

The Application of Rhizobacteria and Indigenous Microorganism on Cow Rumen in Soybean Plants (*Glycine max* L.)

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Abstract—Soybeans are national food crop commodity with a high level of consumption per year. Efforts to increase soybean production are accomplished through fertilizer application. The utilization of chemical fertilizers, however, can negatively impact the environment. One alternative to the application of fertilizer is to provide superior microbes that are beneficial to the plants. This study aimed to determine how the application of rhizobacteria and indigenous microorganism in cow rumen affects the growth of soybean plants. This study method included isolate reculture, identification, and selection for soybean plant application using one factor of a completely randomized design method consisting of five treatment levels. The results indicated that the superior isolates, AJ8, had the highest potential utilization as Indole Acetic Acid hormone producer, while MTA1 isolates were phosphate solvent isolates. The identification results using *Microbact* showed that AJ8 isolates had 99% similarity with *Acinetobacter baumannii*, while MTA1 isolates were identified as *Propionibacterium granulosum*. Application of *Acinetobacter baumannii* AJ8 and *Propionibacterium granulosum* MTA1 bacteria in single treatment has more potential to increase the plant height and root length compared to consortium treatment. The potential use of the superior bacteria discovered still needs further study until the biological fertilizer formulations formed can reduce the utilization of chemical fertilizers.

Keywords—soybean, indigenous microorganism, biological fertilizer, *Acinetobacter baumannii*, *Propionibacterium granulosum*

I. INTRODUCTION

Soybean is a national food crop commodity with the highest level of consumption per year (Ministry of Agriculture Strategic Plans 2015-2019). The inability of a local soybean crop to fulfill the food needs causes the government to conduct soybean import. High soybean demand is imbalanced with soybean slow-growing production. Efforts have been made to elevate the soybean production in Indonesia, one of which is by land improvement with a fertilizing system [1].

Fertilization aims to complete nitrogen, phosphate, potassium nutrient element for crops [2]. However, the

administration of chemical fertilizer is inefficient as not all fertilizer administered can be absorbed by crops. Besides, chemical fertilizer will have an impact on soil fertility declination, leaving harmful substance residue in the environment [3] [4] [5]. Technological innovation role in increasing crop productivity should be necessary existed. One alternative way to conduct this is by the utilization of growth hormone-producing microbes and nutrient provider microbes, such as nitrogen and phosphate as the raw material of biological fertilizer production.

Biological fertilizer is a fertilizer that contains living microorganisms to improve nutrients needed, facilitate organic elements in the soil, and increase the plant growth [6]. Biofertilizer has a high potential as an alternative of chemical fertilizers, as it is more environmentally friendly, easy to apply, non-toxic, and more effective application [7], besides contributing to the production cost reduction [8]. Some superior microbes that have been extensively studied and developed as a biological agent to enhance crop growth are rhizobacteria.

Rhizobacteria is a living microorganism in plant root known as Plant Growth Promotion Rhizobacteria (PGPR) [9]. Some rhizobacteria such as *Agrobacterium tumefaciens*, *Agrobacterium rhizogenes*, *Erwinia herbicola*, *Rhizobium*, *Alcaligenes faecalis*, *Enterobacter cloacae*, *Acetobacter diazotrophicus*, and *Bradyrhizobium japonicum* produce a substance of plant growth booster, such as Indole Acetic Acid (IAA) [10]. IAA hormone is a physiological group of phytohormones that play an important role in plant growth [11] [12] [13]. Approximately 80% of bacteria that colonize the rhizosphere have the ability to synthesize IAA because the root exudate secretes tryptophan. tryptophan compounds are inducers for IAA biosynthetic pathways in bacteria [14]. Biotechnological developments have emerged environmentally friendly alternative products such as compound microbial products to enhance plant growth. The development of inoculant product has been widely applied as biological fertilizer derived from Rhizobacteria, i.e.,

Bradyrhizobium japonicum, *Bacillus subtilis*, *Pseudomonas corrugata*, *Bulkhoderia*, *Serratia marcescens* [15] [16] [17] [18].

PGPR treatment to the environment can improve soil physical, chemical, and biological properties. In addition to rhizobacteria, liquid fertilizer from the fermented beef rumen, namely Indigenous Micro-Organism (IMO) of cow rumen have been widely applied in the field experiment and been able to increase crop growth. IMO contains natural microbes that can adapt to any environmental conditions. Content of organic material from IMO cow rumen can improve soil fertility [19]. Ruminant digestion contains microbes that have phytase enzyme that can decompose phytate into inorganic orthophosphate and simple phosphoric group like monophosphate. Also, cow rumen is known to be profitable in agriculture as possessing high cellulose [20], chitinase [21], and methane monooxygenase [22] enzyme activity. The effectiveness of rhizobacteria and indigenous rumen cow needs to be observed further in the form of separate and mixture culture against crop growth. The purpose of this study was to testify the ability of rhizobacteria superior isolates and indigenous rumen cow in single and consortium on the soybean crops. This study is expected to contribute to the development of bio-inoculant of biological fertilizer, which is more effective in increasing soybean production.

II. METHOD

A. Place and Period

This study was conducted on January-April, 2019 in Microbiology and Greenhouse Laboratory, Department of Biology, Universitas Negeri Malang, Indonesia.

B. Isolate Reculture

Two isolates used in this study were AJ8 as Indole Acetic Acid (IAA) hormone-producing rhizobacteria and MTA1 from cow rumen as phosphate solvent bacteria. Isolate reculture was done by inoculating AJ8 and MTA1 isolate on Nutrient Agar (NA) media as the culture assay using quadrant streak plate method. Two isolates were confirmed based on the morphological characteristics using Gram staining method. The pure isolate was then streaked on the slant agar as culture stock.

C. Bacterial Identification

Bacterial identification was made using *Sistem OxidTM Microbact GNB 24E* (Thermo-Fisher Scientific, Waltham, Massachusetts, USA). Bacterial isolates used for physiological assay were rejuvenated on slant Nutrient Agar in 24 hours. The culture was suspended into the physiological salt solution and homogenized using vortex. 0.1 mL of the solution was added into each well of 12A and 12B kit, then incubated for 24 hours at 37°C [23]. Assay profile result was matched using *Microbact 2000* software to obtain bacterial species identification result.

D. Bacterial Culture Effectiveness Assay on Soybean Crop in The Greenhouse

• Crop preparation

Anjasmoro variety soybean seeds were obtained from Balai Penelitian Tanaman Kacang dan Umbi (Balitkabi), Malang. Soybean seeds were soaked and washed using sterile aqua dest, then planted on 2 cm depth in the polybag. Planting

media used soil which had been sterilized using autoclave at 121°C in 1 atm pressure.

• Bacterial culture preparation

One loop of 24-hour bacterial isolates were inoculated in 50 mL of Nutrient Broth medium and incubated for 24 hours. The culture was given 10 mL, with the total bacterial cell of 10^8 cell/mL around the plant roots.

• Study design

This study was arranged using a Completely Randomized Design with six treatments and four replications. Each treatment administration is explained in Table 1.

TABLE I. EFFECTIVENESS ASSAY BIOLOGICAL

No	Code	Treatment	Replication
1	P1	A negative control without bacterial inoculation	4
2	P2	Positive control with NPK fertilizer	4
3	P3	AJ8 bacteria culture administration	4
4	P4	MTA1 bacteria culture administration	4
5	P5	AJ8 + MTA1	4
Total unit assay			24

• Data Analysis

Crops were observed for 14 days after planting based on the root length, plant height, lateral root number, and wet weight. Plant height was measured from the soil surface until the highest tip of leaves, root length was measured from the base root until the tap root end, lateral root number was calculated based on the root branches, and wet weight was measured using a digital scale. Data obtained was analyzed using ANOVA with *SPSS 21.0* software and compared using Duncan test on 5% significant range [24].

III. RESULT AND DISCUSSION

A. Isolate Reculture

AJ8 and MTA1 isolate grew after incubated for 24 hours on NA media. AJ8 and MTA1 isolate characteristics are listed in Table 2.

TABLE II. ISOLATE MORPHOLOGICAL CHARACTERISTICS

Isolate Code	Colony Characteristics					P Solvent Index (mm)	IAA (ppm)
	Shape	Color	Edge	Elevation	Colony diameter		
AJ8	Rounded	Cream	Flat	Concave	1.11	-	38.39
MTA1	Rounded	White	Flat	Concave	0.39	7.66	-

This study used two isolates chosen from the previous study, i.e., AJ8 and MTA1. AJ8 isolate had Indole Acetic Acid (IAA) hormone production ability with 38.39 ppm, while MTA1 was able to dissolve phosphate with the solvent index of 7.66 mm [25]. Based on the Gram staining result, AJ8 isolate had rounded shape, flat edge, concave elevation, and cream colony. Microscopical observation of Gram staining result indicated that AJ8 was included as coccobacillus Gram-negative bacteria (Figure 1a,b). MTA1 isolate morphologically had rounded shape, flat edge, concave elevation, cream color. MTA1 isolate was also included as basil Gram-positive bacteria (Figure 1c,d).

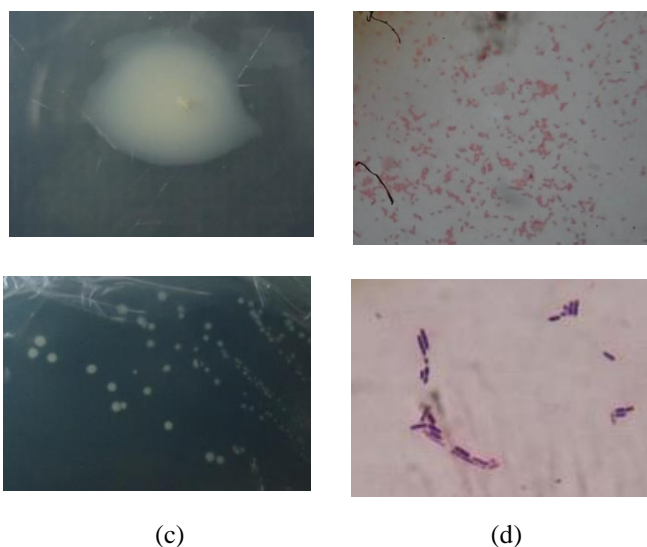


Fig. 1. Morphological characteristics and staining result with 1000x magnification; (a) AJ8 colony morphology, (b) AJ8 microscopic cell, (c) MTA1 colony morphology, (d) MTA1 microscopic cell

B. Bacterial Identification using Microbact

AJ8 isolate identification using *Microbact* had positive test results of motility, lysin, glucose, xylose, citrate, and catalase (Table 3). The profile of test results showed that AJ8 isolate had 99% similarity rate with *Acinetobacter baumannii*.

TABLE III. IDENTIFICATION BACTERIA USING MICROBACT

No	Biochemical Test	Results		No	Biochemical Test	Results	
		AJ8	MTA1			AJ8	MTA1
1	Spore	-	-	18	Malonate	-	-
2	Oksidase	-	-	19	Inositol	-	-
3	Motility	+	+	20	Rhamnose	-	-
4	Nitrate	-	+	21	Sucrose	-	+
5	Lysine	+	-	22	Lactose	-	-
6	Ornithine	-	-	23	Arabinose	-	-
7	H ₂ S	-	-	24	Adonitol	-	-
8	Glukose	+	+	25	Raffinosa	-	-
9	Manitol	-	-	26	Salicin	-	-
10	Xylosa	+	-	27	Arginin	-	-
11	ONPG	-	+	28	catalase	+	+
12	Indole	-	-	29	coagulase	-	-
13	Urease	-	-	30	hemolysis	-	-
14	V-P	-	-	31	Novobiosin sensitive test	-	-
15	Sitrate	+	-	32	Starch hydrolysis	-	-
16	TDA	-	-	33	Casein hydrolysis	-	-
17	Gelatin	-	-				

+ : for positive; - : negative results

A. baumannii is widely reported as having the ability to produce Indole-acetic acid (IAA) hormone by involving IPDC gene through Indole-3-Pyruvic Acid (IpyA) pathway. The role of IAA hormone produced by *A. baumannii* biologically increases microbial interactions with plants, as well as enhancing the crop growth. Some strains of *Acinetobacter* genus are known to be involved in plant growth stimulation. Other strains of *Acinetobacter* show as the plant growth promotion Rhizobacteria (PGPR) [26] [27].

Acinetobacter sp. has a high ability to produce IAA during fermentation with 62.428 ppm [28]. Recent studies reported that *A. baumannii* also controlled IAC gene repression to degrade IAA that allegedly hydrolyzed IAA into two hydroxy-indole-3-acetic acids in subsequent dioxindole-3-acetic acid to the final catechol product. IAA is used as a single carbon source and energy for bacteria [29]. In addition, *Acinetobacter* genus also has the ability to improve the availability of nitrogen elements [30], dissolve mineral phosphate [31], produce siderophore, and assist waste remediation [27]. *Acinetobacter* strain RSC7 is also used as a biofertilizer formulation for *Vigna* plant growth promotor at the seed stage [32].

The positive MTA1 isolation test results using *Microbact* indicated that isolate was identified as *Propionibacterium granulosum*. *P. granulosum* is motile bacteria with the capability of degrading nitrate, glucose, and sucrose, besides producing catalase and synthesizing propionic acid using transcarboxylase enzyme in the fermentation process. *Propionibacterium* is Actinobacteria phylum found everywhere and utilized as a potential biological fertilizer to increase plant growth. Some of *Propionibacterium* capabilities reported are phosphorus and potassium solvent, and nitrogen fixation [33]. *Propionibacteria* are also involved in nitrogen element fixation at legume crops under symbiotic conditions and free-living [34]. *P. granulosum* MTA1 has the ability to dissolve the highest phosphate content either in solid or liquid medium as mentioned in the previous study, contributing the sustainable farming. Bacterial phosphate solvent is considered important in the agricultural field due to the ability to enhance crop growth, decompose organic matter, increase plant nutrient cycle, and reduce chemical fertilizer utilization [35].

C. Bacterial Application on Soybean Crop

AJ8 and MTA1 isolate assay as soybean sprout growth agent was done by inoculating the isolates into Anjasmoro variety soybean sprouts. Soybean sprout growth was observed for 14 days. The analysis results of root length and plant height indicated a significant influence of AJ8 (P3) and MTA1 (P4) isolate treatment. Single bacterial culture treatment can increase the plant height by 20-25% (table 3), while the combination of AJ8 + MTA1 isolate (P5) had a significant effect on the plant height by 15% compared to the negative control (Table 4).

TABLE IV. EFFECT OF AJ8 AND MTA1 BACTERIAL TREATMENT IN SOYBEAN

Treatment	Plant height (cm)	Primer roots length (cm)	Number of lateral roots	Wet weight (g)
K- (P1)	37.83 ^a	5.33 ^a	22.00 ^a	1.74 ^a
K+ (P2)	40.66 ^{ab}	7.60 ^{ab}	23.66 ^a	1.89 ^a
AJ8 (P3)	45.93 ^b	10.66 ^b	23.66 ^a	2.18 ^a
MTA1 (P4)	45.23 ^b	10.10 ^{ab}	22.33 ^a	1.88 ^a
AJ8 + MTA1 (P5)	42.56 ^{ab}	5.50 ^a	23.66 ^a	1.86 ^a

The number followed by different letters in the column showed significantly different results based on the Duncan test at $\alpha = 0.05$

A. baumannii AJ8 treatment (P3) was able to give a significant effect on the root length; however, it had no significant effect on the lateral root number. This was suspected that IAA hormone produced by bacteria only affected the root length. Exogenous generated IAA hormone affects the lateral and adventitious root growth [10]. IAA-induced roots with high range administration between $[10^{-4} - 10^{-6}]$ M will inhibit the primary root length and form the root hair, while primary root growth is stimulated with low IAA administration, i.e. $[10^{-9} - 10^{-12}]$ M.

The consortium treatment of *A. baumannii* AJ8 and *P. granulosum* MTA1 (P5) did not affect the root length, lateral root number, and plant wet weight. Consortium treatment was unlike the single culture with a significant effect on the plant height and primary root length. The consortium bacterial treatment in one culture did not necessarily have a significant influence on plant growth because bacteria produce metabolite compound suspected to inhibit other bacteria activity. Thus *A. baumannii* AJ8 and *P. granulosum* MTA1 bacteria will be more efficiently utilized in a single treatment to enhance the plant growth. This study is the preliminary part limited to the bacterial identification and applications in plants. It is necessary to confirm the bacterial molecular identification, and further studies are needed to discover IAA hormone-producing bacteria and phosphate solvent activity in crops until reaching the seed production phase.

IV. CONCLUSION

The identification result using Microbact™ 24E indicated AJ8 isolate had 99% similarity with *Acinetobacter baumannii* AJ8 and MTA1 isolate was identified as *Propionibacterium granulosum*. *A. baumannii* AJ8 and *P. granulosum* MTA1 in the single treatment had a more potential increase in plant height and primary root length compared to consortium treatment. Further studies are necessary for molecular bacterial identification and application on the crop until reaching the production phase.

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