# POTENTIAL OF BALI'S INDIGENOUS RHIZOBACTERIAL AS PGPR IN SOYBEAN PLANTS

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## ABSTRACT

*Soybean is a strategic commodity with very high demand with huge variations and high value of processed products ranging from feed, food, energy, and industrial raw material. This study was done to determine potential use of Bali's indigenous rhizobacterial to promote growth and increase the yield of soybean under the greenhouse experiment. Five isolates of Bali's indigenous rhizobacterial namely FN1, FN2, FL3, FL4, and FL5 were tested for their effectiveness to promote the growth and increase the yield of soybean. Treatments with isolates of Bali's indigenous rhizobacterial significantly improved the growth of soybean, in which the plant height, diameter of stem, the fresh and dry weight of shoot, the fresh and dry weight of root, content of chlorophyll in the leaf, and the number of nodule significantly (p<0.05) higher on treated plants when compared to control. These treatments were also significantly increased the yield. The number of pod per plant, the weight of pod per plant and content of protein in the seed of treated plants were significantly higher than that of control. Molecular identification based on 16S rRNA gene sequence showed that FN1 and FL5 isolate were similar to Kleibsiella pneumonia with the similarity level at 98%. FN2 isolate was similar to Kleibsiellavariicolawith the similarity level at 99%, FL3 isolate was similar to Proteus mirabilis with the similarity level at 100%, and FL4 isolate was similar to Providenciarettgeri with the similarity level at 99%. These Bali's indigenous rhizobacteria may be further developed as plant growth-promoting agents to increase the yield andprotein content of soybean.*

***Keywords**: acetoin-producing bacteria, yield, protein content, soybean*

## 1. Introduction

Soybean is a strategic commodity with very high demand with huge variations and high value of processed products ranging from feed, food, energy, and industrial raw material (Bantacut, 2017). Soybean is consumed mainly as vegetables and snacks (Konovskyet al., 1994). Soybean has a sweet, nutty flavor and can be eaten as a snack either boiled in saltwater or roasted like peanut seed. Soybean is a rich source of vitamin A, carbohydrates, protein and iron (Kavithamani et al., 2010). Soybeans more nutritious than vegetable green peas (Gu et al, 2003).

Castro et al., (2009) reported that bacterial volatiles such as acetoin can be used for plant-bacteria communicationand as a plant growth promotion triggers.Ryu et al., 2003 reported that bacteria could produce the volatiles compound such as acetoin (3-hydroxy-2-butanone) and 2,3-butanediol have been shown to trigger plant growth enhancement and Induce Systemic Resistance in Arabidopsis. Ryu et al. (2004) reported that several airborne chemicals from certain soil bacteria which are the physically separating PGPR from their host plant have been identified as effective signals for triggering plant growth and ISR. Ryuet al. (2005) proved that treatment by drenching the rhizosphere with either acetoin or 2,3butanediol could increased foliar fresh weight of Arabidopsis by 66% and 43%, respectively when compared to control. Ann et al. (2013) reported that the tobacco seedling treated with 10 ppmacetoin from Bacillus vallismortis EXTN-1 could increase the disease resistance to Pectobacteriumcarotovorum SCC1, the severity of disease was reduced from 90% to 45% when compared to control. This study was done in order to test the effectiveness of Bali's indigenous rhizobacterial to increase the yield and protein content of soybean.

## 2. Research Metodology

### 2.1. Isolation of Bacteria

Bacteria were isolated by using Nutrient Agar (NA) medium. The serially diluted soil samples were platted on the NA medium. After two days of incubation at 32 $^0$C, colonies were subcultured in fresh NA medium.

### 2.2. Identification of Bali's indigenous rhizobacterial

The identification of bacterial isolates was based on the analisys of 16S rRNA. DNA was isolated using the Genejet. DNA amplification was done using 2x Kappa PCR Ready Mix (Kappa Biosystem) with specific primers (63F 5'-CAG GCC TAA CAC ATG CAA GTC-3' and 1387R 5'-GGG CGG WGT GTA CAA GGC-3'). 16S rRNA genes obtained were sequenced using the dye terminator sequence by the V.3.1 cycle sequencing kit. Sequences were compared with a database that was available in NCBI using Blast search engines (http://blast.ncbi.nlm.nih.gov/Blast.cgi). A phylogenetic tree was created using the program MEGA version 6.0, program PAUP version 4.0b, and Maximum Parsimony Method.

### 2.4. Effect of Bali's indigenous rhizobacterial Inoculation on Plant Growth, Yields and Protein Content

Treatment with Bali's indigenous rhizobacterial suspension was done in a greenhouse. This experiment was designed in a randomized block design (RBD) with six treatments namely FN1, FN2, FL3, FL4, FL5 and KT0 (control). Each treatment was replicated four times, thus there were 24 experimental units in this experiment. Each experiment unit consisted of 10 soybean plants. The application of Bali's indigenous rhizobacterial suspension was done once at transplanting time by mixing 2 ml Bali's indigenous rhizobacterial suspension with 50 soybean seed. Soybean seed treatment of Bali's indigenous rhizobacterial suspension and soybean seed without (KT0) were planted in polyethylene bags filled with 5 kg of sterile growth media per bag, consisting of soil and cow manure (3:1, w/w).

The samples for analysis of growth parameters were taken at 15, 30, 45, 60 days after sowing (DAS) to determine the plant height, fresh and dry weight of shoot, fresh and dry weight of root, and leaf chlorophyll content. Leaf chlorophyll content (SPAD unit) was determined with a chlorophyll-meter SPAD-502 (Konica Minolta, Japan). Measurement of yield components such as number of pod per plants and number of seed per pod. Total soluble proteins were extracted using cold phosphate buffer saline (PBS) pH 7.4 (Sambrook and Russell, 2001) in 1 g seed/10 mL buffer ratio. The homogenate was further centrifuged at 14,000 rpm for 30 min. The protein amount in the supernatant was assayed by the dye-binding micro-method of Bradford, using the Roti-Quant reagent from Roth (Karlsruhe, Germany). Total soluble protein content was expressed as mg bovine serum albumin (BSA) per g soybean seeds. All data were subjected to the Analysis of variance (ANOVA) followed by the Duncan's multiple range test (DMRT) at 5% level of significance using SAS software version 6.12 (SAS Institute, Gary, NC, USA).

## 3. Results and Discussion

### 3.1. Identification of Bali's indigenous rhizobacterial

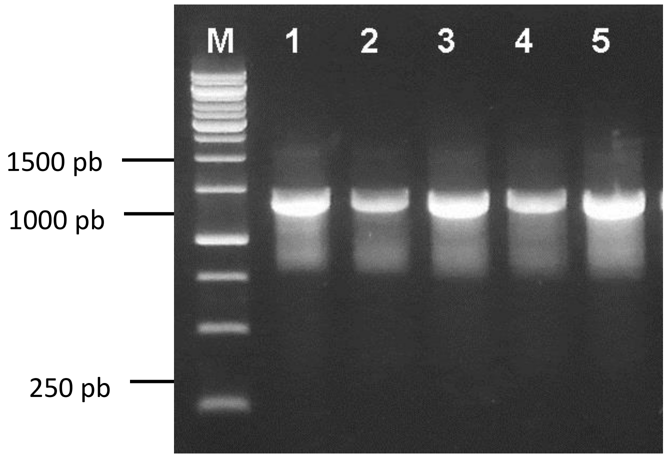


Figure 1. Agarose gel electrophoresis of PCR products obtained by amplifying THE 16S rRNA gene from the genomic DNA of bacterial isolates 1: FN1, 2: FN2, 3: FL3, 4: FL4, and 5: FL5, M: Marker 100 bp DNA ladder.

Molecular identification based on the 16S rRNA gene sequence showed that FN1 and FL5 isolate were similar to *Kleibsiella pneumonia* with the similarity level at 98%. FN2 isolate was similar to *Kleibsiellavariicola*with the similarity level at 99%, FL3 isolate was similar to *Proteus mirabilis* with the similarity level at 100%, and FL4 isolate was similar to *Providenciarettgeri* with the similarity level at 99% (Figure 2).

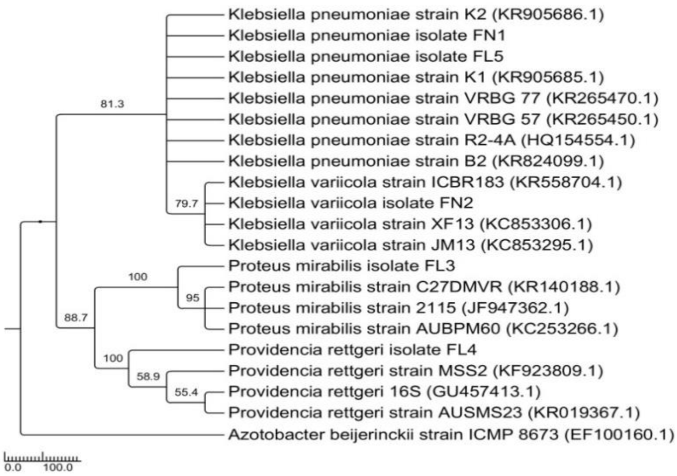


Figure 2. Phylogenetic tree showing the relationships among 16S-rDNA sequences of isolates FN1, FN2, FL3, FL4 and FL5 and the most similar sequences retrieved from databases. The tree was inferred with the Maximum Parsimony method with parameters of Jukes-Cantor. Bootstrap values higher than 70% are shown at the appropriate branching points. The scale indicates the number of nucleotide substitutions per site.

### 3.2. Effect of Bali's indigenous rhizobacterial Inoculation on Plant Growth, Yields and Protein Content

Treatment with *K. pneumonia* A552, *K. variicola* 04, *P. mirabilis*AL3Ba, *P. rettgeri*AL2TT, and *K. pneumonia* 06 significantly increase the plant height, diameter of stem, content of chlorophyll in the leaf (SPAD unit), and number of nodule, dry weight of root, and dry weight of shoot (p<0.05) as presented in Table 1. This result suggested that all five isolates have similar potential as a promoter of soybean growth. Other studies showed that inoculation of strains *Agrobacterium, Burkholderia, Enterobacter,* and *Pseudomonas* could promote the growth of rice (Souza, 2012). Application of *Serratiamarcescens*NBRI1213 significantly increased the plant height, the dry weight 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 the treatment of tomato plants with bacteria resulted in better and bigger growth. The growth of the plant was increased because bacteria produced growth hormones such as auxin, ethylene, cytokinin, and gibberellins which are necessary for plant growth (Husen, 2009)**.** Treatment with *Pantoeaagglomerans*could increase the number of maximum tiller of rice cultivar CicihMedangSelem by 93.27% to 120.19% when compared to control (Khalimi*et al*., 2012). Another study done by Hussain *et al*. (2009) showed that treatment of the root of rice with *Mesorhizobiumciceri*strain CRI-32 increased the number of maximum tillers by 25,34%.

Tabel 1. Effect of Bali's indigenous rhizobacterial Inoculation on plant height, the diameter of the stem, the content of chlorophyll in the leaf(SPAD unit), number of nodules, dry weight of root, and dry weight of shoot.

| Treatments | plant height (cm) | Diameter of stem (mm) | Chlorophyll content (SPAD unit) | Number of nodule | Dry weight of root (gr) | Dry weight of shoot (gr) |
|---|---|---|---|---|---|---|
| Kontrol | 30,25 c | 0,622 a | 38,7 a | 7 a | 1.70 a | 14.60 a |
| *K. pneumonia* A552 | 34,42 b | 1,039 b | 51,2 b | 19 c | 2.15 b | 17.30 b |
| *K. variicola* 04 | 35,12 ab | 1,352 b | 49,7 b | 34 d | 2.25 b | 18.95 c |
| *P. mirabilis*AL3Ba | 34,52 b | 1,350 b | 49,8 b | 18 c | 2.20 b | 18.15 c |
| *P. rettgeri*AL2TT | 37,07 a | 1,854 b | 50,1 b | 18 c | 2.34 b | 19.70 c |
| *K. pneumonia* 06 | 33,82 b | 1,352 b | 47,9 b | 12 b | 1.90 a | 16.25 b |

* 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.

These results are similar to the results of other studies done by Kumar (2011) wherethe replication of *Enterobacter cloacae* PfR8 to the chili 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 a bio-stimulant that could promote plant growth (Koo and Kyung-Suk, 2009). Treatment with two isolates of *Pantoeaagglomerans i.e.* Paj and BS2a could increase the dry weight of the shoot of rice by 377% to 511% (Khalimi*et al*., 2012). Kannan and

Ponmurugan (2010) reported that the treatment of *Azospirillumbrasilense*on rice cultivar CO43 increased the dry weight of root by 41.95%. *Pantoeaagglomerans*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).

Treatment with *K. pneumonia* A552, *K. variicola* 04, *P. mirabilis*AL3Ba, *P. rettgeri*AL2TT, and *K. pneumonia* 06 significantly increase the number of pod per plant, the weight of pod per plant, the water content in seed, and the protein content in seed ($p<0.05$) as presented in Table 2. This result suggested that all five isolates have similar potential as promoter of soybean yields and protein content in seed. 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%. A similar result was also reported by Khorshidi*et al*. (2011) in which the treatment of rice seedling with suspension of *Azosphirillumlipoferum*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 a number of grain per panicle by 29.21%. In general, the treatment of bacteria 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 a stable characteristic of rice. Treatment with *E. cloacae* significantly increased the yield of rice under a greenhouse experiment. All five isolates of *E. cloacae* tested in this study showed similar performances in terms of plant growth promotion and rice yield increment. The percentage of yield increase resulted from *E. cloacae* treatment varied from 22.53% to 26.13%. Treatment with *P. agglomerans*on rice local variety, CicihMedangSelem, 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 the 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 bacteria 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 consistency of the activities of these isolates under field conditions.

Tabel 2. Effect of Bali's indigenous rhizobacterial Inoculation on the number of pod per plant, the weight of pod per plant, the water content in seed, and the protein content in seed

| Treatments | The number of pod per plant | The weight of pod per plant | The water content in seed | The protein content in seed |
|---|---|---|---|---|
| Kontrol | 52.52 a | 25.55 a | 64,99 | 11.06 a |
| *K. pneumonia* A552 | 54.43 a | 28.00 c | 66,41 | 12.97 b |
| *K. variicola* 04 | 60.18 b | 28.21 c | 66,76 | 13.65 c |

| | | | | |
|---|---|---|---|---|
| *P. mirabilis*AL3Ba | 64.10 c | 29.71 d | 67,23 | 14.64 d |
| *P. rettgeri*AL2TT | 61.64 b | 27.71 b | 66,31 | 13.85 c |
| *K. pneumonia* 06 | 53.75 a | 26.10 a | 65,07 | 12.43 b |

* 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

Treatment with *K. pneumonia*A552, *K. variicola* 04, *P. mirabilis*AL3Ba, *P. rettgeri*AL2TT, and *K. pneumonia* 06effectivelypromoted the growth of soybean,

increased the soybean yield by 22.53% to 26.13%, and increased the protein in soybean seed.

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