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Preface

Over the past decades, rapid growth of global economic has lifted millions of people out of poverty. In line with rising population, rapid urbanization, and industrialization, it has also led to increase consumption of resources and generate of waste almost beyond the limits of the ecological carrying capacity.

The coming decades will likely witness of the increasing pressures on industries to shift to more resource-efficient and low-carbon production processes as part of global efforts to sustain growth, conserve resources and slow down the pace of climate change. Countries and regions that successfully manage this transition will get a better position to exploit the opportunities created by the shift towards a low-carbon world economy. It is green industry's initiation, a pattern of industrial development that is sustainable in economic, environment and social.

Universitas Pembangunan Nasional "Veteran" Yogyakarta in conjunction with its global partners is proud to announce the International Conference on Green Agro-Industry, to be held on November 11-14, 2013, at Yogyakarta, Indonesia. The basic aim of the conference is to contribute to the development of highly productive methods and technologies for the various segments of the agro-industries. This conference is designed to provide a forum for the presentation, discussion and debate on state-of-the-art and emerging technologies in the field of agro based industry and any issues related to sustain the environment.

Finally, we would like to express our gratitude to the Rector UPN “Veteran”, Yogyakarta for the financial support, the Dean of the Faculty of Agriculture for hosting, and the Scientific and Steering Committee. We wish to thank the keynote speaker Director of PT Astra Agro Lestari Tbk and Plenary Speakers: Prof. Sakae Shibusawa (Tokyo University of Agriculture and Technology, Japan), Prof. Raj. Khosla, Ph.D. (Colorado State University, USA), Prof. Dr. Nilda Burgos (University of Arkansas, USA) Ir. Toine Hattink (Director of Department of Horticulture, HAS den Bosch, Netherlands) Prof. Dr. Endang Gumbira Sa’id (Bogor Agricultural University, Indonesia) . Nur Iswanto, PhD. (IKAGI, International Society of Sugar Cane Technologists Councillor), Prof. Wijitapure Wimalaratana. (Department of Economics, University of Colombo), Prof. Hassan M. El Shaer (Desert Research Center, Cairo, Egypt), Dr. Mofit Eko Poerwanto (UPN “Veteran” Yogyakarta, Indonesia) as well as participants for their contribution in making the International Conference on Green Agro-Industry.

We wish to thank PT Astra Agro lestari as the major sponsor and all other sponsors for their contribution in making this Conference possible. As a Chairperson, I highly appreciate the great efforts of the members of the organizing committee whose hard work made this seminar a great success.

Yogyakarta, November 11 , 2013

Sri Wuryani

Chairperson, ICGAI 2013

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POTENTIAL OF THERMOTOLERANCE ISOLATES BACTERIA FROM THE LAND THAT AFFECTED BY MERAPI ERUPTION AS A PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR)

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ABSTRACT

Merapi vulcano erupted on October-November 2010 and destroyed any vegetations on the land affected by the eruption. A month after erupted, some plants: bananas, bamboos, grass, keladi starting to growth. There was an association between plant root and microorganisms. The present study was conducted to obtain a thermo tolerance isolates bacteria and to describe the potential of the isolates as a PGPR. The bacterial were isolated from rhizosphere of the start growing plants. The viability isolates were tested gradually on incubation temperature from 55°C to 100°C for 24 hours. The isolates that viable on 100° C, designated as a thermo tolerance bacteria. The parameters which were analysis were IAA production, nitrogenase activity, phosphate solubilization, and pH. A total of 9 thermo tolerance bacterial isolates had been isolated. According to the sequence of their 16S-rDNA, they were identified as *Bacillus* sp. (5 isolates), *Paenibacillus* sp. (2 isolates) and *Arthrobacter* sp. (2 isolates). All of the thermo tolerance isolates were able to produce IAA. The production of IAA ranges from 6.5 mg L^{-1} (in *Bacillus* B1e) to 75 mg L^{-1} (*Bacillus* B1b), where as the capability of isolates to solubilizing phosphate ranges from 0.95 mg L^{-1} to 1.37 mg L^{-1}. All of the isolates had relative low ability to fixed N2.

Key words: *thermo tolerance, bacteria, Merapi-eruption, PGPR*

INTRODUCTION

Merapi volcano erupted on Oktober-Nopember 2010. The eruption ejected a tremendous amaount of volcanic material on and around the volcano. Wide agricultural areas araound the volcano were destroyed by the volcanic materials. A month after eruption, some plant: bananas, bamboos, grass, *keladi* starting to growth. They would be a thermotolerance plants. There was an association between plant root and microorganisms. The associated bacteria must be has same characteristics, thermo tolerance also.

Rhizosphere is the soil found around the root and under the influence of the root. It is a site with complex interactions between the root and associated microorganisms (Sylvia *et al.* 1998). Plant–bacterial interactions in the rhizosphere are the determinants of plant health and soil fertility. Plant growth-promoting rhizobacteria (PGPR) are free-living soil-borne bacteria that colonize the rhizosphere and enhance the growth of plants

(Kloepper 1980). These organisms affect plant growth directly by fixation of atmospheric nitrogen, solubilization of minerals such as phosphorus, or by production of plant growth regulators (phytohormones), or indirectly by improving growth-restricting conditions either via production of antagonistic substances, by inducing resistance against pathogens, and by improving soil properties by leaving organic residues (Khan et al., 2009).

The role of microbial as a PGPR in productivity of crops not fully exploited for the benefit of crop improvement under different agro-ecosystems. The beneficial PGPR in plant growth promotion are very often region-specific besides soil-specific in natural ecosystems (Saharan and Nehra, 2011). PGPR have resulted in positive responses under controlled (laboratory and greenhouse) conditions; however, natural variations make it difficult to predict how PGPR may respond when applied to field conditions.

The present study was conducted to obtain a thermo tolerance isolates bacteria and to describe the potential of the isolates as a PGPR This research very attractive to be done because the thermo isolates have been isolated are indigeneous bacteria from locally region that affected by volcanic eruption. These isolates could be suitable applied in the regions that potentially affected by volcanic eruption, that are to be found in many regions in Indonesia. Volcanic eruption is repeated. The isolates bacteria may be still viable when eruption repeating, because they are thermo tolerance bacteria.

MATERIALS AND METHODS

A. Isolation and identification

The bacterial were isolated from rhizosphere of the start growing plants. The viability isolates were tested gradually on incubation temperature from 55°C to 100°C for 24 hours. The isolates that viable on 100° C, designated as a thermo tolerance bacteria. The isolates were identified based on the 16S rDNA gene sequences. 16S rRNA gene was amplified using the set of primers 27F (*Escherichia coli* position 8-27, 5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492R (*Escherichia coli* position 1510-1492, 5'-GGC TAC CTT GTT ACG ACT T-3'). DNA sequence of the 6S rDNA fragments was compared using BLASTN at http://www.ncbi.nlm.nih.gov/BLAST/.

B. Determination of IAA production (Gravel et al., 2007)

Auxin (IAA) production by the isolated bacteria was initially determined through colorimetric analysis using Salkowski's reagent. Liquid cultures were prepared in Nutrient Broth supplemented with L-tryptophan. The culture was mixed vigorously with Salkowski's reagent. The mixture was incubated at room temperature for 20 min and the absorbance was measured at 535 nm. The concentration of IAA was evaluated by comparison with a standard curve.

C. Determination of Nitrogenase Activity (Belimov *et al.*, 1995).

Nitrogenase activity was analyzed by acetylene reduction assay (ARA) method. Liquid cultures were prepared in Jensen's N-free. Atmosphere containing 10 % acetylene (v/v), which was achieved by removing air and replacing with equal volume of acetylene,

were added to the vials. At 24 hour intervals the gaseous phase was injected into a gas chromatograph (GC) equipped with flame ionization detector (FID) and a Porapak N column, in order to assay ethylene concentration.

D. Determination of phosphate solubilization (Grover, 2003; Nenwani *et al.*, 2010) and pH

Liquid cultures were prepared in Pikovskaya's medium were added Ca3(PO4)2. Soluble P of the culture was analyzed by chlorostannous reduced molybdophosphoric acid blue method. The blue color intensity of the solution was measured at 600 nm. The pH of the cultures was measured by pH meter.

RESULT AND DISCUSSION

A. Isolation and identification

A total of 11 thermo tolerance bacteria had been isolated from rhizosphere of bamboos or grass. Based on the examination on the similarities of the cells and colony morphology of isolates, nine have been selected. The identification of isolates bacterial are presented in table 1.

Table 1. Thermo tolerance bacteria isolated from a rhizosphere of bamboos or grass

Strain no.	Code	Identify by 16S rRNA sequence	Isolates sources
1.	B1b	*Bacillus*	Rhizosphere of bamboos
2.	B1c	*Arthrobacter*	Rhizosphere of bamboos
3.	B1L	*Arthrobacter*	Rhizosphere of bamboos
4.	R32	*Paenibacillus*	Rhizosphere of grass
5.	R36	*Paenibacillus*	Rhizosphere of grass
6.	R310	*Bacillus*	Rhizosphere of grass
7.	Rtn36	*Bacillus*	Rhizosphere of grass
8.	Btn59	*Bacillus*	Rhizosphere of bamboos
9.	B1e	*Bacillus*	Rhizosphere of bamboos

According to the sequence of their 16S-rDNA, they were identified as *Bacillus* sp. (5 isolates), *Paenibacillus* sp. (2 isolates) and *Arthrobacter* sp. (2 isolates). *Bacillus* and *Paenibacillus* are gram-positive, aerobic or facultativeaerobic, they are spore-forming bacteria therefore *Bacillus* and *Paenibacillus* survival when Merapi erupted. *Bacillus* and *Paenibacillus* commonly found in rhizosphere. Garbeva et al., 2003 (in Antoun and Prevost) showed that the majority (95%) of gram positive bacteria are *Bacillus* and *Paenibacillus* species. *Arthrobacter* is gram positive bacteria minority in soil.

B. Determination of IAA production

Figure 1 represents the IAA production of isolates at 0, 2 and 4 days of incubation. All the isolates showed differential capacities to produced phytohormones Indole-3-acetic acid (IAA). The highest amount of IAA was produced by the strain *Bacillus* B1b. The IAA production of the isolates ranging from 6.5 mg L^{-1} (in *Bacillus* B1e) to 75 mg L^{-1}

(*Bacillus* B1b). Egamberdieva (2008) reported that ranging from 0.9 mg L^{-1} to 1.75 mg L^{-1} of IAA was produced by *Bacillus* strain, isolated bacteria from rhizosphere of wheat, whereas Erturk et al. (2010) showed that *Bacillus* and *Paenibacillus* were found to produce IAA in concentration of 33.6 and 32.8 mg L^{-1}, respectively.

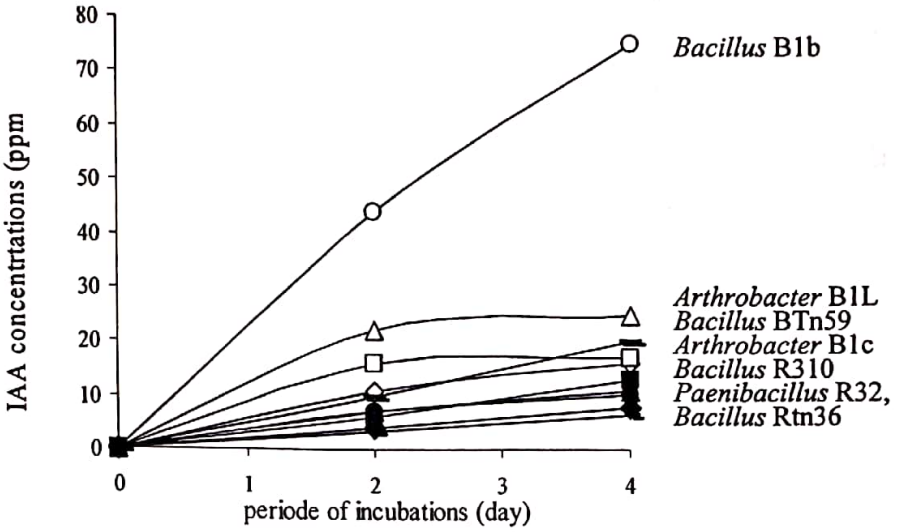

Figure 1. Production of phytohormones by thermo isolates

The major factor considered to be responsible for growth promotion by plant growth-promoting bacteria is auxin production. *Bacillus* sp. has been widely studied as plant growth promoting rhizobacteria producing phytohormones. Effect of the bacterial strains on the growth of the plants was studied by inoculating *S. tuberosum* sprouts and comparing various growth parameters of inoculated plants with non-inoculated treatments. Bacterial inoculation improved the plant growth by increasing shoot length approximately 40% and root length up to 42%, approximately 66% increase in the number of leaves, as compared to the control plants. Enhanced mineral and nutrient uptake ability due to bacterial inoculation promotes plant growth (Ahmed and Hasnain, 2010). Auxins act as long-distance signals controlling many developmental processes of the plants either directly or indirectly. Stimulatory effects of auxin-producing bacteria on root morphogenesis and development have been reported in various studies, which results in enhanced root surface area and increased root elongation. Increase in root length and surface area stimulates efficient water and nutrient uptake, which in turns effects the overall development and growth of the plants (Etesami et al., 2009). (Kidoglu et al., 2007).

C. Determination of nitrogenase activity

Figure 2 and 3 represents the nitrogenase activity and cells population of the thermo isolates. The result show that *Paenibacillus* R32, *Bacillus* R310 and *Bacillus* Bie had better nitrogen fixation ability than the other isolates. The nitrogenase activity of *Paenibacillus* R32, *Bacillus* R310 and *Bacillus* Bie were 0.61; 0.88 dan 0.54 µmol etilen/ml culture/day respectively, whereas the ability of the other isolates ranging from 0.15 to 0.17 µmol etilen/ml culture/day. Cell population of *Paenibacillus* R32, *Bacillus*

R310 and *Bacillus* Bie were log 6.3; 7.5 dan 7.9, respectively; whereas population of the other isolates ranging from log 6.6 to7.95.

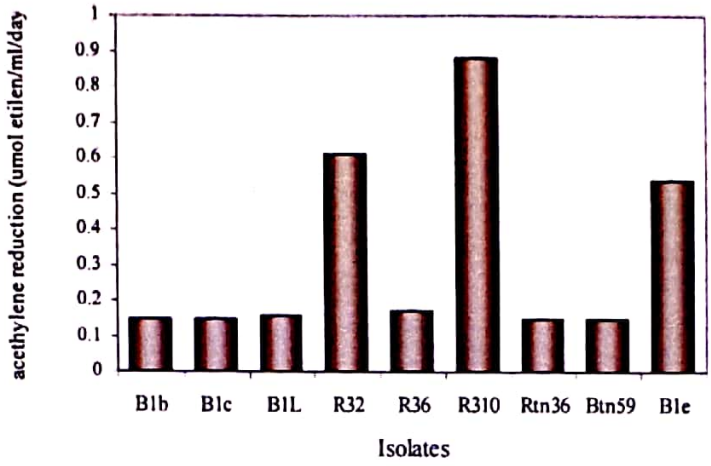

Figure 2. Nitrogenase activity of the thermo isolates

Nitrogen is required in large quantities by most agricultural plants throught their growth period. Many different N_2 fixing bacteria have been isolated from the Rhizosphere. Soil microorganisms can provide nutrients to plants through the fixation of atmospheric N_2. Numerous studies have shown that different species of bacteria fix atmospheric N_2 and consequently affect growth and yield of various crops (Khalid et al., 2009).

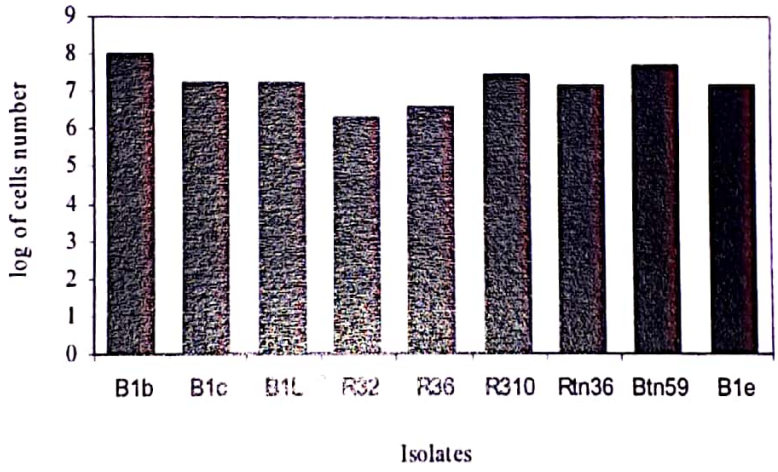

Figure 3: Cells number of the isolates

D. Determination of phosphate solubilization

Figure 4 and 5 represents the phosphate solubilization ability of the selected thermo isolates and pH changes at four weeks of incubation. Based on the examination on growth of the thermo isolates on Pikovskaya's solid medium, five isolates have been selected. The highest amount of soluble phosphate was produced by *Bacillus* B1b. After 4 weeks of incubation, a 1. 37 mg/l of soluble phosphate was produced by *Bacillus* B1b, and the pH values of the cultures were reduced from 7 to 4. The phosphate

solubilization was due to the acidification of the culture by bacterium. Phosphate solubilizing bacteria such as species of *Bacillus* and *Paenibacillus* have been applied to soils to specifically enhance the phosphorus status of plants (Brown 1974)

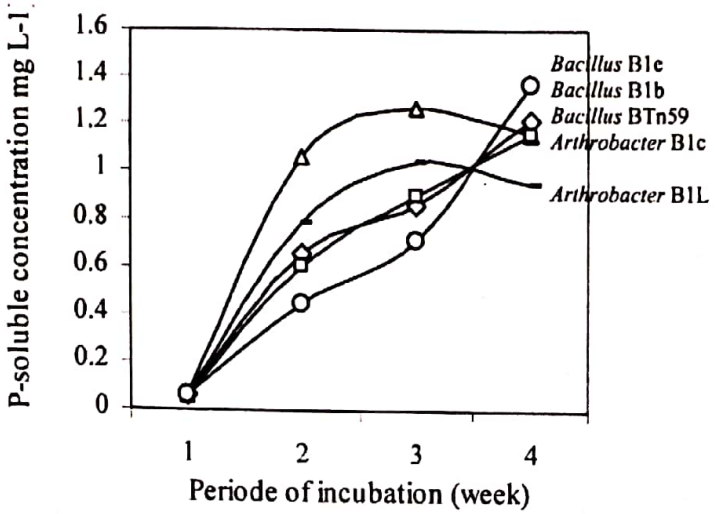

Figure 4. Production of soluble phosphate by selected thermo isolates

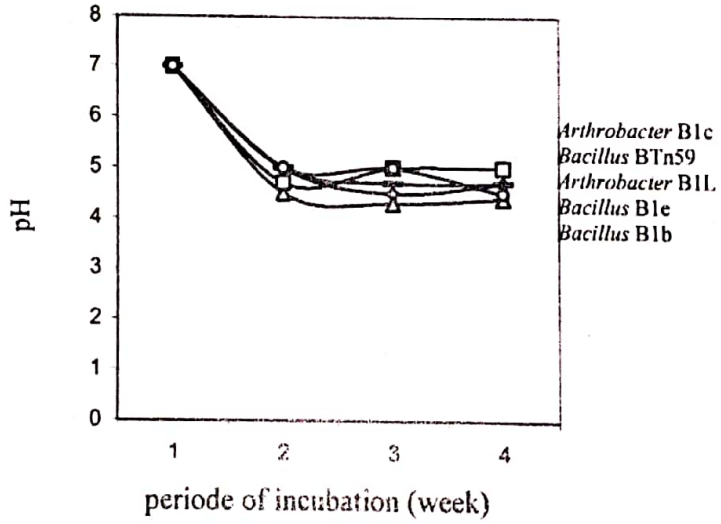

Figure 5. Changes of pH values of the cultures

Plant growth is frequently limited by an insufficiency of phosphates, which are considered one of the most important growth-limiting environmental factors. The low solubilities of common phosphates, such as $Ca_3(PO_4)_2$ hydroxyapatite and aluminum phosphate cause low phosphate availability. However, because some bacteria can solubilize insoluble phosphates, they may promote plant growth (Rodríguez and Fraga, 1999). Several reports have suggested that PGPR can stimulate plant growth through their P-solubilizing activity. The assimilation of nutrients, such as N, P, and K, in plants increased in response to inoculation with P-solubilizer (*Bacillus megaterium*) and K-solubilizer (*Bacillus mucilaginous*). Inoculation with a phosphate-solubilizing *Bacillus* strain M3 significantly improved P, Fe, and Mn contents of the leaves of raspberry (*Rubus idaeus*), suggesting that *Bacillus* M3 alone or in combination with some other

strains had the potential to increase the nutrition of raspberry plants, in addition to growth and yield (Khalid et al., 2009). The results suggest that among the nine isolates, the *Bacillus* B1b isolate had the highest ability to produce of IAA. Based on the ability of the isolates to produce IAA, the *Bacillus* B1b was the most potentially as a PGPR.

CONCLUTION

Nine thermotolerance isolates bacteria have been isolated from the land that affected by merapi eruption. They were identified as *Bacillus* sp. (5 isolates), *Paenibacillus* sp. (2 isolates) and *Arthrobacter* sp. (2 isolates). All of the thermo tolerance isolates had ability to produce IAA and solubilize insoluble phosphates. Based on the ability of the isolates to produce IAA, the *Bacillus* B1b was the most potentially as a PGPR.

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