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Research article

Optimizing Plant Growth-Promoting Rhizobacteria to Improve the Relationship Between Above and Below Ground Biodiversity

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Abstract.
Biodiversity plays a crucial role in ensuring environmental sustainability. Plant-rhizosphere microbiome interactions are ecologically important because they can alter the biological, chemical, and physical properties of the soil, ultimately affecting plant productivity. To maintain soil function, bio-ameliorant technologies, new modern composting crop residues, and limited use of synthetic pesticides have all been used to boost microbial activity in the soil. Soil management biotechnology is the use of microbes to maintain soil function, resulting in increased plant productivity. It offers a novel way to deal with a wide range of issues that are currently unsolved by conventional technology. It is thought that an improved relationship between above and below ground biodiversity can contribute to the re-establishment of essential biological functions. The Indonesian Research Institute for Biotechnology and Bioindustry (IRIBB), PT Riset Perkebunan Nusantara, has been working on developing plant growth-promoting rhizobacteria (PGPR) as a bio-ameliorant to improve the relationship between above and below ground biodiversity for sustainable management and increased plant productivity.

Keywords: bio-ameliorant, biodiversity, biotechnology, rhizosphere, soil microbes

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1. Introduction

Business development efforts in the plantation and agriculture sector currently have considerable challenges. In addition to scarce cultivable land and climate change, some issues that must be addressed are environmentally friendly demands, increase fertilizer price, overuse of pesticide, high labor costs, stagnant productivity, stagnant selling prices for plantation products, and decline of profits per hectare. In this regard, an integrated breakthrough is required to lessen the impacts of the conditions toward agricultural actors, either in the scale of a company or an independent farmer, and eventually achieve a sustainable profitability.

A biotechnological approach is then introduced in the practices of soil management providing a novel path on solving problems that remain unanswered by current conventional technology. Utilization of soil microbes is an effective method for stabilizing soil aggregates [1], increasing nutrient uptake, controlling soil-borne pathogens, and accelerating solid organic wastes decomposition [2] with less pollutant added to the environment. Bio-ameliorants are basically microorganisms that can improve nutrient availability to plants. They are thought to be an important part of sustainable agriculture because they can significantly decrease the number of chemical fertilizer application. Furthermore, microbial inoculants can enrich the microbial biodiversity in the rhizosphere thus increasing soil microbial activity [3]. Therefore, it is hypothesized that by increasing rhizospheres microbial activity through bio-ameliorant application, nutrient solubilization can be improved, leading to less chemical fertilizer application. Plant rhizosphere is a soil area adjacent to a plant's root which is satiated with soil microbial activity [4] resulted from bacteria and fungi as the dominant groups [5] demonstrating crucial role on rhizosphere's ecology. Soil microorganisms that live in the rhizosphere, some of which are aggressive plant roots colonizer and some are plant growth promoter, are designated as plant growth promoting rhizobacteria (PGPR) [6].

Soil microorganisms especially bacteria and fungi play an important part on providing vital service to all ecosystems, such as cycling the nutrient and organic matter in the soil, sequestering carbon, regulating greenhouse gasses [7], bioremediating of toxics and pollutants [8], and increasing the amount soil nutrient availability for crops. In the tropical region, the diversity of the soil microorganism (bacteria and fungi) is greater than that of other soil organism. Improvement of biological diversity of both above (crops) and below-ground (soil microbial) properties can benefit the environment not only at ecosystem but also agricultural level. This paper discusses the development technology and commercialization implemented at the Indonesian Research Institute for Biotechnology and Bioindustry (IRIBB), PT Riset Perkebunan Nusantara, on developing plant growth-promoting rhizobacteria (PGPR) i.e., *Burkholderia cenocepacia*, *Azotobacter beijerinckii*, *Pseudomonas fluorescens*, and *Trichoderma polysporum* as a soil bio-ameliorant to improve the relationship between above and below ground biodiversity.

2. Methodology

2.1. Development of soil bio-ameliorant technology

The results of our laboratory's research (2009-2011) generated one prototype of a soil bio-ameliorant product, namely AggreStab. Development of an up-scaled AggreStab production technology was carried out using the core paradigm for market-driven

goals that are simple, cheap, sourced from abundant materials, and efficient output. The pilot-scale production technology of AggreStab soil bio-ameliorant (2011-2016) is an intermediate step before entering commercial scale which started from laboratory, greenhouse, and field experiments as a series of its technology developments. Briefly, the main stages to produce soil bio-ameliorant were: (i) inoculating the bacteria into a specific medium as a starter and its propagation into industrial medium capacity, (ii) incorporating the cultured microbes onto a pasteurized-minerals and (iii) packaging.

2.2. Exopolysaccharide of PGPR bacteria

The main exopolysaccharide bacteria used as active ingredient of a soil bio-ameliorant is *B. cenocepacia*. The bacteria produce exopolysaccharide in average of 5.03 mg ml^{-1}. Moreover, *B. cenocepacia* is capable in fixing atmospheric N_2 non-symbiotically that measured with acetylene reduction analysis (ARA) with the value of 0.73 µmol g^{-1}. Also, it can produce indole acetic acid (IAA) hormone of 78.9 ppm and can survive on pH 3–7 for 6–12 months [9]. Total population of exopolysaccharide bacteria in the mineral matrix were estimated using total plate count method in ATCC 14 media.

2.3. Propagation PGPR as an active ingredients of soil bio-ameliorant

Burkholderia cenocepacia was prepared by the following steps: inoculation of the bacteria into 500 ml shake flasks containing 150 ml the American Type Culture Collection (ATCC) No. 14 medium which incubated on orbital shaker at 150 rpm, 30°C for 7 days. Following incubation, the total cell count of exopolysaccharide bacteria should achieve 10^8 cell ml^{-1} which were counted using total plate count method on solid ATCC No. 14 medium. The medium consists of (L^{-1}): 0.8 g K_2HPO_4, 0.2 g KH_2PO_4, 0.1 g $CaSO_4.2H_2O$, 0.2 g $MgSO_4.7H_2O$, 2.0 mg $FeCl_3$, $Na_2MoO_4.2H_2O$ (trace), 0.5 g yeast extract, 20 g sucrose, 15 g bacto agar, and final pH 7.2 [10].

Azotobacter beijerinckii. Burk's N-free medium comprises of (L^{-1}): 0.41 g KH_2PO_4,10 g glucose, 0.52 g K_2HPO_4, 0.05 g Na_2SO_4, 0.2 g $CaCl_2$, 0.005 g $FeSO_4.7H_2O$, 0.1 g $MgSO_4.7H_2O$, 0.0025 g $Na_2MoO_4.2H_2O$. Agar concentration for semi- and solid medium were 1.8 g and 15 g, respectively. pH was adjusted to 7.0 ± 0.1 before autoclaving at 121°C for 15 min [11][12].

Pseudomonas fluorescens. King's B medium consists of 10 g proteose peptone (DIFCO), 1.5 g anhydrous K_2HPO_4, 15 g glycerol, 5 mL $MgSO_4$ (1 M; sterile), antibiotics (as needed). Add H_2O to first three ingredients to bring volume to 1 L. Adjust the pH

to 7.0 with HCl. Autoclave and then add 5 mL of sterile 1 M $MgSO_4$ [13], whereas *T. polysporum* fungus was propagated on potato dextrose agar (DIFCO).

2.4. Description of the PGPR product ``AggreStab''

AggreStab was produced in a shape of 2-5 mm granule that mixed with minerals and amino acids as its carrier and was packed in a 25 kg bag (Fig 1). AggreStab contains a PGPR culture of non-pathogenic and endophytic bacteria *Burkholderia cenocepacia*, *Pseudomonas fluorescens*, *Azotobacter beijerinckii* (10^6-10^8 cfu g^{-1}), and *Trichoderma polysporum* (10^4-10^6 propagule g^{-1} of product). Utilizing mineral and amino acids materials, AggreStab is considered slow-release soil bio-ameliorant, environmentally friendly, and harmless to human as well.

Figure 1: AggreStab soil bio-ameliorant in granular form with one-year stable quality guarantee.

Field experiments were carried out in plantation (sugarcane and oil palm) and food (paddy and corn) crops as well as horticulture (potatoes, chili, garlic, red onion, cabbage, tomatoes, and leek). However, this paper will only focus on crop plantations field experiments which were carried out at PT Astra Agro Lestari (Central Kalimantan), PT Bumitama Gunajaya Agro (Central Kalimantan), and PT Buana Karya Bakti (South

Kalimantan). The treatments included a combination of NPK and AggreStab applications with a reduction of NPK fertilizer dosages.

3. Result and Discussion

Our previous research indicated that the use of multi-isolate inoculum was more efficient in terms of soil nutrient improvement, availability of aggregates, and stable aggregate formation than that of a single isolate. The selected bacteria species can stimulate the formation of stable soil aggregate and enhanced water and nutrient retention. Some of them are capable of improve the solubilization of other nutrients with the intention of increasing fertilizer utilization efficiency on marginal soils.

Burkholderia cenocepacia, *P. fluorescens*, *A. beijerinckii*, and *T. polysporum* are the active ingredients that are endophytic for target plants, can reduce heavy metals, able to grow at pH 3-7, and able to increase water retention 11.2-61.6% [1]. In addition, in vitro analysis shows that the PGPR bacteria can inhibit Ganoderma species by using a cell lysis mechanism of the young growth mycelium of Ganoderma (Fig 2.). Moreover, *T. polysporum* can inhibit the growth of mycelium *Fusarium solani* and *F. oxysporum* by 65.5-67.9%; *Ganoderma* by 50-78.7%; and *Rigidoporus lignosus* by 74.1-87.4%.

Basic research to quantify and evaluate the physical, chemical, and biological processes in soil involving soil micro-organisms must continue to be carried out to provide in-depth scientific research on soil quality improvement and address problems related to soil quality. The results of this study on the role of exopolysaccharides in increasing soil aggregate, especially as a chelating agent, indicating that exopolysaccharide *B. cenocepacia* contains O-H (hydroxyl) and C=O (carbonyl) groups as showed FTIR spectrum analysis in the range of 3403-3400 cm^{-1} and 1651-1636 cm^{-1}. Furthermore, the analysis at 1126 cm^{-1} further confirmed that those groups have α and β configurations [14].

Since 2015, the soil bio-ameliorant AggreStab has being produced commercially at 20 tons day^{-1} capacity. An up-scaled plant has been constructed at the workshop of the Indonesia Research Institute for Biotechnology and Bioindustry, PT Riset Perkebunan Nusantara. In 2017-2019 the development technology of soil bio-ameliorant was supported by Project Management of the Ministry of Research and Technology, the Republic of Indonesia, to develop the commercial market. Unlike chemical fertilizer, AggreStab soil bio-ameliorant will only be produced after the users order the product since microbes of AggreStab soil bio-ameliorant have a shelf-life of about 12 months.

The application of AggreStab has shown that stable soil aggregate is formed thus increase the soil fertility. It also able to cut off chemical fertilizer application up to 50. The other benefits of AggreStab are:

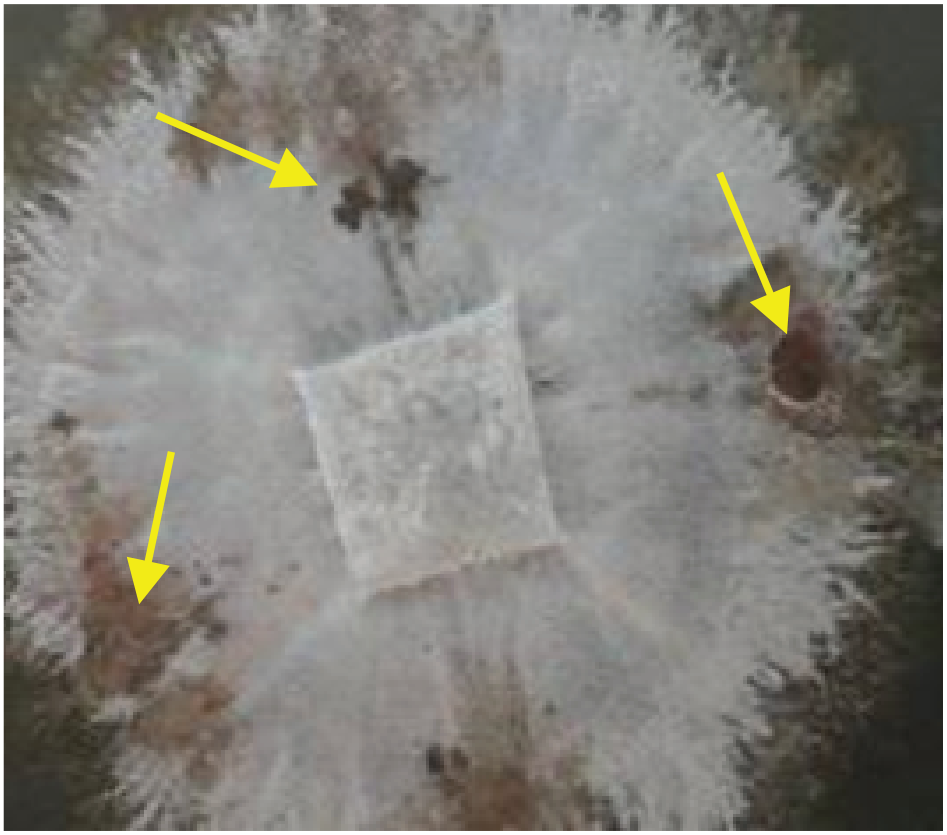

Figure 2: The PGPR of *B. cenocepacia* can inhibit Ganoderma species by using a cell lysis mechanism (arrow).

1. Application cost reduction by 10-30% saving on fertilizer cost.
2. Environmentally friendly practice.
3. Applicable to any crop.

Finally, AggreStab application can cut off costs on storage, labor and transportation as the extra benefi. Although the benefits offered are remarkable, the implementation of AggreStab bio-ameliorant has some constraints. Being a new product type, it raised doubt about its effectiveness compared to conventional products. There are a vast number of different microorganisms in bio-ameliorant products. They tend to be heavily promoted, which is a great need for a standard for accurate and straightforward ways of measuring their effectiveness. Therefore, the successful commercialization of such a product needs a smart strategy to be taken. This product has been legally registered at the Ministry of Agriculture with registration number 398.OL/Kpts/SR.310/B/10/2020 for commercial production and marketing purposes.

Application of soil bio-ameliorant in the form of exopolysaccharide-producing bacteria to make soil aggregate more stable by bio-augmentation technique has been widely accepted. The field experiment has been organized using randomized complete block design. Field experiment was examined on the basis of mature oil palm production.

The application of 1.5 kg of AggreStab tree^{-1} year^{-1} combined with a 75% NPK fertilizer gave better yields of fresh fruit bunches weight and N uptake of oil palm leaves when compared to treatment with 100% dosage of NPK. The use of soil bio-ameliorant with the active ingredient exopolysaccharide PGPR bacteria combined with a reduction in the dosage of NPK fertilizer by 25-50% is predicted to cut fertilizer application costs by 8.6-48.9% ha^{-1} year^{-1} (specific location) with a production yield higher than or equal to the 100% dosage of NPK standard fertilizer treatment (Table 1).

TABLE 1: Observation of production and yield of AggreStab application at demo plots in Central Kalimantan and South Kalimantan.

Treatments	Fresh Fruit Bunch (Ton ha^{-1})					
	PT Astra Agro Lestari, Tbk (Soil texture)	PT Astra Agro Lestari, Tbk (Peat soil)	PT Bumitama Gunajaya Agro (Soil texture) Mature-plant	PT Bumitama Gunajaya Agro (Soil texture) Immature-plant	PT Buana Karya Bakti Soil Texture)	Average
Control	24.3	10.8	20.9	6.96	11.67	14.92
AggreStab	26.6	15.4	22.4	8.05	13.52	17.19
Increase in ton	2.3	4.6	1.53	1.09	1.86	2.28
Increase in percent	9.5	42.6	7.3	15.7	15.9	15.3
	Yield of palm oil					
	PT Astra Agro Lestari, Tbk	PT Astra Agro Lestari, Tbk	PT Bumitama Gunajaya Agro	PT Bumitama Gunajaya Agro	PT Buana Karya Bakti	Average
Yield of palm oil (%)	24	24	22	19	19	22
Control (kg)	5,832	2,592	4,591	1,322	2,216	3,311
AggreStab (kg)	6,384	3,696	4,928	1,530	2,570	3,821
Increase in kg	552	1,104	337	207	353	511
Increase in percent	9.5	42.6	7.3	15.7	15.9	15.4

This microbes-based technology must be thoroughly studied and enhanced to achieve the expected results in order to be fully adopted by the farmers and the stakeholders in crop plantation and agriculture. Therefore, quantified commercial production, strain improvement, and other research topics are being considered. Furthermore, governments and federal agencies should promote the PGPR-based soil bio-ameliorant as environmentally friendly crop improvement alternatives. Moreover, entrepreneurs should put more infestation into their businesses in the PGPR industry and financial assistance for new companies. Apart from that, a large number of efforts

is required to educate farmers and consumers in order to achieve public awareness on the advantages of using soil bio-ameliorant.

4. Conclusion

Crop plantation and agriculture, among the various sectors present within a country, play a critical role in the survival and the fulfilment of the demands of exports and a growing population. As a form of below-ground biodiversity, soil organisms provide a wide range vital services to all ecosystems, such as cycling the nutrient and organic matter in the soil, sequestering carbon, and regulating greenhouse gasses.

In Indonesia, a lack of intensive microbial activity in marginal soils can be overcome by introducing beneficial microbes as soil bio-ameliorants. AggreStab soil bio-ameliorant containing indigenous PGPR inoculants from Indonesia is a cost-effective, eco-friendly, and renewable products and it can reduce the use of chemical fertilizers to promote sustainable practices within agriculture and plantation systems. AggreStab production technology was developed using efficient principles of bioprocess technology. This product has legally been registered to The Ministry of Agriculture for commercial production and marketing. Through appropriate management of PGPR as a soil bio-ameliorant in agriculture and plantation, the cultivation efficiency in plantation crops, food crops, and horticulture could be increased by reducing conventional fertilizer dosages and maintaining soil fertility.

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