rhizobacteria belongs to group of *Serratia* spp. can increase the avaibality of P, fix the N (Gholami, *et al.* 2008) and produce indole acetic acid (El-Azeem *et al.*, 2007). In present study, we proved that the root system and the intake of N, P and K were obviously higher on the plants with *E. cloacae* treatment. Indole acetic acid is known as plant growth hormone that stimulates the growth of lateral roots and root hairs.

Other study showed that the number of panicles per hill could be increased by 92.13% to 110.95% by the treatment of *P. agglomerans* (Khalimi *et al*., 2012). A study done by Hussain *et al*. (2009) showed that the rice plants treated with *Rhizobium leguminosarum* strain LSI-30 could increased the number of panicles per hill by 27.33%. Khalimi *et al*. (2012) reported that treatment with *P. agglomerans* on rice increased the number of grains per panicle by 11.95% to 24.95%. Similar result was also reported by Khorshidi *et al*. (2011) in which the treatment of rice seedling with suspension of *Azosphirillum lipoferum* for 12 h increased the number of grains per panicle by 5.28%. Hussain *et al*. (2009) used the suspension of *Rhizobium phaseoli* to soak rice seedling and proved that this treatment increased number of grain per panicle by 29.21%. In general, treatment of rhizobacteria did not significantly increase the weight of 1000 grains (Khalimi *et al*., 2012; Khorshidi *et al*., 2011; Alam *et al*., 2001). This is probably due to the weight of 1000 grains is stable characteristic of rice.

Treatment with *E. cloacae* significantly increased the yield of rice under green house experiment. All five isolates of *E. cloacae* tested in this study showed similar performances in term of plant growth promotion and rice yield increment. Percentage of yield increase resulted from *E. cloacae* treatment varied from 22.53% to 26.13%. Treatment with *P. agglomerans* on rice local variety, Cicih Medang Selem, significantly increased the yield per hill, in which the weight of grains per hill on rice treated with isolates Pj, Bs and PB were 114.03%, 134.33% and 154.17% higher than that of control (Khalimi *et al*., 2012). Khorshidi *et al*. (2011) reported that treatment of rice seedling with the suspension of *Pseudomonas fluorescens* for 12 h increased the yield by 33.3%. Although the percentage of yield increase in the present study is lower than previous studies with other type of rhizobacteria and other cultivars of rice, however all five isolates of *E. cloacae* tested in this study are promising agents that can be developed as indigenous plant growth promoting agent to increase the rice yield in Bali, Indonesia. For this purpose, the field trial is necessary to evaluate the effectiveness and consistence of the activities of these isolates under field condition.

Table 6: Number of panicles per hill, weight of filled grains per hill and weight of 1000 grains

Treatment	Number of panicles per hill	Weight of filled grains per hill (g)	Weight of 1000 grains
Control	31.58 b*	58.54 b	27.19 a
Al2N	36.75 a (16.37%)	72.04 a (23.06%)	27.11 a
Al3Bt	37.28 a (18.05%)	71.73 a (22.53%)	27.54 a
Al9Sa	37.16 a (17.67%)	73.60 a (25.73%)	27.67 a
Al6G	37.25 a (17.95%)	73.83 a (26.12%)	27.61 a
Al7Kla	37.42 a (18.49%)	73.85 a (26.13%)	27.30 a

* Values in the same column followed by the same letters are not significantly different ($p>0.05$) according to the Duncan's Multiple Range Test at 5% level.

** Values in the parenthesis indicated the percentage of increase when compared to control.

4. Conclusion

Five isolates of *Enterobacter cloacae* namely Al2N, Al3Bt, Al9Sa, Al6G and Al7Kla effectively promoted the growth of rice cultivar Ciherang, a high yielding variety that commonly grown in Bali. These five isolates of *E. cloacae* improved the root system of rice, increased the macro nutrients content in the leaf, increased the content of chlorophyll in the leaf, and increased the number of tillers per hill. Treatment with the isolates of *E. cloacae* increased the rice yield by 22.53% to 26.13%.

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