

STUDIES ON FUNCTIONAL BACTERIA OF INDONESIAN TROPICAL FOREST PLANTS FOR BIOREHABILITATION OF DEGRADED LANDS¹

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ABSTRACT

Forest degradations have left vast amount of damaged and abandoned lands in Indonesia. In this paper, we present our approaches in rehabilitation of adverse soils using functional bacteria isolated from plant species of Indonesian tropical rain forests. For these purposes, we collected bacteria from various bio-geo-climatically different forests and conducted bioassays to test these bacterial abilities in improving plant growth. Repeated seedling-based studies on *Shorea* spp., *Alstonia scholaris*, *Acacia crassicaarpa*, and *Agathis lorantifolia* have revealed that many bacteria were able to promote plant growth at early stage in the nursery. Various plant responses towards inoculations suggested that although forest soils maintain highly diverse and potent bacteria, it is necessary to select appropriate approaches to obtain optimum benefits from these plant-bacteria interactions. Our ideas and futures studies for further management of these plant-bacteria interactions for biorehabilitation are also discussed.

Keywords: Plant-bacteria interaction, growth promotion, adverse soil

I. INTRODUCTION

Recurrence of massive forest fire, illegal logging, mining, and mismanaged activities conducted individually or institutionally have led to vast amounts of destroyed and converted forest areas worldwide. Indonesian forests have been exploited for more than 20 years now, resulting in more than 20 million hectares of deforested areas (Subiakto *et al.*, 2005). Stressed/adverse soils are often associated with nutrient limitation, and extremely low pH condition is associated with high Al concentration in the soil solution. Tilak *et al.* (2005) reported only 1% of the total P present in the soil is available to plants due to

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its chemical bonding and low solubility. These complex problems as well as a severe leaching on the forest floor, and/or presence of toxic elements have caused low survival rate of transplanted seedlings into these adverse areas. Thus, rehabilitation on such degraded, abandoned, and adverse lands remains a trivial effort.

Attempts to improve soil condition such as that applied in agricultural sectors by adding inorganic fertilizer and lime to increase the soil pH are inefficient and can pollute the environments because forests usually cover considerably vast areas that include mountains, rivers, and villages. Albeit rehabilitation efforts have been recently intensified and accelerated to encounter the global issue of the human impact towards the environment, these actions seem suffer draw back and become less effective without a proper identification and solution to the real problems.

Rising reports on the beneficial effects of functional bacteria on plant growth have inspired many researchers to understand this host-bacteria interaction. In subtropical regions, applications of plant growth-promoting rhizobacteria (PGPR) for improving seedling growth in the nursery for forest management have been increasing (Chanway, 1997; Garcia *et al.*, 2004). However, investigations on functional bacteria from Indonesian forests have not been adequately studied. Bacteria play a pivotal role in maintaining nutrient cycle, preserving nutrient for plant growth, and improving soil structure and fertility (Lucy *et al.*, 2004).

In this paper, we discuss our approach in utilizing rhizobacteria for plant growth promotion. Rhizobacteria were collected from various forest conditions and plant species in Indonesia and tested for growth promotion on *Acacia crassicarpa*, *Shorea selanica*, *Shorea leprosula*, *Agathis lorantifolia*, and *Alstonia scholaris* in the nursery.

II. MATERIALS AND METHODS

A. Isolation and Screening Strategy

Roots are an important reservoir for bacteria. Root/rhizosphere is a “hot zone” where active nutrient circulation takes place. In our study, most bacteria were isolated from root (or nodules) and soils of the host plants. We used culture-based method in isolations of bacteria with various media that are based on our interests: (1) for trapping oligotroph: yeast mannitol agar, and Winogradsky’s mineral mixture-based nitrogen-free medium solidified with 0.3% gellan gum (WMM; Hashidoko *et al.*, 2002); (2) for trapping auxotroph: nutrient agar, and potato dextrose agar. The use of WMM not only allowed the emergence of epiphytes but also endophytic bacteria.

The orderly strategy for studying functional bacteria for plant growth promotion generally follows illustration in Figure 1. References therein describe the methods in detail.

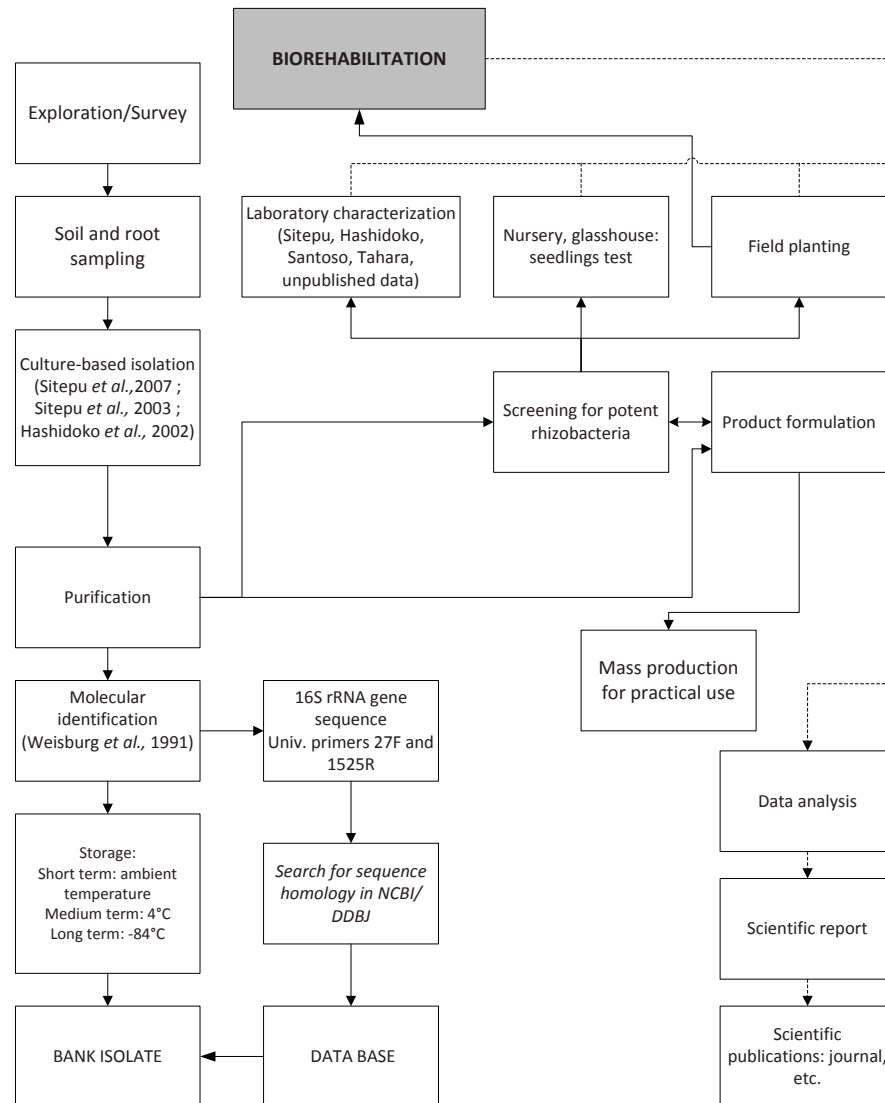


Figure 1. Orderly strategy in investigating functional bacteria of Indonesian tropical forest plants for biorehabilitation of degraded lands.

B. Sampling Sites

Exploration and isolation of functional bacteria have been done from biogeoclimatically different forest conditions ranging from abandoned/degraded to conserved ecosystems in Indonesia. The ecosystems ranged from adverse soils to conserved ecosystems: (1) former coal mining (Figure 2.a and b), cement mining, and degraded peat swamp forest (Figure 2.c and d) to represent degraded forest and adverse soils; (2) nursery to represent a controlled condition; (3) plantation forest of BKPH (Bagian Kesatuan Pemangkuan Hutan or forest management unit at regency level) Gunung Kencana to represent a monoculture/oligoculture man-made system; (4) experimental forests of Carita, Haurbentes, and Cikole to represent maintained and diverse vegetation compositions; and (5) arboretum Nyaru Menteng to represent a conserved ecosystem.

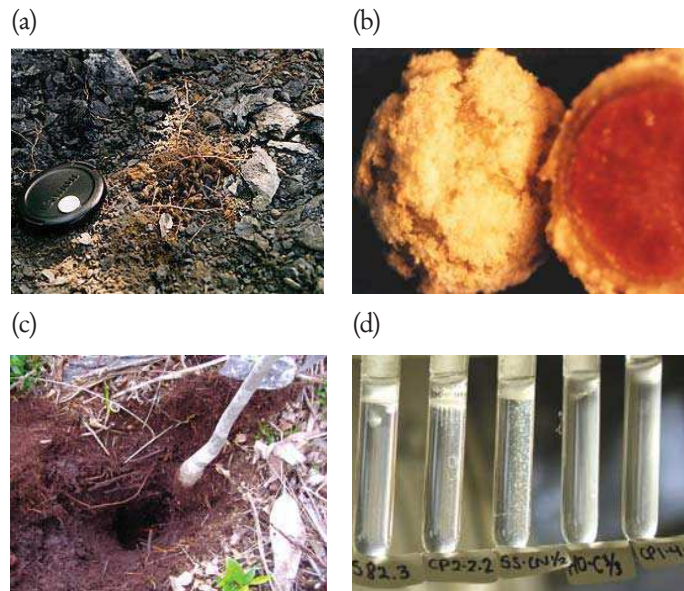


Figure 2. Orderly strategy in investigating functional bacteria of Indonesian tropical forest plants for biorehabilitation of degraded lands.

III. RESULTS AND DISCUSSION

A. Isolation of Bacteria from Diverse Forest Conditions

Using different types of media, 411 bacterial isolates were trapped from various forest plants grown in natural and pot conditions; and from various

forests types in Sumatra, Java, and Kalimantan, Indonesia. A study by Singh *et al.* (2004) suggested that bacteria and plant are actually interdependent for nutrient supply. Plants select their bacterial partners based on their functions rather than taxonomic (Wieland *et al.*, 2001). This may rationalize our findings of diverse genera of associative bacteria isolated regardless of tree species.

Bacterial identification showed that gram-negative bacteria predominantly isolated from host plants indicate the common nature of the plant rhizosphere (Table 1). Predominance of gram-negative bacteria in the rhizosphere has been previously reported by Olsson and Persson (1999) and Donata-Correa *et al.* (2004) who investigated rhizosphere bacteria of tagasaste and barley plants.

Table 1. Bacteria isolated from various forest types in Indonesia

No.	Total Bacterial isolates	Interaction with host	Isolation media	Forest type	Provinces
1.	14	Association	WMM	Lowland experimental forest, Liamkiwa	South Kalimantan
2.	82	Association	WMM	Lowland peat swamp Dipterocarpaceae-dominated forest maintained as arboretum in Nyaru Menteng	Central Kalimantan
3.	15	Association	NA	Lowland community peat swamp forest	West Kalimantan
4.	7	Association	NA	Lowland degraded peat swamp forest, Kalamangan	Central Kalimantan
5.	28	Association	WMM	Pongpok Landak Nursery of PT. Perhutani/pot soil	West Java
6.	29	Association	WMM	Experimental forest Carita	Banten, Java
7.	70	Association	WMM	Nursery of FNCRDC*	West Java
8.	11	Association	WMM	Experimental forest Haurbentes	West Java
9.	6	Association	WMM	Nursery Hokkaido University**	Hokkaido
10.	35	Symbiotic	YMA	Upland experimental forest, Cikole	West Java
11.	59	Symbiotic	YMA	Former coal mining area, PTBA	South Sumatra
12.	46	Symbiotic	YMA	Plantation forest of BKPH Gunung Kencana, Perum Perhutani	Banten
13.	9	Symbiotic	YMA	Upland adverse soil of former cement mining, Cibadak	West Java

Note : WMM: Winogradsky's mineral mixture-based nitrogen-free medium solidified with 0.3% gellan gum; YMA: yeast mannitol agar, NA: nutrient agar; PDA: potato dextrose agar. *: 4 strains were phylloplane bacteria; **: bacteria isolated from seedling rhizoplane of Indonesian origin. BKPH: Bagian Kesatuan Pemangkuan Hutan or forest management unit at regency level, PT Perhutani or state-owned forest enterprise; PTBA: Bukit Asam Mining Company; FNCRDC: Forest and Nature Conservation Research and Development Center.

Isolated bacteria were maintained and stored in Forest Microbiology Laboratory, Forestry Research and Development Agency (FORDA), Indonesia and/or Ecological Chemistry Laboratory, Hokkaido University, Japan.

B. Screening of Growth Acceleration Capacity by Symbiotic Bacteria on Seedlings in Glasshouse Condition

Most rhizobia inoculated improved growth of *A. crassicarpa* seedlings in controlled condition in glasshouse (Figure 3; Data not shown). Rhizobia that accelerated height growth more than 100% compared to non-inoculated seedlings are presented in Table 2. The most improved growth was achieved by seedlings inoculated with rhizobial strain S.8.4, as much as 254% (Sitepu *et al.*, 2003). *Acacia crassicarpa* is a legume species having better qualities and adaptabilities towards more diverse conditions compared with other acacias, such as *A. mangium*, *A. auriculiformis*, *A. leptocarpa*, *A. aulocarpa*, *A. cincinnata*, *A. uraria* and *A. hylonola* (various sources in Kapisa *et al.*, 2000).

Effectiveness of symbiosis led to a better nitrogen fixation by rhizobial inoculant. Dowling and Broughton (1986) said that eventual growth performance of inoculated plants was a resultant of many factors including rhizobial genomic, plant genomic and environment. This study by Sitepu *et al.* (2003) allowed selections of the most prominent rhizobia for accelerating *A. crassicarpa* growth which is significant for nursery management from economical and ecological viewpoints.

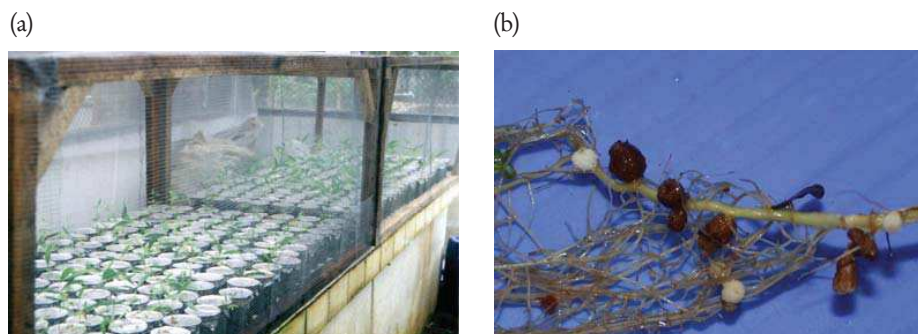


Figure 3. Screening of potent rhizobia for growth promotion of *Acacia crassicarpa* seedling. (a): simple screen house that protected *A. crassicarpa* seedlings from pest attack; (b): Symbiosis performed between inoculated rhizobia and *A. crassicarpa* as indicated by nodules.

Table 2. Symbiotic bacteria isolated from post coal mining areas and their efficiency in improving growth of *Acacia crassicarpa* seedlings

No.	Bacterial isolates	Source of isolation	Tested host	Symbiotic performance (%)	Height growth acceleration (%)
1	S6.2	<i>Paraserianthes falcataria</i>	<i>Acacia</i>	42.27	107.46
2	S6.4	<i>Paraserianthes falcataria</i>	<i>crassicarpa</i>	72.07	183.22
3	S7.3.2	<i>Acacia auriculiformis</i>		53.30	135.51
4	S8.4	<i>Paraserianthes falcataria</i>		100.0	254.21
5	S9.2	<i>Acacia mangium</i>		45.55	115.80
6	S10.3.1	<i>Acacia mangium</i>		56.50	143.64
7	S11.2	<i>Acacia mangium</i>		55.28	140.52
8	S22.1	<i>Acacia mangium</i>		42.84	108.90
9	S34.1	<i>Acacia auriculiformis</i>		52.43	133.28
10	S38.2	<i>Acacia mangium</i>		45.16	114.80

C. Screening of Growth Acceleration Capacity by Rhizobacteria on Seedlings in Glasshouse Conditions

Bacteria have given immediate effect on seedling growth since the first month after inoculation. Dipterocarp seedlings responded variably to bacterial inoculation and most of the inoculated seedlings showed growth promotion. The growth promotion reached up to 41.4% and the most prominent PGPR strains from these studies are presented in Table 3. Only few of the tested bacteria gave negative effect on seedling growth. It has been pointed out that the negative effect is often resulted from phytotoxin produced by these bacteria (Brown and Rovira, 1999).

Inoculations of PGPR to the dipterocarp seedlings showed significant effect on their height growth rather than their diameter. Their ability to grow taller than other seedlings will help the seedlings to survive in nature in a competition with other ground storey vegetation. Dipterocarp seedlings require moderate light intensity in the forest floor in natural conditions, but full exposure to such light will kill the seedlings.

Table 3. Rhizobacteria and their efficiency in improving growth of dipterocarp seedlings

No.	Bacterial isolates	Source of isolation	Tested host	Height growth acceleration (%)
1	<i>Erwinia</i> sp. FL11.2.2	<i>Shorea leprosula</i>	<i>Shorea selanica</i>	35.57
2	<i>Alcaligenaceae</i> FL2.4.2	<i>Hopea mengarawan</i>	<i>Shorea selanica</i>	33.56
3	<i>Burkholderia</i> sp. FL3.5.1	<i>Hopea odorata</i>	<i>Shorea selanica</i>	19.46
4	<i>Pseudomonas</i> sp. FL41	<i>Shorea selanica</i>	<i>Shorea leprosula</i>	29.01
5	<i>Burkholderia</i> sp. FL44	<i>Hopea mengarawan</i>	<i>Shorea leprosula</i>	27.48
6	<i>Rhizobium</i> sp. FL5.1	<i>Hopea mengarawan</i>	<i>Shorea leprosula</i>	30.53
7	NI HM3	<i>Hopea mengarawan</i>	<i>Shorea selanica</i>	28.71
8	<i>Sphingomonas</i> sp. R5b	<i>Shorea balangeran</i>	<i>Shorea selanica</i>	32.67
9	<i>Sphingomonas</i> sp. SB3	<i>Shorea balangeran</i>	<i>Shorea selanica</i>	40.59
10	<i>Burkholderia</i> sp. FL37	<i>Shorea selanica</i>	<i>Shorea selanica</i>	27.23
11	<i>Enterobacter</i> sp. FL13.2.1	<i>Shorea leprosula</i>	<i>Shorea leprosula</i>	31.60
12	<i>Burkholderia</i> sp. CK32	<i>Shorea balangeran</i>	<i>Shorea leprosula</i>	41.40
13	<i>Burkholderia</i> sp. JW6	<i>Shorea selanica</i>	<i>Shorea leprosula</i>	23.50
14	<i>Azospirillum</i> sp. JW13	<i>Shorea selanica</i>	<i>Shorea leprosula</i>	32.00
15	NI CK4	<i>Shorea balangeran</i>	<i>Shorea leprosula</i>	30.70

D. Screening of Growth Acceleration Capacity by Double Inoculation of Rhizobacteria and Arbuscular Mycorrhizal Fungi in Glasshouse Conditions

Double inoculations of arbuscular mycorrhizal fungi (AMF) accelerated height and diameter growth of *Alstonia scholaris* seedlings up to 79 and 20.8%, respectively compared to non-inoculated seedlings (Table 4). Our studies suggested that individual inoculation of either AMF or bacteria gave most significant effect on height growth, whereas double inoculation gave most significant effect on diameter growth (Data not shown).

Table 4. Double inoculation of bacteria and AMF and growth acceleration on *Alstonia scholaris*

Source of isolation	Inoculant strains (AMF + Bacteria)	Tested host	Growth acceleration (%)	
			Height	Diameter
-	Non-inoculated	<i>Alstonia scholaris</i>	0.00	0.00
<i>Shorea selanica</i>	<i>Gigaspora</i> sp. + <i>Burkholderia</i> sp. JW6		79.0	20.8
<i>Combretocarpus rotundatus</i>	<i>Glomus</i> sp. + NI Cr.R1.2		77.1	14.5
<i>Shorea selanica</i>	<i>Glomus</i> sp. + NI JW3a		60.0	15.9

Double inoculation of AMF and rhizobacteria increased N and P content of *Agathis lorantifolia* seedlings 6.5 months after inoculation (Figure 4; Miftahuliyah, 2006 unpublished data). The results showed that inoculations regardless of composition of AMF and/or bacteria gave a better nutrient content to the host plants. The most improved N and P contents (12.8 and 2.08 mg/plant) were achieved by the seedlings inoculated with a combination of *Gigaspora* sp. and bacteria strain Aa.R2.1. On the other hand, N and P content of non inoculated seedlings were significantly lower than those of all inoculated seedlings by 10.8 and 0.31 mg/plant, respectively. A better nutrient content indicated that microbes were functional even in a limited growth space such as polybag. Mycorrhizal fungi assisted N and P uptake of the plant roots (Smith *et al.*, 1986). Several studies have demonstrated the beneficial effect of combined inoculations, for example, dual inoculations of plant growth-promoting rhizobacteria (PGPR) together with ectomycorrhizal fungus for optimum growth of seedlings (Frey-Klett *et al.*, 1999). Garbaye (1994) suggested mechanism by which mycorrhization helper bacteria (MHB) may promote mycorrhization including stimulation and facilitating of the fungal growth and mycorrhizal establishment, initiation of root-fungus affinity, as well as receptivity of the roots for fungal infection.

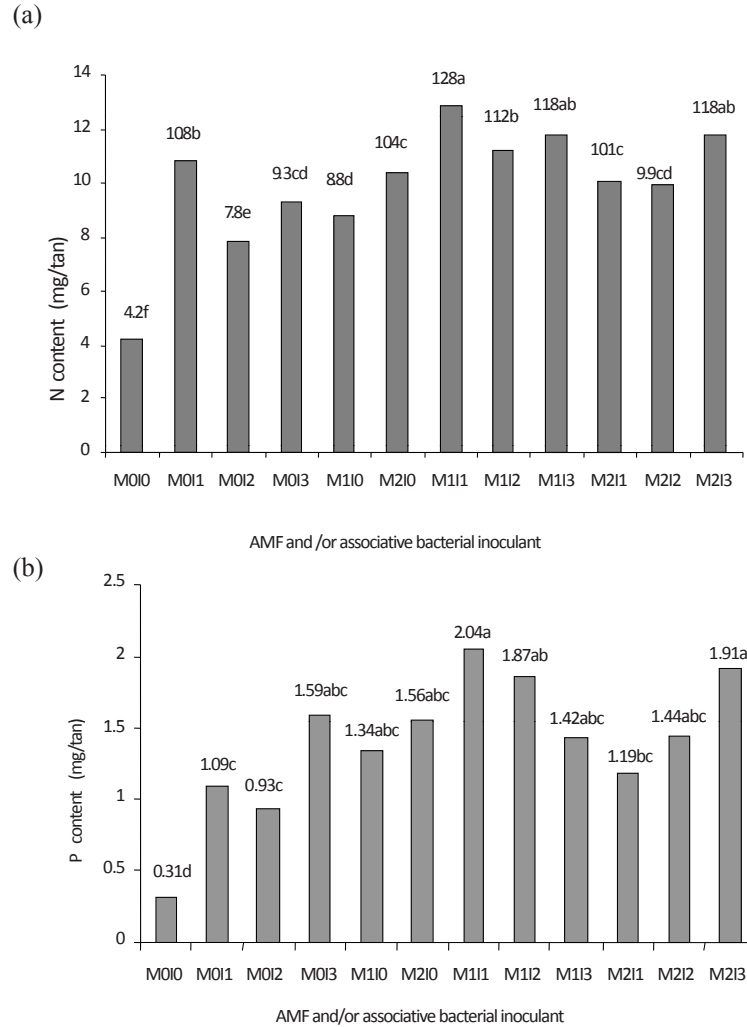


Figure 4. N and P content of *Agathis loranthifolia* seedlings inoculated with AMF and rhizobacteria (Miftahuliyah *et al.*, 2006 unpublished data). Note: M0I0 (non-inoculated); M0I1 (without AMF + bacteria strain Aa.R2.1); M0I2 (without AMF + bacteria strain Gr.R2.1); M0I3 (without AMF + bacteria strain Nt.R2.2); M1I0 (*Gigaspora* sp + without bacteria); M2I0 (*Glomus clarum* + without bacteria); M1I1 (*Gigaspora* sp + bacteria strain Aa.R2.1); M1I2 (*Gigaspora* sp + bacteria strain Gr.R2.1); M1I3 (*Gigaspora* sp + bacteria strain Nt.R2.2); M2I1 (*Glomus clarum* + bacteria strain Aa.R2.1); M2I2 (*Glomus clarum* + bacteria strain Gr.R2.1); M2I3 (*Glomus clarum* + bacteria strain Nt.R2.2).

E. What is necessary in bioreforestation using functional bacteria

Our screening works in laboratory and glasshouse/nursery provide preliminary, which is yet an important information on the effective PGPR. Most bacteria induced growth promotion effect on tested plants. We have found that growth performance of plants after inoculation is a resultant of bacteria-host-environment interaction. The consequence of this interaction is a rather specific case therefore screening work should not be overlooked for obtaining optimum growth promotion effect. In a relatively small pot condition, however, particularly at the initial stage, competition is likely so high for nutrition, water as well as niche. It is clear that much more complex mechanisms are on functioning in the bacteria-plant interaction than what we have already understood.

In the case of legume-rhizobia symbiosis, Kijne (1992) reported the rate of biological fixation from this symbiosis that reached 40% of the global fixation rate or approximately 70 tons of available nitrogen per year. The author further suggested that this type of symbiosis could provide exclusive nitrogen supply available for plant uptake and replace the use of urea fertilizer. Although these studies have been done extensively, particularly in the agriculture sector, Brockwell *et al.* (1995) and Thies *et al.* (1992) reported that an introduction of foreign rhizobial inoculant to a new soil environment often failed to conduct efficient symbiosis due to a severe competition with the native rhizobia and other soil inhabitants. Therefore, searching for local biopotential of rhizobia from each of the targeted sites for biorehabilitation should be implicated.

Utilization of PGPR in agriculture is different from that in forestry because of the different practices in these two research disciplines. In agriculture, rhizobacteria are commonly applied as bacterial paste coated on annual crop seeds, and the seeds are directly planted in the fields; but in the forestry, rhizobacteria are often applied to perennial seedlings firstly grown in nursery before transplanting to the fields (Lucy *et al.*, 2004). Application of these functional bacteria isolated from various forest types in Indonesia for preparation of seedlings in the nursery is one alternative to obtain ecological and economical benefits. This approach would be more preferable than the excessive inputs of inorganic fertilizers, such as of NPK fertilizers. Nowadays, the urge to find alternative to reduce environmental loads due to chemicals utilization have widely drawn attentions. Kloepper (2003) raised the problem of nitrate contamination of ground water and phosphate run-off that has led to eutrophication of ponds and rivers.

Seedlings usually suffer low survival rate after the transplanting into adverse soils. Inoculated seedlings by PGPR (including rhizobia) or consortium inoculant would lead to increase of the survival rate after the transplanting

into the fields. A further study to test the preliminary nursery results will be implicated in our future works. In the adoption process of the seedlings to adverse environments in a complex and natural situation, more important and interesting nature of PGPR-plant interaction may be discovered.

From a practical viewpoint, biorehabilitation is dependent upon soil managements by providing sufficient nutrient for plants to grow well. It is then necessary to focus on rhizosphere soil because PGPR have abilities to alter and regulate such micro-scale of soil environments. These studies would provide a better understanding in planning a practical biorehabilitation strategy.

Preparation of seedling inoculated with bacteria before the outplanting is relatively easy for nursery application, environmentally benign, and inexpensive. The use of indigenous plant species over introduction of exotic ones is another important consideration for bioreforestation. Local trees are more adaptable to site local condition and their timber and non-timber productions are highly saleable and priceless. In a global account, regenerated forest may contribute to responding to the current global issues of carbon sink destruction as well as greenhouse emission that accelerate global warming and exacerbate El Nino weather impact.

IV. CONCLUSIONS

1. Four hundreds and eleven bacteria isolates were trapped from diverse hosts of various forest conditions in Indonesia including degraded, nursery, man-made, experimental, and conserved forests.
2. Various responses of seedlings due to inoculations from our repeated studies which emphasized the importance of screenings stage of rhizobacteria for optimum growth improvement. Inoculations gave positive, no or negative effect on seedling growth.
3. Bacteria functioned immediately after inoculation, promoting various growth performances of the tested seedlings. This stage is very critical in screening effective rhizobacteria and understanding the ecological role of these bacteria in their interaction with trees.
4. Double inoculation of rhizobacteria and AMF increased N and P content of *Agathis lorantifolia* seedlings 6.5 months after inoculation. The most improved N and P contents (12.8 and 2.08 mg/plant) were achieved by the seedlings inoculated with a combination of bacteria strain Aa.R2.1 and *Gigaspora* sp.
5. Biorehabilitation of adverse soils is dependent upon soil managements, and it is necessary to focus on rhizosphere because PGPR have abilities to alter and regulate rhizosphere. Our understanding of such micro-

scale environment would assist us in formulating a practical strategy for biorehabilitation.

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