



HOKKAIDO UNIVERSITY

Title	Screening of rhizobacteria from dipterocarp seedlings and saplings for the promotion of early growth of Shorea selanica seedlings
Author(s)	Sitepu, Irnayuli R.; Aryanto; Ogita, Noriko et al.
Citation	Tropics, 16(3), 245-252 https://doi.org/10.3759/tropics.16.245
Issue Date	2007-05-31
Doc URL	https://hdl.handle.net/2115/35166
Rights	© 2007 日本熱帯生態学会
Type	journal article
File Information	16_245.pdf



Screening of rhizobacteria from dipterocarp seedlings and saplings for the promotion of early growth of *Shorea selanica* seedlings

Irnayuli R. SITEPU¹, ARYANTO², Noriko OGITA¹, Mitsuru OSAKI³, Erdy SANTOSO², Satoshi TAHARA¹ and Yasuyuki HASHIDOKO^{1,*}

¹ Lab. of Ecological Chemistry, Division of Applied Bioscience, Graduate School of Agriculture, Hokkaido University, Kita-9, Nishi-9, Kita-Ku, Sapporo 060-8589, Japan

² Lab. of Forest Microbiology, Forest & Nature Conservation R&D Center, Jalan Gunung Batu No.5, Bogor-16610, Indonesia

³ Development of Plant Function, Division of Biological Resources and Production, Graduate School of Agriculture, Hokkaido University, Kita-9, Nishi-9, Kita-Ku, Sapporo 060-8589, Japan

*Corresponding author: Yasuyuki HASHIDOKO. Tel: +81-11-706-3840, Fax: +81-11-706-4182, E-mail: yasu-h@abs.agr.hokudai.ac.jp

ABSTRACT Low-cost forest management using highly effective microorganisms to promote seedling and tree growth is an important issue in reforestation in Indonesia and other tropical rain forest zones. To survey effective plant growth-promoting rhizobacteria (PGPR) for dipterocarp seedlings and/or saplings, forty-four bacterial strains were isolated from rhizoplane of several Dipterocarpaceae seedlings and saplings in Carita plantation forest located in the western part of Java Island followed by test for their effectiveness on the growth of *Shorea selanica*. Some isolated rhizobacteria demonstrated growth-promoting activity on dipterocarp seedlings. In the primary bioassay, these rhizobacteria indicated positive, negative or no effect on the plant growth, and 16S rRNA gene sequences of some important isolates were determined for their tentative identification. The most prominent strain isolated from *Shorea leprosula* was *Erwinia*. Other effective isolates were tentatively identified as, *Rhizobium*, *Enterobacter*, *Duganella*, an Alcaligenaceae bacterium, an Oxalobacteraceae bacterium, and some yeasts.

Key words: tropical forest rehabilitation, plant growth promoting rhizobacteria, low-input forest management, Dipterocarpaceae

INTRODUCTION

In year 2000, it was estimated that forest cover in Indonesia decreased to approximately 105 million hectares from the total of 118 million ha in 1990 at the annual rate of 1.3 million ha (Phat et al. 2004). This rate contributes 10% to the 13 million ha of annual deforestation rate in tropical forests on the earth

(Kobayashi, 2004). Forests are continually degraded through various means including legal and illegal logging, fire, shifting cultivation, and social economic instability of the forest community as well as mismanagement by forest implementers. This situation does occur not only in Indonesia but also other tropical countries, and thus reforestation is an urgent priority in the world for conservation and rehabilitation of the forest resources. A major problem in rehabilitation of tropical rain forest is soil infertility. In Indonesia, low pH and severe leaching limit nutrient availability (Powell, 1994). Fertilizing and liming in deforested areas to improve soil conditions are not economically efficient due to relatively long term necessity for economic return by perennial timber trees. Hence, low-input and sustainable forest management is an important issue in reforestation, and bio-fertilization is one of possible technical approaches.

Shorea selanica that belongs to Dipterocarpaceae is a native species and an important timber tree in tropical forest throughout Indonesia, Malaysia, Brunei and the Philippines (Subiaktio & Sakai, 2002). Dipterocarpous trees form ectomycorrhizas, and the tablets of ectomycorrhizal fungal spores are, for example, commercially available in markets (Turjaman et al. 2005). However, dipterocarp mycorrhizas will become functionally effective seven months after inoculation in *S. leprosula*, *S. acuminata*, *Hopea odorata* and *S. pinanga* (Lee, 1990; Yazid et al. 1994; Turjaman et al. 2005). Mycorrhizal fungi, on the other hand, do not reside alone in the rhizosphere (Garbaye, 1994; Frey-Klett et al. 1999; Brulé et al. 2001; Poole et al. 2001). In fact, the most abundant inhabitants of the rhizosphere and rhizoplane of plant are bacteria because this niche is rich in low molecular weight of absorbable carbon compounds as root exudates which are essential for bacterial metabolisms (Garbaye, 1994). Some of these rhizoplane

bacteria also play significant role to promote tree growth. The roles of these functional bacteria, so-called plant growth-promoting rhizobacteria (PGPR), can be various depending on the bacterial strains and seedling genetic traits. For loblolly and slash pine seedlings, Enebak et al. (1998) reported that PGPR improved their stand densities. Indirect effects of PGPR to plant growth by assisting mycorrhizal formation (termed mycorrhizal helper bacteria, MHB) have also been reported. *Pseudomonas fluorescense* BBc6R8 promoted the symbiosis of *Laccaria bicolor* S238N-Douglas fir (*Pseudotsuga menziesii*) and the bacterial effect was shown to be the greatest one when the fungus is under unfavorable conditions (Garbaye, 1994; Brule et al. 2001). *Paenibacillus* sp., *Burkholderia* sp., and *Rhodococcus* sp. stimulated ectomycorrhizal infection at different stage of ectomycorrhizal lateral root formation between *Laccaria rufus* and *Pinus sylvestris* (Poole et al. 2001), *P. monteirii* and *P. resinovorans* promoted symbiosis of *Pisolithus alba* in the soil and *Acacia holosericea* and *P. monteirii* increased the fungal biomass in the soil (Founoune et al. 2002).

The objective of this study was to screen PGPR from the rhizoplane of naturally growing dipterocarp seedlings and saplings in Carita plantation forest. This research is part of our wider efforts to contribute to the improvement of adverse soil, for bioreforestation using native, functional rhizobacteria.

MATERIALS AND METHODS

Isolation of rhizoplane bacteria

Rhizoplane bacteria were isolated from two adjoining

localities, nursery and plantation forest in Carita Research Forest in the western part of Java Island, Indonesia. Carita plantation forest is a planted forest, and various stages of *Shorea* spp. and *Hopea* spp. (0–40 years) are grown. The soil at the sampling site is categorized as alluvial soil (Ultisol) with pH ranged from 4.1–5.5. Focusing on N-fixing bacteria, we used Winogradsky's mineral mixture-base soft gel for primary isolation of rhizospheric bacteria inhabiting root surface of dipterocarp saplings. Approximately, 1-cm-long fine fibrous roots were collected from approximately 2-year-old *Shorea selanica*, *S. leprosula*, *Hopea mengarawan* and *H. odorata* saplings, naturally emerging from underneath mature stands (Table 1). The fine roots were also collected from the seedlings in the nursery pots. The samples were washed several times with sterile water to remove adhering soil, and then incubated for 24 hours at room temperature in the N-free, soft gel medium of Winogradsky's mineral mixture which contained 1% sucrose as a sole carbon source and was solidified with 0.3% gellan gum (Hashidoko et al. 2002). After removing the root, the medium was further incubated for 48 hours. The bacterial colonies in the soft gel medium were transferred to the modified Winogradsky's mineral mixture agar medium containing 0.005% yeast extract as extra component, and each distinguishable colony was purified on the same medium. Forty-four out of 75 isolates were selected based on their growth performance on the modified Winogradsky's agar medium (data not shown). These isolates were maintained in the gellan gum-Winogradsky's medium at room temperature thereafter (Hashidoko et al. 2004).

Table 1. Bacterial strains collected from two adjoining localities in Carita Research Forest.

<i>A. Saplings in the Plantation Forest</i>	
Host	Identification
<i>Hopea mengarawan</i>	NI no. 1, <i>Rhizobium</i> sp. no. 2, NI no. 3, NI no. 4, NI no. 5, NI no. 6, NI no. 7, <i>Stenotrophomonas</i> sp. no. 8, NI no. 9, Alcaligenaceae bacterium no. 10, <i>Rhizobium</i> sp. no. 11
<i>Hopea odorata</i>	NI no. 12, NI no. 13, NI no. 14, <i>Burkholderia</i> sp. no. 15, NI no. 16, NI no. 17, NI no. 18, NI no. 19
<i>Shorea leprosula</i>	NI no. 20, <i>Erwinia</i> sp. no. 21, NI no. 22, <i>Pseudomonas</i> sp. no. 23
<i>Shorea selanica</i>	NI no. 41, NI no. 42, NI no. 43, NI no. 44
<i>B. Seedlings in the Nursery</i>	
Source	Identification
<i>Shorea selanica</i>	<i>Sphingomonas</i> sp. no. 24, NI no. 25, <i>Enterobacter</i> sp. no. 26, <i>Pseudomonas</i> sp. no. 27, NI no. 28, NI no. 29, NI no. 30, Oxalobacteriaceae no. 31, NI no. 32, NI no. 33, NI no. 34, NI no. 35, NI no. 36, NI no. 37, NI no. 38, NI no. 39, NI no. 40

Note. NI: non-identified strain.

Characterization of rhizoplane bacteria based on 16S rRNA gene sequence

Total DNA was prepared using a commercial DNA-preparation kit, Isoplant II[®] (Wako Pure Chemical Industries Co. Ltd., Osaka, Japan) according to the instruction. Partial 16S rRNA gene region (1.5 kbp) was amplified by PCR using the HotStarTaq[™] kit (Qiagen, Hilden, Germany) with the 27F and 1525R primer set (Weisburg et al. 1991; Hoo et al. 2004). The reaction conditions were as follows: hot starting at 95°C for 15 min, 30 cycles of 94°C for 1 min, 56°C for 1 min, and 72°C for 1 min, then 1 cycle of 72°C for 10 min.

Amplified product of approximately 1.5 kbp was partially sequenced using BigDye[®] Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, USA) with the primers choices of 926F (5'AAACTCAAAGGAA TTGACGG3'), 518R (5'GTATTACCGCGGCTGCTGG3'), 1112F (5'GTCCCGCAACGAGCGCAAC3') and 1080RM (5'ACGAGCTGACGACA3') under the conditions of 1 cycle of 96°C for 5 min and 25 cycles of 96°C for 30 s, 50 °C for 15 s, 60°C for 4 min using the ABI PRISM[®] 310 Genetic Analyzer (Applied Biosystems, California, USA). The most probable genus of the isolate was determined by the BLASTN search system.

Seedling preparation and inoculation

Seeds of *S. selanica* were surface-sterilized with 5% sodium hypochlorite for 2 min and followed by rinsing 10 times with sterile water, and pre-germinated on a sterile (2 atm, 121°C for 30 min) mixture of sand and mineral soil (3:1, v/v). The mineral soil was originated from the surrounding arboretum. Seedlings of approximately 8–10 cm in height at approximately 10 days after germination were individually transplanted into pots that contained 1.6 liter of the non-fertilized, sterile soil, and at the same time the isolated bacteria were inoculated as follows.

Prior to inoculation, the 44 strains were subcultured individually on a 20 ml of nutrient broth agar (NBA) media made from 0.8% (w/v) commercial nutrient broth and 2.5% agarose (w/w) in a 9-cm-diameter petri dish at room temperature. The bacterial colony-emerging agar plate where only one strain of bacterium had been inoculated firstly divided with a sterile scalpel into 6 identical pieces. Each 1/6 part, approximately 3 ml of NBA medium containing 24 mg NB broth per a piece, was smashed in the pot soil (1.6 l) to provide 10⁸⁻⁹ bacterial cells. Inoculation was replicated to 6 seedlings. Seedlings for control were treated with the same volume of NBA medium without bacterium. The seedlings were placed in a glass house in Forest and Nature Conservation

Research and Development Center, Bogor, Indonesia and sprinkled with tap water everyday.

Each replicate was grouped into one block making 6 blocks and randomization was conducted in each block by using Excel 2001[®] (Microsoft, Redmond, USA). Height and diameter were measured for 6 consecutive months. Aboveground dry weight (70°C for 5 days) and leaf number were recorded as post harvest parameters. Data were subjected to analysis of variance using a statistic software, SPSS[®] version 10.0 (SPSS Inc., Chicago, USA). Pairwise significant differences were tested by the Fisher's LSD-test.

Analysis of N, C, and P of the aboveground mineral composition and soil

The milled aboveground of all 6 replicates were combined and after oven-drying at 105°C for 24 hours were analyzed for their total C (Walkley-Black C method) (Walkey & Black, 1934), N (Kjeldhal) (Bremner & Mulvaney, 1982) and P (Olsen) (Olsen & Sommers, 1982). Similarly, the soils in the pot of all 6 replicates were combined and analyzed. The quantification of the elements in the soil and aboveground tissues mentioned above is preliminary experiment, because both soil and aboveground from one series of treated pots were eventually mixed up for the chemical analyses.

RESULTS

Response of *Shorea selanica* seedlings to bacterial inoculation

Some seedlings of *S. selanica* showed significant growth variation ($P < 0.05$) as a response to inoculation of rhizospheric bacteria after 3 month-incubation (Fig. 1). The increase of growth performance among the treatments, varied substantially by 2.5–7.1 cm and 0.6–1.7 mm for height and diameter, respectively. The first month after the inoculation, most inoculated seedlings had already shown better growth performance compared to those of control (without bacteria; $P < 0.05$). Particularly, seedlings inoculated with bacterial strain no. 10 isolated from *H. mengarawan* sapling and strains no. 15 and 21 isolated from *H. odorata* and *S. leprosula*, respectively, showed the best effect (Fig. 1a) and a similar trend was again observed for the second month (Fig. 1b); however, in the third month (Fig. 1c) the seedlings showed a different growth trend as follows: inoculated with strains no. 21 still maintained the best growth achievement but no. 15 diminished its growth promotion ($P < 0.05$) by 20.2 and 17.8 cm for the height, respectively. On the other

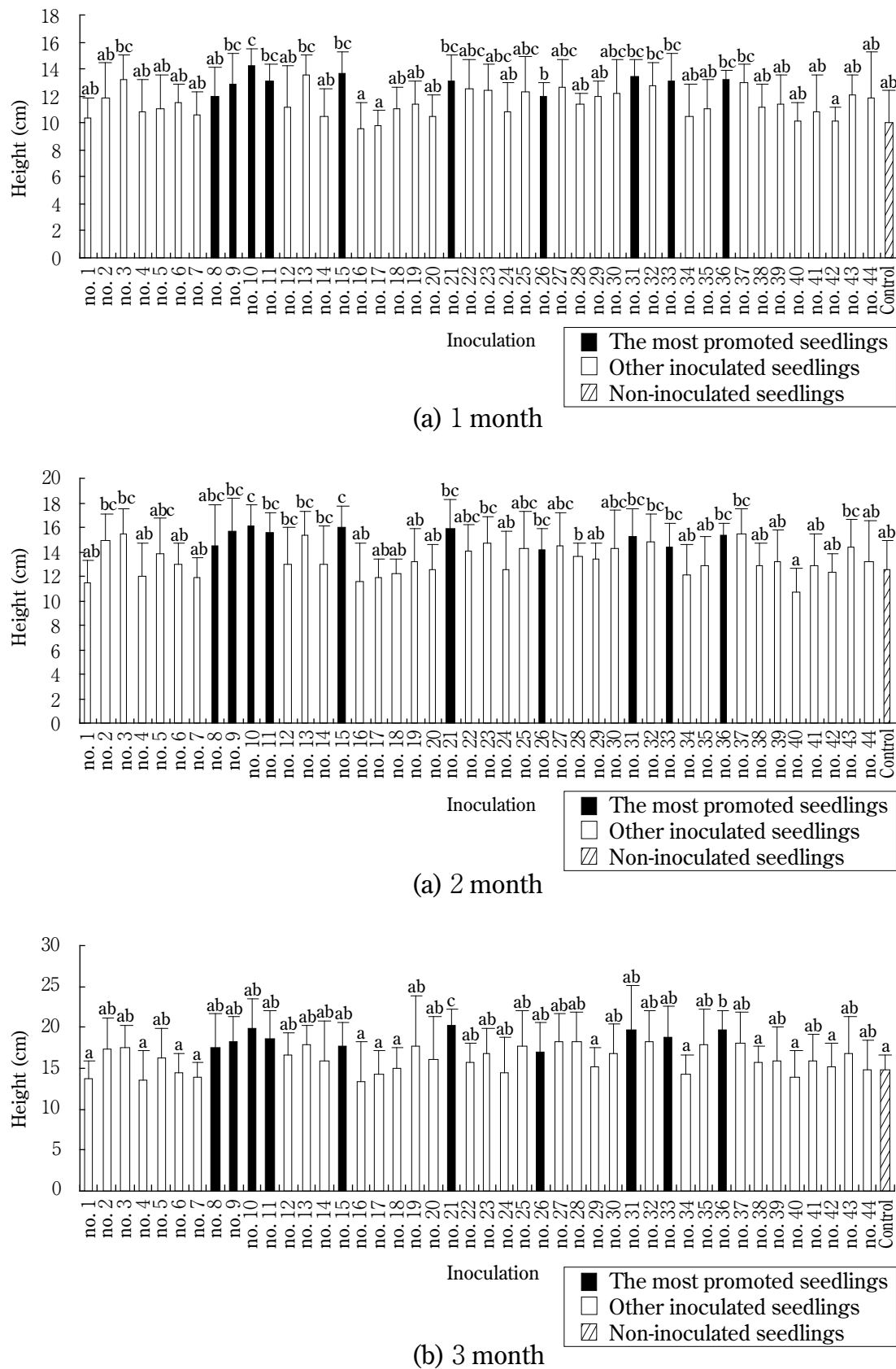


Fig. 1. Height of *S. selanica* after inoculation.

Note. no.1–44 refer to bacterial strain. Height of 1–3 months are significantly different ($P < 0.05$) and columns with the same letter are not significantly different by LSD (average of 6 replicates).

hand, inoculation did not give significant difference for plant biomass (shoot and root) 6 months after the inoculation.

Tentative identification of PGPR

Some bacteria which showed growth-promoting effect on *S. selanica* seedlings were identified at genus levels. The isolates no. 2 (GenBank accession no. DQ091231) and no. 11 (DQ091234) were identified as *Rhizobium* spp., respectively. Isolate no. 8 (DQ091232) was close to *Stenotrophomonas* sp. On the other hand, no. 15 (DQ091235) was *Burkholderia* sp., whereas no. 10 (DQ091233) was an Alcaligenaceae bacterium close to genus *Achromobacter*, and no. 31 (DQ091241) was an Oxalobacteraceae bacterium relative of *Sinobacter* and *Duganella*, all belonging to β -proteobacteria. The isolates no. 21 (DQ091236), 23 and 27 (DQ091239, DQ091240), and 26 (DQ091238) were tentatively identified as an *Erwinia* sp., two *Pseudomonas* spp. and an *Enterobacter* sp. of γ -proteobacteria, respectively.

N, C, and P of the aboveground mineral composition and soil

N, C, and P contents of the samples of best 10 and

worst 5 in their growth performance are presented in Table 2. Inoculated with no. 9 and 36, both unidentified microorganisms, are a true N fixer or an N fixation stimulator because of low C/N for the host seedlings. Higher P content in the leaves of Oxalobacteraceae no. 31 and unidentified no. 33 indicated their P solubilizer traits, but these isolates did not show any solubilization activity toward insoluble inorganic P *in vitro*, suggesting that they were rather mycorrhizal helper microorganisms.

DISCUSSION

In our sampling site Carita, we were able to isolate several proteobacteria from the rhizosphere of dipterocarp saplings (Table 1). Most soils of tropical forests are nutrient-poor and are typically acidic which often limit tree growth. For instance, only 0.1% of the total P present is available to the plants because of its chemical fixation and low solubility (Tilak et al. 2005) and total nitrogen content is also low. Under such conditions, PGPR offer a biological nutrient provision by solubilizing the insoluble inorganic phosphorous of soil and fixing atmospheric nitrogen and make them available to the plants. In adverse soil, rhizobacteria are likely to be depending

Table 2. Content of N and P in the leaves and soil of selected 6-month-old *Shorea selanica* seedlings inoculated with rhizospheric bacteria from dipterocarp saplings and seedlings.

Strain	Base/ Homology (%)	Leaf				Soil			
		C mg/g	N mg/g	C/N	P mg/g	C mg/g	N mg/g	C/N	P µg/g
<i>Stenotrophomonas</i> sp. no. 8	853/98	512	6.46	79	1.13	1.1	0.1	16	47.9
Unidentified no. 9	-	519	8.32	62	1.25	1.0	0.1	14	42.3
Alcaligenaceae no. 10	525/97	508	7.27	70	1.24	0.9	0.1	13	48.8
<i>Rhizobium</i> sp. no. 11	482/96	516	7.27	71	1.24	0.9	0.1	13	45.1
<i>Burkholderia</i> sp. no. 15	838/95	518	7.21	72	1.21	0.8	0.1	11	46.2
<i>Erwinia</i> sp. no. 21	376/94	484	6.03	80	1.21	0.9	0.1	13	58.0
<i>Enterobacter</i> sp. no. 26	1556/97	503	5.60	90	1.13	1.1	0.1	15	54.3
Oxalobacteraceae no. 31	643/95	504	6.57	77	1.52	1.1	0.1	15	55.7
Unidentified no. 33	-	502	6.55	77	1.47	1.0	0.1	14	60.7
Unidentified no. 36	-	510	9.66	53	1.23	1.0	0.1	14	48.9
Unidentified no. 1	-	491	6.89	71	1.07	0.9	0.1	12	38.0
Unidentified no. 4	-	501	5.73	87	1.20	0.8	0.1	11	48.1
Unidentified no. 7	-	489	7.66	64	1.29	1.0	0.1	14	54.5
Unidentified no. 16	-	509	6.98	73	1.08	0.8	0.1	11	41.9
Unidentified no. 40	-	479	8.27	58	1.22	1.0	0.1	14	51.8
Control no. 45	-	492	7.17	69	1.15	0.8	0.1	11	36.0

Note. All of the isolates here are unicellular microorganisms. Best ten seedlings in their growth performance (10 from the top) are shown in bold characters. Worst five and control seedlings (6 from the bottom) were shown in plane font.

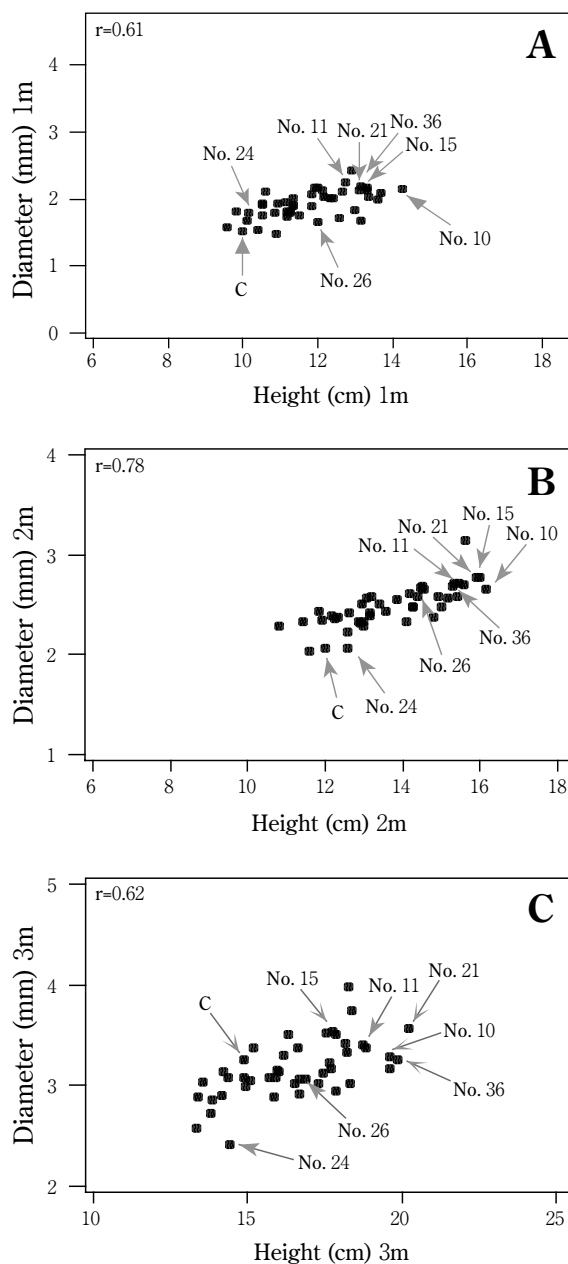


Fig. 2. Growth performance of bacterium-inoculated *S. selanica* seedlings during 1–3 month growth.

Growth promoting effects of the rhizobacteria after inoculation were shown by the growth performance of the *S. selanica* seedlings indicated by diameter of the main stem at 2–5 cm from the pot soil surface and height of the seedlings. There was a positive correlation between the height and diameter: (A) at 1 month after inoculation, (B) at 2 month after inoculation and (C) at 3 month after inoculation. Note: the arrow "C" indicating position of the control seedling.

upon their carbon sources from the host plants, because plant root in adverse soil actively exudes organic acids and/or sugars. Due to this reason, many of rhizobacteria in adverse soil are oligotrophs and/or free-living diazotrophs, and many of which may improve the growth of the host plants, particularly those in seedling and/or

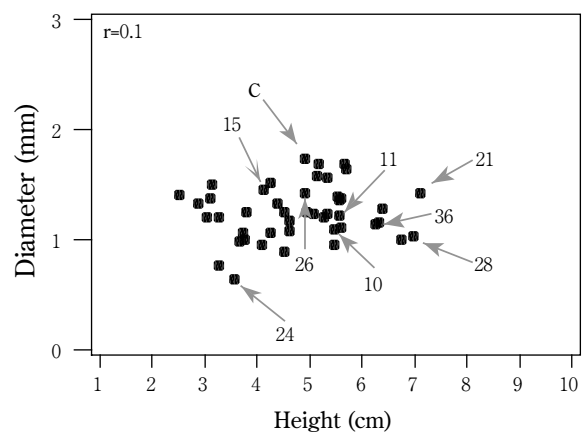


Fig. 3. Growth promoting effect of bacterial inoculants shown by growth values during 2-month-incubation (third month–first month).

Height of the seedlings was regarded as a practical and reliable parameter for the growth stimulation, because height reflected growth performance during 1 to 3 month after the inoculation [value as shown by (3-months grown seedling) minus (1-month old seedling)] compared to the control but diameter during the seedling growth was remarkably high in the control seedling. Seedlings focused as better and worse growth performances were indicated by arrows, along with the control seedlings. Note the arrow "C" indicating position of the control seedling.

sapling stages.

Microorganism that most assisted N-accumulation in the leaves was unidentified isolate no. 36 (probably non-proteobacteria). Although the total N-content in no. 36-inoculated *S. selanica* seedlings was analyzed only once, N-providing effect of the isolate was clearly shown as the relatively high storage of N-content both in leaf and soil (Table 2) at the end of the pot experiment. On the other hand, a higher P-content in the leaves was observed in the seedlings that had been inoculated with a β -proteobacterium of Oxalobacteraceae no. 31 (Table 2). Since all of the 6-month-old *S. selanica* seedlings possessed ectomycorrhizas in their roots over 50% of the frequency (the average of mycorrhizal infection rate was 67%, and ranged from 55–74%), P-content reflects MHB activity of the inoculants, unless the bacterium is an acid-producing one (Rodriguez et al. 2004). The positive effect of Oxalobacteraceae bacterium no. 31 may suggest that MHB-like activity of the rhizosphere bacterium is due to their ability to supply ammonia on the hyphoplane of ectomycorrhizal fungi.

Among 44 isolates that had been inoculated to *S. selanica* seedlings, the best performance for positive effect on the overall growth of the test plant was shown by *Erwinia* sp. no. 21 isolated from *S. leprosula* sapling (Figs. 1, 2 and 3). Bacteria belonging to genus *Erwinia* are Gram-negative and rod-shaped, facultative anaerobic,

γ -proteobacteria, and many of species are known as phytopathogens, but gall-forming *E. herbicola* pv. *gypsophilae* is also known as a PGPR due to its active cytokinin production (Lichter et al. 1995). In other strains (Fig. 1), PGPR-like effect on seedling growth may involve production of plant growth hormone-like substance(s) because the ability to produce the plant hormone, such as indole-3-acetic acid is widespread among rhizosphere bacteria (Fett et al. 1987; Ahmad et al. 2005). Rhizosphere is rich in root exudates which are used to synthesize indole-3-acetic acid as secondary metabolite by microorganisms residing in this zone. Our current research showed that 30 out of 71 microorganisms isolated from rhizoplane and rhizosphere of dipterocarp saplings from Central Kalimantan peat soil were indole-3-acetic acid-producing bacteria (unpublished data). Indole-3-acetic acid is the most common product of L-tryptophan pathway by organisms including PGPR (Ahmad et al. 2005). Height of the seedlings was regarded as a practical and reliable parameter for the observation of growth stimulation effect due to bacterial inoculation especially during first 3 months as shown in this study. The growth of diameter, on the other hand, was slow and thus was not a reliable parameter in this instance.

Utilization of PGPR isolated from native trees for tree planting is one option to fulfill both ecological and economical benefits, and therefore would be more preferable than the use of synthetic chemical fertilizer for growth promotion. Thus, study to investigate the interaction amongst such PGPR-like bacteria and other rhizoplane/rhizospheric microorganisms should be implicated in our future approach. Natural forest floor is occupied by living organisms including tree roots and diverse soil microbes and their interactions lead to rhizo-biocomplex formation (Hashidoko, 2005). Rhizo-biocomplex is biologically potential weapon for improving the elemental circulation in the rhizosphere.

To the best of our knowledge, this is the first demonstration for the role of rhizospheric bacteria showing a PGPR-like behaviour on dipterocarpous seedlings. Our future approach will include a growth test of the rhizobacteria-inoculated seedlings under a stressed soil condition in order to investigate further performance of these potential PGPR in a complex and natural situation. Further screening of more active PGPR-like bacteria in tropical forests should be done as one of bioresources searching, along with studies on effective inoculation and transplanting techniques for combination of dipterocarps and PGPRs.

ACKNOWLEDGEMENT The authors are grateful to the financial support by grant-in-aid for scientific research (No. 13460143 and No. 16208032 to Y.H.) from Japan Society for Promotion of Science (JSPS).

REFERENCES

- Ahmad, F., Ahmad, I. & Khan, M.S. 2005. Indole acetic acid production by the indigenous isolates of *Azotobacter* and fluorescent *Pseudomonas* in the presence and absence of tryptophan. *Turkish Journal of Biology*, **25**: 29–34.
- Brulé, C., Frey-Klett, P., Pierrat, J.C., Courrier, S., Gerard, F., Lemoine, M.C., Rousselet, J.L., Sommer, G. & Garbaye, J. 2001. Survival in the soil of the ectomycorrhizal fungus *Laccaria bicolor* and the effects of mycorrhiza helper *Pseudomonas fluorescens*. *Soil Biology and Biochemistry*, **33**: 1683–1694.
- Bremner, J.M. & Mulvaney, C.S. 1982. Nitrogen–Total. In: *Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties, 2nd ed.* (eds. Page, A.L., Miller, R.H. & Keeney, D.R.), pp. 595–624. American Society of Agronomy, Madison, Wisconsin. USA.
- Chabot, R., Antoun, H., Kloepper, J.W. & Beauchamp, C.J. 1996. Root colonization of maize and lettuce by bioluminescent *Rhizobium leguminosarum* biovar *phaseoli*. *Applied and Environmental Microbiology*, **62**: 2767–2772.
- Enebak, S.A., Wei, G. & Kloepper, J.W. 1998. Effect of plant growth-promoting rhizobacteria on loblolly and slash pine seedlings. *Forest Science*, **44**: 139–144.
- Fett, W.F., Osman, S.F. & Dunn, M.F. 1987. Auxin production by plant-pathogenic pseudomonads and xanthomonads. *Applied and Environmental Microbiology*, **53**: 1839–1845.
- Founoune, H., Duponnois R., Ba, A.M., Sall, S., Branget, I., Lorquin, J., Neyra, M. & Chotte, J.L. 2002. Mycorrhiza helper bacteria stimulate ectomycorrhizal symbiosis of *Acacia holosericea* with *Pisolithus alba*. *New Phytologist*, **153**: 81–89.
- Frey-Klett, P., Churin, J.L., Pierrat, J.C. & Garbaye, J. 1999. Dose effect in the dual inoculation of an ectomycorrhizal fungus and a mycorrhizal helper bacterium in two forest nurseries. *Soil Biology and Biochemistry*, **31**: 1555–1562.
- Garbaye, J. 1994. Helper bacteria: a new dimension to the mycorrhizal symbiosis. *New Phytologist*, **128**: 197–210.

- Hashidoko, Y., Tada, M., Osaki, M. & Tahara, S. 2002. Soft gel medium solidified with gellan gum form preliminary screening for root-associating free-living bacteria inhabiting the rhizoplane of plants. *Bioscience, Biotechnology and Biochemistry*, **66**: 2259–2263.
- Hashidoko, Y., Osaki, M. & Tahara, S. 2004. Gel for microbial stock. Patent in Japan No. 2004–136506.
- Hashidoko, Y. & Tahara, S. 2005. Rhizo-biocomplex: an important concept to regulate rhizospheric environment for sustainable crop production. In: *Proceedings of International Conference on Sustainable Crop Production in Stress Environments: Management and Genetic Options* (eds. Tomar, V.S. & Behl, R.K.), pp. 101–109, JNKVV, Jabalpur, India, February 9–12, 2005.
- Hoo, H., Hashidoko, Y., Islam, M.T. & Tahara, S. 2004. Relatively high threshold level of Mg^{2+} cation required for cell growth of a rhizoplane bacterium *Sphingomonas yanoikuyae* EC–S001. *Applied and Environmental Microbiology*, **70**: 5214–5221.
- Kennedy, I.R., Pereg–Greg, L.L., Wood, C., Deaker, R., Gilchrist, K. & Katupitiya, S. 1997. Biological nitrogen fixation in non-leguminous field crops: Facilitating the evolution of an effective association between *Azospirillum* and wheat. *Plant and Soil*, **194**: 65–79.
- Khalid, A., Arshad, M. & Zahir, Z.A. 2004. Screening of plant growth-promoting rhizobacteria for improving growth and yield of wheat. *Journal of Applied Microbiology*, **96**: 473–480.
- Kobayashi, S. 2004. Landscape rehabilitation of degraded tropical forest ecosystems. Case study of the CIFOR/Japan project in Indonesia and Peru. *Forest Ecology and Management*, **201**: 13–22.
- Lee, S.S. 1990. The mycorrhizal association of the Dipterocarpaceae in the tropical rain forests of Malaysia. *Ambio*, **19**: 383–385.
- Lichter, A., Manulis, S., Sagee, O., Gafni, Y., Gray, J., Meilan, R., Morris, R.O. & Barash, I. 1995. Production of cytokinins by *Erwinia herbicola* pv. *gypsophilae* and isolation of a locus conferring cytokinin biosynthesis. *Molecular Plant–Microbe Interactions*, **8**: 114–121.
- Olsen, S.R. & Sommers, L.E. 1982. Phosphorus. In: *Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties, 2nd ed.* (eds. Page, A.L., Miller, R.H. & Keeney, D.R.), pp. 403–430. American Society of Agronomy, Madison, Wisconsin, USA.
- Phat, N.K., Knorr, W. & Kim, S. 2004. Appropriate measures for conservation of terrestrial carbon stocks—analysis of trends of forest management in Southeast Asia. *Forest Ecology and Management*, **191**: 283–299.
- Poole, E.J., Bending, G.D., Whipps, J.M. & Read, D.J. 2001. Bacteria associated with *Pinus sylvestris*–*Lactarius rufus* ectomycorrhizas and their effects on mycorrhiza formation *in vitro*. *New Phytologist*, **151**: 743–751.
- Powell, M.H. 1994. Selecting and testing nitrogen fixing trees for acid soils. Agroforestry for the Pacific Technologies. A publication for Agroforestry Information, Winrock International Service. Morrilton, Arkansas, USA. NFTA. 10, p. 7.
- Rodriguez, H., Gonzalez, T., Goire, I. & Bashan, Y. 2004. Gluconic acid production and phosphate solubilization by the plant growth-promoting bacterium *Azospirillum* spp. *Naturwissenschaften*, **91**: 552–555.
- Subiakto, A. & Sakai, C. 2002. Securing planting stock supply of meranti. Forest Initiatives Newsletter. Forest Liaison Bureau, Indonesia. p. 7.
- Tilak., K.V.B.R., Ranganayaki, N., Pal, K.K., De, R., Saxena, A.K., Nautiyal, C.S., Mittal, S., Tripathi, A.K. & Johri, B.N. 2005. Diversity of plant growth and soil health supporting bacteria. *Current Science*, **89**: 136–150.
- Turjaman, M., Tamai, Y., Segah, H., Limin, S.H., Cha, J.Y., Osaki, M. & Tawaraya, K. 2005. Inoculation with the ectomycorrhizal fungi *Pisolithus arhizus* and *Scleroderma* sp. improves early growth of *Shorea pinanga* nursery seedlings. *New Forests*, **30**: 67–73.
- Walkey, A. & Black, I.A. 1934. An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Science*, **37**: 29–38.
- Weisburg, W.G., Barns, S.M., Pelletier, D.A. & Lane, D.J. 1991. 16S Ribosomal DNA amplification for phylogenetic study. *Journal of Bacteriology*, **173**: 697–707.
- Yazid, S.M., Lee, S.S. & Lapeyrie, F. 1994. Growth stimulation of *Hopea* spp. (Dipterocarpaceae) seedlings following ectomycorrhizal inoculation with an exotic strain of *Pisolithus tinctorius*. *Forest Ecology and Management*, **67**: 339–343.

Received 20nd June 2006Accepted 16th Nov. 2006