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Isolation and Selection of Endophytic Bacteria Consortia From Medicinal Plant (*Andrographis Paniculata*) As Plant Growth Promotimg Agents

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Abstract: The endophytic bacteria has an important role on the plant, such as a plant growth promoter with the production of plant growth regulators such as IAA and GA3, otherwise it can supply the plant nutrient. They are necessary for the growth and development of plants. The main objective of this study was to obtain effect of endophytic bacteria consortia from king of bitter as plant growth promoter *Andrographis paniculata*. This research used a randomized block design, 25 treatments and 3 replications. Treatment consisted of 1 control and 24 isolates of bacteria consortia. The results showed that 24 endophytic bacterial consortia were obtained that isolated from bitter plant. Endophytic bacteria consortia were contained in root tissues 10^5-10^6, shoot tissues 10^3-10^4 and stem tissues 10^3-10^5 CFU mL^{-1}. In general consortia isolated from the root tissue had higher population than the leaves and stems tissue. The highest populations of endophytic bacteria consortia were produced by 5 MA (1.1×10^6). Endophytic bacteria consortia significantly increased growth, shoot (7.86%), roots (10-120%) and biomass dry weight. The highest percentage of growth plants were indicated by 5MD, 20BB, 20BD and 20CD, 100%, respectively. The highest number (6.5) and length of leaf (6.5 cm) at 5MD and width of leaf 20 MD (1.65 cm). The highest shoot dry weight in 20 BD (0.26 g $plant^{-1}$), root (0.32 g $plant^{-1}$) and biomass dry weight (0.54 g $plant^{-1}$) in 20BA isolate.

Key words: *Andrographis paniculata*, endophytic bacteria, isolation, growth promoter

INTRODUCTION

Endophytic microbe is microbes that live in the tissues and has advantages on the host plant (Hallmann *et al*., 1997; Kloeppe *et al*., 1999). These microbes form an aggregate on the surface of the plant and interact with the plants in the form of specific relationship starting from free relationship, pathogens to the substantial relationship such as commensalism, mutualism and symbiosis (Andrews and Harris, 2000). One of the endophytic microbes that has positive relationship with its host plant is bacteria.

Endophytic bacteria have several mechanisms that can stimulate the growth and health of plants (Taghavi *et al*., 2009). The improvement of the growth and health of the plants cannot be separated from the role of endophytic bacteria. They supply nutrient elements through the process of fixing the nutrient elements from the air (Hirano and Upper, 2000), improve the mobilization of P, trapped Fe (Ryan *et al*., 2008), fight against plant pathogens through the induction of systemic resistance and produce secondary metabolic compounds that are antagonists (Kloepper and Ryu, 2006; Sturz and Nowak, 2000), as well as reduce plants biotic or abiotic stress without pathogenicity (Lughtenber and Kamilova, 2009).

Other than that, endophytic bacteria can produce phytohormon such as Indole Acetic Acid (IAA) (Pedraza *et al*., 2004), cytokinin (Ergun *et al*., 2002) and Gibberellic Acid (GA_3) (Kharwar *et al*., 2008), which are required by plants for growth and development. Research in rice indicated that giving endophytic bacteria stimulates growth and production (Gusmaini *et al*., 2007).

The king of bitter is one of medicinal plants that have significant benefits for human health as hepatoprotector (Rao *et al*., 2004), anti-oxidants (Lin *et al*., 2009), anti-diabetes (Zhang *et al*., 2009) and anti-microbial agents (Xu *et al*., 2006). Whole plants or shoot parts of king of bitter is harvested. Yield of king of bitter in Indonesia ranged from 0.64-1.2 ton ha^{-1} dried herbs (Yusron *et al*., 2007).

Endophytic bacteria is needed to produce biomass, such as king of bitter. It can be expected not only to improve the production of its biomass but also to reduce inputs supply of fertilizer and pesticides, so that cultivation of king of bitter will be more economical and produced safer herbs for human consumption.

In Indonesia, king of bitter have been found growing wild at various environment, from low to highland from dry to humidland. In Java, it can be found from the West,

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Central to East Java, therefore it is necessary to explore endophytic bacteria from various environment to be use as isolates collection in increasing biomass production of king of bitter.

This study was aimed to isolate and select endophytic bacteria consortia derived from king of bitter plants growing in various environment to increase its biomass production.

MATERIALS AND METHODS

The research was carried out in the laboratory of Microbiology and the green house of Indonesian Spices and Medicinal Crops Research Institute in Bogor (Balittro) Indonesia, from June 2011 to January 2012.

Exploration of king of bitter plant: Exploration of endophytic bacteria was carried out on the king of bitter that grew wild in various environmental conditions, i.e., in Pasuruan and Madiun (East Java), Blora (Central Java) and from the cultivated plant collections in Balittro Bogor (West Java). The collected bacteria consortia were isolated and selected by growing them on king of bitter from Cimanggu garden. Parameters of environment were observed such as rainfall, average daily temperature and characteristic of soil chemistry from each site where king of bitter were collected.

Isolation of endophytic bacteria from king of bitter plant: One plant accession were collected from each site so that 4 collection were obtained from four sites. From each plant accession, isolation was taken from 3 plant parts, namely: Leaves, stems and roots. Each bacterial consortia was isolated by growing it on two media concentrations of Soya Agar Trytone (TSA), 5 and 20%, so that 24 isolates of consortia (4 plant accessions×3 parts of plants×2 media types) altogether were obtained. The isolation methods of endophytic bacteria were as follows: (1) Plant parts (roots, stems and leaves) were cleaned from impurities by using running water and then dried by wiping it with sterile filter paper. The 1 g of cleaned material was sterilized by soaking 70% ethanol for 30 sec, followed by soaking in sodium hypochlorite (1-2%) for 1-2 min, then rinsed with sterile water four times; (2) Each of sterilized of king of bitter plant (roots, stems and leaves) parts was crushed by using sterile mortar and 10 mL of distilled water were added. Water solution was then diluted up to 10^2-10^5. The 0.1 mL of diluted solution was taken and placed into petridish that already contained TSA media of 5 and 20% and then sealed by using plastic seal. The sealed petri dish was incubated at 30°C for 24-48 h. The growth of endophytic bacteria colony from king of bitter plant were observed daily and calculated.

Selection of endophytic bacteria consortia: The growth of isolated endophytic bacteria were then examined on the inoculated seeds of king of bitter plant accessions of Cimanggu Balittro in experimental pot using randomized block design, with 25 treatments and 3 replications. The treatments consisted of: (1) Control (without the endophytic bacteria), (2) 5 MA (isolated from Madiun king of bitter roots on 5% TSA), (3) 5 MB (isolated from Madiun king of bitter stems in 5% TSA), (4) 5MD (isolated from Madiun king of bitter leaves in 5% TSA), (5) 5 PA (isolated from Pasuruan king of bitter roots in 5% TSA), (6) 5 PB (isolated from Pasuruan king of bitter stems in 5% TSA), (7) 5 PD (isolated from Pasuruan king of bitter leaves in 5% TSA), (8) 5 BA (isolated from Blora king of bitter roots in 5% TSA), (9) 5 BB (isolated from Blora king of bitter stems in 5% TSA), (10) 5 BD (isolated from Blora king of bitter leaves in 5% TSA), (11) 5 CA (isolated from Bogor king of bitter roots in 5% TSA), (12) 5 CB (isolated from Bogor king of bitter stems in 5% TSA), (13) 5 CD (isolated from Bogor king of bitter leaves in 5% TSA), (14) 20 MA (isolated from Madiun king of bitter roots in 20% TSA), (15) 20 MB (isolated from Madiun king of bitter stems in 20% TSA), (16) 20 MD (isolated from Madiun king of bitter leaves in 5% TSA), (17) 20 PA (isolated from Pasuruan king of bitter roots in 20% TSA), (18) 20 PB (isolated from Pasuruan king of bitter stems in 20% TSA), (19) 20 PD (isolated from Pasuruan king of bitter leaves in 20% TSA), (20) 20 BA (isolated from Blora king of bitter roots in 20% TSA), (21) 20 BB (isolated from Blora king of bitter stems in 20% TSA), (22) 20 BD (isolated from Blora king of bitter leaves in 20% TSA), (23) 20 CA (isolated from Bogor king of bitter roots in 20% TSA), (24) 20 CB (isolated from Bogor king of bitter stems in 20% TSA), (25) 20 CD (isolated from Bogor king of bitter leaves in 5% TSA).

Before inoculation, the king of bitter seeds of Cimanggu accessions were soaked for 2×24 h by using water at room temperature, sterilized with 70% alcohol for 30 sec, followed by soaking in sodium hypochlorite solution (1-2%) for 1-2 min, then rinsed with sterile water. The sterilized seeds were planted into pot/tray which already contained sterilized zeolite. The inoculation of seeds was executed by pouring the suspension of the endophytic bacteria to plant media with the population density of 10^{10} cells mL^{-1}.

Data statistic analysis: The data was analyzed statistically by using ANOVA and treatment differences were separated using LSD at 5% level of probability.

RESULTS AND DISCUSSION

Exploration of king of bitter: Exploration in Madiun was performed on the king of bitter plants that grew up under shade while in Pasuruan, taken from wild plants that grew around an open courtyard. In Blora, the exploration was taken from plants that grew under the teak forest and in Bogor, taken from the experimental garden on open land.

The environmental conditions of the 4 exploration sites indicated that there are differences in agroclimatic condition such as elevation, precipitation, temperature and soil fertility (Table 1). The elevation of Pasuruan, Blora, Bogor and Madiun were 33, 70, 240 and 750 m above sea level (asl), respectively. It showed that king of bitter can grow in a wide range of environmental conditions, from lowland to medium height land in open to shade conditions.

Differences were also found in soil chemical properties. Soil conditions of the Pasuruan site is better than the three other sites in order Blora, Madiun and Bogor. It can be seen from the pH, organic matter and total bases that can be switched (Table 2). Differences in agroclimate affect the growth, leaf appearance and the content of andrographolide (Table 3). On Pasuruan soil that is relativelly fertile, the appearance of the king of bitter plant is quite good and the content of andrographolide is (1.5%) higher than the king of bitter plant growing wild in Madiun (0.04%) and Blora (0.04%).

The king of bitter grows wild in Blora and Madiun that have similar soil chemical properties Pasuruan, the variation of its vegetation is low compared to Pasuruan. This may be due to the low rainfall and daily temperatures that are relatively high in Blora. The environment is dry and relatively hot (32°C and 70 masl) so the soil becomes hard and lumpy and probably the nutrient absorption process of plant is insufficient. The growth environment of king of bitter plants in Madiun is also relatively extreme with the altitude of 750 m asl and the temperature is about 25°C that leads to less optimal growth of plant.

According to Pujiasmanto *et al.* (2007), king of bitter can grow at at elevation 180-861 m above sea level with the temperature of environmental conditions 20.32-26.93°C, relative air humidity 80-85% and precipitation 2053.2-3555.6 mm year^{-1}. King of bitter can grow on soil with nutrient content of moderate N, low P, moderate K, low Mg, very low to low Ca, low to moderate organic C and acid pH to very acid. King of bitter plants are found more in the medium height plains compared to low and high plains. The highest andrographolide content obtained from king of bitter growing in the medium height land (2.27%), while in the lowlands (1.37%) and high land (0.89%).

The growth of cultivated king of bitter plant originating from Bogor is quite good and the content of andrographolide is higher than from the other sites. Environmental conditions for both rainfall and air temperature support its growth, although it was in the lowlands and with the chemical properties of the soil that is less fertile, but due to the cultivation treatment to the plant the appearance of king of bitter plant is better and its andrographolide content is higher than that from Pasuruan, Madiun and Blora (Table 3).

The characteristics of king of bitter plant also showed the differences in shape, size and number of leaves/g. The king of bitter plants from Bogor have larger and heavier leaves, where in 1 g of leaves contained 7 leaves. The size of wild king of bitter plants from Madiun, Pasuruan and Blora is smaller and lighter and the numbers of leaves in 1 g are 13, 17 and 17, respectively (Table 4). It points out that in addition to genetic factors, the environmental factors also affected the plant growth and the secondary metabolite content.

Isolation of endophytic bacteria from king of bitter: The number of endophytic bacteria isolated from explorated king of bitter plant from Pasuruan, Madiun, Blora and Bogor were 24 consortia. The results of this isolation showed that each plant part contained endophytic

Table 1: Agroclimate condition of king of bitter exploration sites

Site	Altitude (m asl)	Average daily temperature (°C)	Rainfall (mm year^{-1})	Note
East Java				
1. Village: Kare	750	25	Moderate 1000-1500	Wildly grow in dike
Sub district: Kare				
Madiun	33	32		
2. Village: Ranuklindungan				
Sub district: Grati Pasuruan	33	32	Moderate-high 1500-2000	Wildly grow yard
Central Java				
Village: Ngliron	70	32	Low<1000	Wildly grow up under teak forest
Sub district:Randublatung				
Blora				
West Java				
Village: Menteng				
Sub district:	240	30	High±2000	Cultivated area
Centre bogor				

bacteria isolates and the bacteria contained in the consortia from each part of the plant are generally different. The number of bacteria also different i.e., endophytic bacteria consortia were contained in king of bitter tissue ranges 10^3-10^6 CFU mL^{-1}, at root tissues 10^5-10^6 and shoot tissues 10^3-10^4 and stem tissues 10^3-10^5 CFU mL^{-1} (Table 5). The results indicated that endophytic bacteria can be isolated either from wild or cultivated plants. This result was line with Zinniel *et al.* (2002), who was able to isolate endophytic bacteria derived from 4 species of cultivated plant and 27 species of wild plants in Nebraska.

Table 2: Characteristic of soil chemistry at king of bitter exploration sites

Variables	Madiun	Pasuruan	Blora	Bogor
pH H20	5.95	7.11	6.15	4.40
KCl	5.27	6.54	5.34	4.13
C-org (%)	1.91	3.05	3.16	1.53
N-total (%)	0.20	0.27	0.29	0.18
C/N ratio	9.55	11.30	10.90	8.50
P_2O_5 available (ppm)	5.81	8.20	3.62	5.36
(cmol kg^{-1})				
Ca	10.03	43.22	39.92	2.36
Mg	2.33	4.32	7.32	0.31
K	1.05	1.92	1.23	0.12
Na	0.28	0.22	0.20	0.14
Total	13.69	49.68	48.67	2.93
CEC (cmol kg^{-1})	13.06	26.08	50.38	15.29

Table 3: Andrographolide content of king of bitter obtained from exploration sites

Site	Andrographolide (%)
Madiun	0.04
Pasuruan	1.51
Blora	0.04
Bogor	2.16

Table 4: Characteristic of leaves of king of bitter from exploration

Site	Shape and size of leaf	No. of leaf g^{-1}	Colour of leaf
Madiun	Round, large	13	Green
Pasuruan	Round, small	17	Green
Blora	Acute, small	17	Green
Bogor	Pointed, longish	7	Green

Each plant consists of one or more of the endophytic microbes consisting of bacteria and fungus (Strobel, 2003; Strobel and Daisy, 2003). In almost any vascular plants that grow in tropical climate contain the endophytic bacteria either bacteria or fungus (Firakova *et al.*, 2007; Zhang *et al.*, 2006). The endophytic microbes present on the whole parts of plant such as leaves, roots, stems and the skin on angiospermae plants (Banerjee *et al.*, 2009).

The exploration site of the host plant also affected the obtained bacteria populations. The population of bacteria on the roots of the plant are higher than those on the stems and leaves. The results of this research showed that the highest bacteria population of the 24 consortia are obtained from the roots of plant that originated from 5MA (10^6 CFU mL^{-1}). This is in line with the results of Mendes *et al.* (2007), that the population of isolated endophytic bacteria from the root is higher than the population from stem on the sugarcane plant. Apparently, the environmental condition in Madiun supports the development of endophytic bacteria better compared to Bogor, Pasuruan and Blora.

The bacteria that are present in the root are generally varied. Both positive and negative gram were found in one consortia from the root. The bacteria from the roots and stems are dominated by positive gram bacteria. The close vicinity and direct contact of the roots to the soil made the diverse bacteria taht exist have greater chance to get into the roots of the plant. Microbes in the soil can enter and live in the roots and form the population ranging from 10^5-10^7 CFU g^{-1} fresh weight

Table 5: Morphological Characteritics of endophityc bacteria colonies isolated from king of bitter

Isolate code	Population (CFU mL^{-1})	Colony colour	Colony form	Colony size	No. of gram + (-)
5 MA	1, 1×10^6	Light-yellow, white	Round	Small-medium	4 (2)
5 MB	1, 3×10^5	White clear	Round	Small-large	4 (0)
5 MD	2, 3×10^4	White, yellow	Round	Small-medium	4 (0)
5 PA	3, 2×10^5	White	Round	small	5 (0)
5 PB	4, 3×10^4	Yellow, white	Round	Small-large	4 (0)
5 PD	3, 3×10^4	Light-yellow	Round	Medium-large	3 (0)
5 BA	1, 7×10^5	White	Round	Small-medium	3 (3)
5 BB	2, 0×10^3	Brown, light-yellow	Round	Small-medium	5 (0)
5 BD	4, 3×10^3	White	Round	Small-medium	3 (2)
5 CA	5, 0×10^5	White	Round	Small-large	5 (0)
5 CB	2, 6×10^5	Light-yellow, white	Round	Small-medium	3 (0)
5 CD	5, 0×10^4	White	Round	Small-medium	3 (0)
20 MA	1, 1×10^6	White, light-yellow	Round	Small-large	6 (0)
20 MB	3, 2×10^5	White	Round	Small-medium	2 (3)
20 MD	1, 3×10^5	White, yellow	Round	Small-medium	3 (0)
20 PA	2, 6×10^5	White	Round	Small	6 (1)
20 PB	6, 0×10^4	White	Round	Small-large	3 (2)
20 PD	3, 3×10^4	White	Round	Medium-large	3 (2)
20 BA	2, 2×10^5	White	Round	Small-medium	3 (2)
20 BB	3, 7×10^3	White, brown	Round	Small-medium	4 (3)
20 BD	7, 3×10^3	White, yellow	Round	Small-medium	4 (0)
20 CA	5, 8×10^5	Light-yellow, white	Round	Small-medium	5 (0)
20 CB	2, 8×10^5	White clear	Round	Small-large	4 (0)
20 CD	5, 7×10^4	White	Round	Small-medium	4 (0)

Table 6: Growth of king of bitter after inoculation by endophytic bacteria at 1.5 MAPs

Treatments	Leaf No.	Leaf length (cm)	Leaf width (cm)	Seed germination (%)
5 MA	5.40^{b}	5.40^{b}	1.50^{a-d}	66.67^{b-c}
5 MB	6.00^{a-b}	6.20^{a-b}	0.97^{e}	58.33^{c-d}
5 MD	6.50^{a}	6.50^{a}	1.20^{c-e}	100.00^{a}
5 BA	5.80^{a-b}	5.80^{a-b}	1.30^{b-e}	83.33^{a-b}
5 BB	6.17^{a-b}	6.17^{a-b}	1.35^{a-d}	91.67^{a}
5 BD	6.17^{a-b}	6.17^{a-b}	1.29^{b-e}	91.67^{a-b}
5 PA	6.00^{a-b}	6.00^{a-b}	1.28^{b-e}	75.00^{a-c}
5 PB	6.00^{a-b}	6.00^{a-b}	1.37^{a-d}	83.33^{b}
5 PD	6.17^{a-b}	6.00^{a-b}	1.18^{d-e}	91.67^{a-b}
5 CA	6.17^{a-b}	6.17^{a}	1.52^{a-c}	91.67^{a-b}
5 CB	6.00^{a-b}	6.00^{a-b}	1.49^{a-d}	83.33^{a-b}
5 CD	6.17^{a-b}	6.17^{a-b}	1.37^{a-d}	83.33^{a-b}
Control	3.00^{c}	3.00^{c}	0.61^{f}	33.33^{d}
20 MA	6.00^{a-b}	6.00^{a-b}	1.43^{a-d}	91.67^{a-b}
20 MB	6.00^{a-b}	6.00^{a-b}	1.50^{a-d}	75.00^{a-c}
20 MD	6.17^{a-b}	6.00^{a-b}	1.65^{a}	83.33^{a-b}
20 BA	6.17^{a-b}	6.17^{a-b}	1.34^{a-d}	91.67^{a-b}
20 BB	6.00^{a-b}	6.00^{a-b}	1.40^{a-d}	100.00^{a-b}
20 BD	5.40^{b}	5.42^{b}	1.56^{a-b}	100.00^{a-b}
20 PA	6.00^{a-b}	6.00^{a-b}	1.35^{a-d}	75.00^{a-c}
20 PB	6.25^{a-b}	6.25^{a-b}	1.52^{a-d}	75.00^{a-c}
20 PD	6.40^{a}	6.40^{a}	1.48^{a-d}	83.33^{a-c}
20 CA	6.00^{a-b}	6.00^{a-b}	1.55^{a-b}	100.00^{a}
20 CB	6.00^{a-b}	6.20^{a-b}	1.43^{a-d}	83.33^{a-b}
20 CD	6.00^{a-b}	6.00^{a-b}	1.48^{a-d}	100.00^{a}
CV (%)	7.86	7.87	11.84	17.38

No. followed by the same letters in each column are not significantly different at 5% LSD

(Hallmann, 2001). The root area is one of the areas that provides nutrients for bacteria through nutrient exudates from the roots, so that bacteria can live and grow better. Nutrition is important for the life of microbes that is usually in the form of sugars such as fructose and sucrose (Mercier and Lindow, 2000). Organic compounds have significant role in shaping soil bacterial communities and significant impact on plant growth (Shi *et al*., 2011).

Selection of endophytic bacteria consortia: Selection of endophytic bacteria consortia as plant growth promoting bacteria were shown by theirs influence on plant growth, development and biomass yield.

Effect endophytic bacteria consortia on plant growth: All endophytic bacteria inoculant significantly increased the percentage of plant growth, number, length and width of leaves of king of bitter compared with control (Table 6). Diverse responses shown by the seeds of king of bitter pointed out the various ability of endophytic bacteria in stimulating the germination of king of bitter seeds. The highest eaf number (6.5) and leaf length (6.5 cm) at Five MD isolate, while leaf width (1.65 cm) at 20 MD isolate. 5MA had the lowest value of leaf number and leaf length and 5 MB in leaf width than another inoculant. Endophytic bacteria applied on king of bitter seeds could increase germination, the percentage of germinated seeds were higher compared than control. The percentage of the growing sprouts vary ranging from 50-100%. The king of better grew 100% at 5 MD, 20 BB, 20 BD, 20 CA and 20 CD isolates (Table 6).

These isolates (MD, MB and MD) were isolated from leaf, root and stem of king of bitter, the wild life from Madiun at pH (5.96) and poor nutrient. Twenty BB and 20 BD isolates were isolated from stem and leaf tissues of king of bitter plant that live in Blora at low soil fertility (poor nutrient) and acid pH. Isolates of 20 CA and 20 CD were isolated from root and leaf tissues of king of bitter plant that live in Bogor at low soil fertility (poor nutrient) and very acidic pH. On the environmental conditions are less than optimal endophytic bacteria able to survive, as well as in other environments, endophytic bacteria can survive on other environmental conditions. These indicate that the environment which to grow plants affect on the properties of bacteria.

The similarly, all endophytic bacteria consortia were inoculated at king of bitter showed significantly effect to increase plant height and root lenght. The highest plant height at 20 MB (6.47 cm) and root length at 20BB (8.98 cm). On the contrary, strains 5PA had the lowest value of plant height and 5BB had the lowest value of root length than another inoculant (Fig. 1).

Endophytic bacteria were able to improve the growth in observed plants and did not show negative effect on the growth of king of bitter seedlings. It was stated by Yu *et al.* (2010) that if surrounding plants were infected by pathogens, but the plant did not show infection

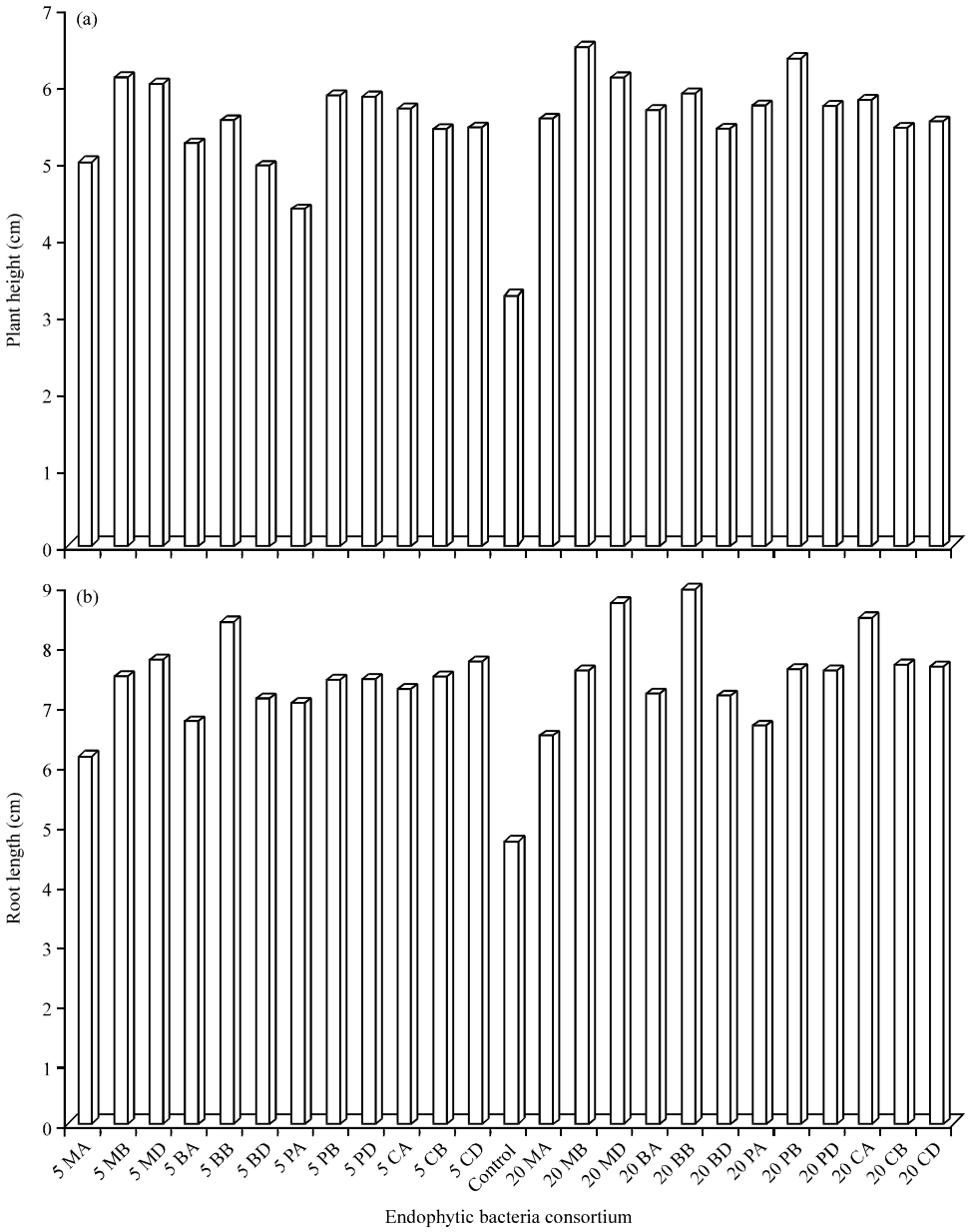

Fig. 1(a-b): Effect of endophytic bacteria consortia on (a) Plant height and (b) Root length of *Andrographis paniculata*

symptoms meant that endophytic microbes within the plant were able to produce anti-microbial compounds naturally. Some strains of bacteria that have been tested through their anti-microbial activity were able to produce the compounds such as alkaloids, peptides and phenol.

Melliawati *et al.* (2008) have isolated endophytic bacteria from forest plants containing active substances (steroids), the compound is able to inhibit the growth of pathogen microbes. In addition, the fact that the growth of king of bitter plant inoculated by the endophytic bacteria was better than control, might be related to the chemical substances produced by endophytic bacteria. It has been reported that endophytic bacteria phytohormon such as IAA (Pedraza *et al.*, 2004), cytokinin (Ergun *et al.*, 2002) and Gibberellin Acid (GA_3) (Kharwar *et al.*, 2008), that played important roles in promoting plant growth. Endophytic bacteria derived from plants that grow in the peatland were able to improve the production and growth of rice, as well as the content of the three phytohormones,

Table 7: Effect of endophytic bacteria consortia on yield of king of bitter at 1.5 MAPs

Treatments	Shoot dry weight (g)	Root dry weight (g)	Total of biomass dry weight (g)
5 MA	0.24^{a-c}	0.16^{b-g}	0.39^{b-g}
5 MB	0.17^{c-e}	0.12^{c-g}	0.29^{g-i}
5 MD	0.24^{a-b}	0.20^{b-c}	0.44^{a-c}
5 BA	0.14^{e}	0.12^{d-g}	0.25^{hi}
5 BB	0.20^{a-e}	0.13^{c-g}	0.33^{c-i}
5 BD	0.17^{b-e}	0.13^{c-g}	0.30^{f-i}
5 PA	0.15^{d-e}	0.09^{g}	0.23^{ij}
5 PB	0.20^{a-e}	0.15^{b-g}	0.36^{c-g}
5 PD	0.22^{a-d}	0.19^{b-e}	0.41^{c-f}
5 CA	0.21^{a-d}	0.11^{e-g}	0.33^{c-i}
5 CB	0.21^{a-d}	0.14^{c-g}	0.35^{c-g}
5 CD	0.18^{a-e}	0.15^{c-g}	0.32^{d-i}
K	0.04^{f}	0.10^{f-g}	0.13^{j}
20 MA	0.19^{a-e}	0.15^{b-g}	0.34^{c-i}
20 MB	0.19^{a-e}	0.13^{c-g}	0.32^{d-i}
20 MD	0.23^{a-c}	0.17^{b-f}	0.39^{c-f}
20 BA	0.23^{a-c}	0.31^{a}	0.54^{a}
20 BB	0.22^{a-d}	0.18^{b-e}	0.42^{b-e}
20 BD	0.26^{a}	0.22^{b}	0.48^{a-b}
20 PA	0.23^{a-c}	0.14^{c-g}	0.37^{c-g}
20 PB	0.23^{a-c}	0.16^{b-g}	0.38^{c-g}
20 PD	0.23^{a-c}	0.15^{b-g}	0.37^{c-g}
20 CA	0.25^{a-b}	0.13^{c-g}	0.43^{a-d}
20 CB	0.24^{a-b}	0.13^{c-g}	0.38^{c-g}
20 CD	0.24^{a-b}	0.14^{c-f}	0.38^{c-g}
CV (%)	18.10	24.13	14.98

No. followed by the same letter in each column are not significantly different at 5% LSD

IAA, cytokinin and GA_3 (Gusmaini *et al*., 2007). This information might be the preliminary finding for other medicinal plants that grow in Indonesia which is known as having abundant natural resources.

Effect of endophytic bacteria consortia on king of bitter biomass yield: The results showed that endophytic bacteria application had significant effect on on biomass yield at king of bitter. Increasing of shoot and root dry weight and total dry biomass king of bitter. The highest shoot dry weight at the 20BD (0.26 g plant^{-1}), root dry weight (0.31 g plant^{-1}) and total of biomass dry weight (0.54 g plant) at 20 BA (Table 7). The two isolates (20 BD and 20 BA) were isolated at king of bitter tissues from Blora.

Inoculation of endophytic bacteria improved biomass, 84% in roots, 38% in shoots and 48% in leaves (Rogers *et al*., 2012). Likewise, Taghavi *et al*. (2009) reported that endophytic bacteria improved root development. This improvement may be associated with phytohormone and chemical compounds that regulated the metabolism of plant growth that produced by endophytic bacteria.

All tested isolates of endophytic bacteria were able to improve the growth and biomass production compared to control (Table 7). The increased shoots and roots dry weights ranged from 7-86 and 10-120%, respectively. The formation of the colony was an important factor for optimization of the role of bacteria in the plant. Endophytic bacteria that can form colonies faster, will also quickly adapt to host plants and thus can perform better. The process of colonization was important to predict how the bacteria interact with plants and wether they can established in the host plants environment after application of bio fertilizer or biocontrol agents (Compant *et al*., 2010).

CONCLUSION

The twenty four isolates of endophytic bacteria consortia have been succefully isolated from four sites and three plant parts namely, roots, stems and leaves of king of bitter plant. Endophytic bacteria consortia were contained in king of bitter tissue ranges 10^3-10^6 CFU mL^{-1}, at root tissues 10^5-10^6 and shoot tissues 10^3-10^4 and stem tissues 10^3-10^5 CFU mL^{-1}. The population of endophytic bacteria consortia isolated from root tissues were higher compared to the stems and leaves. The highest population was obtained from roots of king of bitter at 5 MA (10^6 CFU mL^{-1}).

Endophytic bacteria consortia promoted king of bitter growth, as shown by the increasing in the percentage of plants growth, plant height, the number, length and width of the leaf, root length and biomass yield. Endophytic bacteria consortia significantly increased growth, shoot (7.86%), roots (10-120%) and biomass dry weight. The highest plant growth percentage (100%) were found in 5MD, 20BB, 20BD and 20CD. The highest leaf number (6.5) and length (6.5 cm) in 5 MD and leaf width in 20 MD (1.65 cm). The highest shoot dry weight in 20BD (0.26 g plant^{-1}), root (0.31 g plant^{-1}) and total of biomass dry weight (0.54 g plant^{-1}) in 20BA.

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