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Isolation of Endophytic Bacteria from Tomato and Their Biocontrol Activities against Fungal Diseases

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Endophytic bacteria have gained attention due to their interesting features related to plant growth and health stimulation. The objective of this research was to determine the populations and spectrum of indigenous root endophytic bacteria from tomato (*Lycopersicon esculentum)* and the biocontrol activity of the bacteria for plant protection. The isolation procedure of these endophytic bacteria was done using surface-sterilization method using alcohol and sodium hypochlorite (NaOCl). General medium trypsic soy agar (TSA) was used as the growth medium for isolation. The total population density of endophytic bacteria recovered from tomato roots ranged from 1.0 to 4.4 (in $\log_{10}$ scale) CFU g^{-1} fresh root weight. A total of 564 strains of endophytic bacteria were isolated from tomato plants grown in West Java, Indonesia. Endophytic bacterial strains were identified based on their fatty acid profile using FAME-GC-MIDI system. Fifty species and 32 genera of endophytic bacteria were found in association with tomato root. The most abundant endophytic bacterial genera were *Bacillus* spp. and *Pseudomonas* spp. One hundred and eighty one bacterial strains were tested for their *in vitro* antagonism towards *Rhizoctonia solani, Fusarium oxysporum* f.sp. *radicis-lycopersici*, and *F. oxysporum* f.sp. *lycopersici*. Fourteen strains showed antagonism *against R. solani*, nine strains against *F. oxysporum* f.sp. *radicis-lycopersici* and seven strains against *F. oxysporum* f. sp. *lycopersici*. The close relationship between endophytic bacteria and their hosts make them ideal candidates for biological control and plant growth promotion.

Key words : *Bacillus* spp., endophytic bacteria, *Pseudomonas* spp., tomato

Bakteri endofit telah menarik perhatian karena potensinya untuk peningkatan pertumbuhan dan kesehatan tanaman. Tujuan penelitian ini adalah untuk mengetahui populasi dan spektrum bakteri endofit yang berasal dari akar tanaman tomat dan potensinya sebagai agen biokontrol untuk perlindungan tanaman. Prosedur isolasi bakteri endofit dilakukan dengan menggunakan metode sterilisasi permukaan dengan alkohol dan sodium hipoklorit (NaOCl), dan medium *trypsic soy agar* (TSA) sebagai media pertumbuhan untuk bakteri. Strain bakteri endofit diidentifikasi berdasarkan profil asam lemaknya dengan menggunakan FAME-GC-MIDI system. Kepadatan total populasi bakteri endofit yang diisolasi dari akar tomat berkisar 1.0-4.4 (pada skala $\log_{10}$) CFU g^{-1} berat akar segar. Sebanyak 564 strain bakteri endofit berhasil diisolasi dari tanaman tomat dari daerah Jawa Barat, Indonesia. Hasil identifikasi menunjukkan 50 spesies dan 32 genera bakteri endofit ditemukan berasosiasi dengan akar tomat. Genus bakteri endofit paling banyak ditemukan adalah *Bacillus* spp. dan *Pseudomonas* spp. Sebanyak 181 isolat bakteri endofit diuji antagonismenya secara *in vitro* terhadap cendawan patogen. Sebanyak 14 isolat bakteri menunjukkan reaksi antagonisme terhadap *Rhizoctonia solani*, 9 strain terhadap *Fusarium oxysporum* f.sp. *radicis-lycopersici*, dan 7 strain terhadap *F. oxysporum* f. sp. *lycopersici*. Hubungan yang sangat dekat antara bakteri endofit dan tanaman inangnya menjadikan bakteri endofit sangat berpotensi sebagai agen biokontrol dan pemacu pertumbuhan tanaman.

Kata kunci: *Bacillus* spp., bakteri endofit, *Pseudomonas* spp., tomat

Endophytic bacteria colonize healthy plant roots without causing apparent disease. More recently, endophytic bacteria have gained attention due to their interesting features related to plant growth and health stimulation. Studies on endophytic bacteria have been conducted. Some of the bacteria are known to increase nutrient availability, produce growth hormones, convey stress tolerance, induce systemic resistance, or deter plant pathogens (Hallmann *et al*. 1997; Buchenauer 1998). Several reports demonstrated that endophytic bacteria are plant-associated bacteria that colonize and persist in various healthy plants, such as fruits, vegetables, stems and roots (Mclnroy and Kloepper 1995; Sturz *et al*. 1997).

Bacterial endophytes can be isolated from surface-sterilized plant tissue or extracted from internal plant tissue. Endophytic microorganisms enter plant tissue primarily through the root zone; however, aerial portions of plants, such as flowers, stems, and cotyledons, may also be used for entry (Quadt-Hallmann and Kloepper 1996). Specifically, the bacteria enter tissues via germinating radicles,

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secondary roots, stomata, or as a result of foliar damage. Endophytes inside a plant may either become localized at the point of entry or spread throughout the plant (Quadt-Hallmann *et al*. 1997). Variations in the populations of endophytic bacteria are attributed to plant source, plant age, tissue type, time of sampling, and environment. Generally, bacterial populations are larger in roots and lower in the stems and leaves (Hallmann *et al*. 1997).

Endophytic bacteria may be considered a new source of biocontrol agents (Backman and Sikora 2008). Some reports have demonstrated that bacterial endophytes are able to improve plant growth and reduce disease symptoms caused by plant pathogens such as *Fusarium oxysporum* subsp. vasinfectum on cotton, *Verticillium albo-atrum*, and *Rhizoctonia solan*i on potato (Hallmann *et al*. 1997), *Clavibacter michiganensis* subsp. sepedonicum on potato (Van Buren *et al*. 1993), and plant-parasitic nematodes (Munif *et al*. 2000; Vertrivelkalai *et al*. 2010). When compared with rhizobacteria, endophytic bacteria have distinct advantages: 1) they colonize an ecological niche similar to that of vascular plant pathogens, i.e. vascular wilt pathogens and sedentary plant parasitic nematodes; 2) they face less competition with other microorganisms; 3) they have access to sufficient supply of nutrients; 4) they are less exposed to rhizosphere's abiotic and antibiotic stress factors; 5) their bioactive metabolites are better translocated within the host plant (Hallmann *et al*. 1997; Buchenauer 1998). The exploitation of the beneficial properties of endophytic bacteria requires a basic knowledge of the endophytic bacteria, such as distribution within the plant tissue, diversity, population dynamics, and characterization. The objective of this research was to determine the variations and types of indigenous endophytic bacteria from tomato plants grown in West Java, Indonesia, and its biological control potential against fungal disease.

MATERIALS AND METHODS

Isolation of Endophytic Bacteria. A total of 20 tomato plants were uprooted at random from tomato fields in the districts Bogor, Sukabumi, and Bandung, West Java, Indonesia. The tomato plants were approximately two months old. The tomato roots were transported to the laboratory for immediate processing. Tomato roots were washed under running tap water to remove adherent soil particles and then blotted dry on tissue paper. To simplify root processing, the root system was divided into three parts: top, middle, and bottom. The top section was discarded, whereas the middle and bottom parts were examined. The root was weighed and then surface sterilized with 3% and 6% sodium hypochlorite containing 0.01% Tween 20 for 3 min, followed by four rinses in sterile 0.01 M potassium phosphate buffer (PB) at pH 7.0 (80 g NaCl, 2 g KCl, 11.5 g Na_2HPO_4, 2 g KH_2PO_4). To confirm complete surface-sterilization (sterility check), the surface sterilized roots were imprinted on tryptic soy agar (TSA). If bacterial growth occurred within 48 h, samples were discarded. The tomato roots were then macerated with a sterile mortar and pestle in three times PB (w/v). The macerate was decanted into sterile conical flasks and shaken for 30 sec. A dilution series was made and 100 µL of each dilution was plated onto 1/10 strength TSA with a Drigalski spatula (plate spreader). Petri plates were incubated at 24 °C for 2-3 d and colony forming units (CFU) were calculated. Three replicates were made per dilution. On each Petri plate a zone containing approximately 30 bacterial colonies was marked. All bacterial colonies from this zone were transferred and purified on full strength TSA. The bacterial colonies were stored in tryptic soy broth (TSB) plus 20% glycerol at -20 °C.

Identification of Endophytic Bacteria. All bacterial strains were identified based on their fatty acid profile using fatty acid methyl ester (FAME) analysis by gas-chromatography and the software Microbial Identification System (MISI Microbial ID, Newark, Delware, USA). The extraction procedure for whole-cell fatty acid from endophytic bacteria was done following the method described by Sasser (1990).

Harvesting. Bacteria from stock cultures (-80 °C) were pre-cultivated for 24 h on tryptic soy broth agar (TSBA) and then streaked on TSBA for identification. Each strain was streaked with a loop onto TSBA in four zones with increasing dilution and incubated at 28 °C for 24 h. The bacteria from zone 2 and 3 were harvested. A loopful of bacteria (about 50 mg) was used to inoculate medium tube.

Saponification. Lipids were saponified by adding 1.0 mL of 15% NaOH in 50% methanol Reagent 1 (sodium hydroxide/certified ACS 45 g, methanol /reagent grade 150 mL, and deionized distilled water 150 mL) into each sample tube and the tube was sealed with a teflon-lined cap, then mixed for 5 sec. The tube was heated in a waterbath at 100 °C for 30 min, and the content was mixed again.

Methylation. The samples were methylated by

adding 2 mL of 6 N HCl in 50% methanol (Reagent 2: 6.0 N hydrochloride acid 325 mL, methanol (reagent grade) 275 mL), mixed for 5 sec, placed in a waterbath 80 °C for 10 min, and then rapidly cooled in water.

Extraction. The FAMEs were extracted from this solution by adding 1 mL of methyl tert-butyl ester (MTBE) hexane mix (50:50, v/v) (Reagent 3: hexane (HPLC grade) 200 mL, methyl tert butyl ether (MTBE) 200 mL), rotated for 10 min. The top, organic phase was transferred with a Pasteur pipette to test tubes.

Washing. The organic phase was then washed using 3.0 ml of diluted NaOH (Reagent 4: sodium hydroxide 10.8 g, deionized distilled water 900 mL). The organic phase was then transferred to 2 mL vials for subsequent analysis by gas chromatography using an HP 5890 (Hewlett Packard, MIS, ID, Newak, Delware, USA).

***In vitro* Antagonism of Endophytic Bacteria towards Plant Pathogenic Fungi.** A total of 181 bacterial endophytes were tested for antibiosis against fungal pathogens *R. solani*, *F. oxysporum* subsp. *radicis-lycopersici*, and *F. oxysporum* subsp. *lycopersici*. Isolates *R. solani* and *F. oxysporum* subsp. *lycopersici* were obtained from the fungal collection of the Institut fuer Pflanzenkrankheiten, University of Bonn, while isolate *F. oxysporum* subsp. *radicis-lycopercisi* was obtained from the fungal collection of the Humboldt University of Berlin. The fungi were grown on potato dextrose agar (PDA) at pH = 6.4 and stored in potato dextrose broth plus 20% glycerol at -80 °C.

In vitro antibiosis was tested on PDA using the dual-culture technique. Fungal plugs (d = 10 mm) of 6 d old cultures were placed at the center of each Petri dish and the endophytic bacteria were streaked in two lines approximately 2 cm from the fungus. The bacterial strains were precultured on tryptic soy agar (TSA) for 2 d at 24 °C. Plates containing fungus alone served as controls. Petri dishes were maintained at 25 °C until radial growth in the control reached the border of the plate. Antibiosis were scored as ' + ' in the presence of an inhibition zone or as ' - ' in the absence. The experiment was replicated twice.

RESULTS

Population of Endophytic Bacteria. The population densities of indigenous endophytic bacteria in tomato roots between the plants varied. The lowest endophytic population was 1.8 (in $\log_{10}$ scale) CFU g^{-1} fresh root after surface sterilization with 3% NaOCl and 1.0 (in $\log_{10}$ scale) CFU g^{-1} fresh root after surface sterilization with 6% NaOCl. The highest endophytic populations were 4.4 (in $\log_{10}$ scale) CFU g^{-1} fresh root after surface sterilization with 3% NaOCl and 3.0 (in $\log_{10}$ scale) CFU g^{-1} fresh root surface sterilization with 6% NaOCl (Table 1).

Spectrum of Endophytic Bacteria from Tomato Roots. A broad range of bacterial genera and species were recovered from tomato roots in West Java. In total, 564 isolates of bacteria were identified by FAME-GC and MIDI system. Fatty acid profile is likely to become more widely used and will lead to easier identification through comparison to the literature as well as reference strains (Sasser 1990). Based on the similarity index (SI), isolates were identified at the species level for SI > 0.4, at the genus level for SI 0.2-0.4, and remained unidentified for SI < 0.2. Fifty one bacterial species comprising 32 genera were found in tomato roots from West Java, Indonesia. The most abundant endophytic bacterial species were *B. megaterium* (14.31%) followed by *P. putida* (12.44%) and *Serratia marcescens* (10.85%) (Table 2).

***In vitro* Antagonism of Endophytic Bacteria towards Fungal Pathogens.** A total of 181 strains of endophytic bacteria were studied for their *in vitro* antagonistic activity towards *R. solani*, *F. oxysporum* subsp. *radicis-lycopersici*, and *F. oxysporum* subsp. *lycopersici* on PDA. Fourteen strains (7.7 %) showed antagonism against *R. solani*, nine strains (5.0 %) against *F. oxysporum* subsp. *radicis-lycpersici*, and seven strains (3.9 %) against *F. oxysporum* subsp. *lycopersici* (Table 3). The level of antagonism expressed as size of the inhibition zone varied from weak (<1 cm) to medium (1 to 2 cm) and strong (>2cm) (Rocha *et al.* 2009).

Isolates of endophytic bacteria with pronounced antagonism towards *R. solani* included strains of *Bacillus megaterium, Burkholderia cepacia, Comamonas oxydovorans, Enterobacter intermedius, Pseudomonas chlororaphis, P. mendocina, P. putida, P. stutzeri, P. savastanoi, P. syringae,* and *S. marcescens.* Whereas, bacterial species with antagonism towards *F. oxysporum* subsp. *radicis-lycopersici* and *F. oxysporum* subsp. *lycopersici* included *Bacillus megaterium, Burkholderia cepacia, P. chlororaphis, P. putida*, and *S. marcescens.* In this dual culture assay, of 181 endophytic bacteria strains could inhibit *R. solani*, *F. oxysporum* subsp. *radicis-lycopersici*, and *F. oxysporum* subsp. *lycopersici* in varying degrees (Fig 1). The inhibitory rates of the total bacterial strains were less than 10% (Table 3).

Table 1 Population density of endophytic bacteria isolated from tomato from West Java, Indonesia, following surface-sterilization with sodium hypochlorite (NaOCl)

Sample	Origin of samples	3 % NaOCl			6 % NaOCl		
		Sterility check[a)]	Log CFU/g fresh root[a)]	Bacterial isolates[c)]	Sterility check[a)]	Log CFU fresh root[b)]	Bacterial isolates[c)]
T1	Bogor	+	-	-	-	1.6	10
T2	Bogor	+	-	-	-	n.d[(d)]	-
T3	Bogor	-	3.3	29	-	1	6
T4	Bogor	-	3.4	30	-	1.7	30
T5	Bogor	-	2.8	35	-	1.5	30
T6	Bogor	-	3.6	31	+	-	-
T7	Bogor	+	-	-	-	2.2	11
T8	Bogor	-	3.8	30	-	1.9	12
T9	Sukabumi	-	3.8	30	-	1.2	16
T10	Sukabumi	-	3.5	19	-	2.2	12
T11	Sukabumi	-	4	32	-	1.0	18
T12	Sukabumi	-	4	38	-	2.9	30
T13	Sukabumi	+	-	-	+	-	-
T14	Sukabumi	-	4	32	-	2.3	30
T15	Bandung	+	-	-	-	n.d	-
T16	Bandung	+	-	-	+	-	-
T17	Bandung	-	3.6	24	-	n.d	-
T18	Bandung	-	2.9	21	-	n.d	-
T19	Bandung	+	-	-	-	3.0	-
T20	Bandung	+	-	-	-	2.5	8
Total		12		351	17		213
							564

[a)] - : Samples free from surface contamination, + : Samples with surface contamination
[b)] Means of the population density of two replications
[c)] - : Bacterial isolates were not taken, root samples were contaminated
[d)] n.d : Non detectable; population density below detection limit

DISCUSSIONS

Sodium hypochlorite is commonly used as a surface disinfectant for recovering bacterial endophytes, mainly because it is non-toxic and easy to handle. The concentration of NaOCl varied between 0.5 and 5% and the incubation time between 3 and 90 min. For example, Misaghi and Donndelinger (1990) treated cotton plants with 1.0% NaOCl in 0.05 Triton X-100 for 90 min and with 75% ethanol for 5 min, followed by 0.5% NaOCl in 0.05 Triton X-100 for 60 min. Sturz *et al*. (1997) isolated endophytic bacteria from red clover and potato. The root tissue of red clover and potato tuber were rinsed with 95% ethanol, then surface sterilized with 5% NaOCl for 3 min, followed by 2% solution of hydrogen peroxidase for 3 min, and then three washes with sterile distilled water.

Well defined isolation procedures for recovering

Table 2 Species diversity of endophytic bacteria recovered from tomato roots in West Java, Indonesia using FAME-GC (in percent of 564 isolates)

No	Species	Percent of total isolates	No	Species	Percent of total isolates
1.	*Acidovorax avinae*	0.46	27.	*Lactobacillus fermentum*	1.84
2.	*Agrobacterium radiobacter*	3.46	28.	*Macrococcus lutens*	0.69
3.	*Alcaligenes xylosoxydans*	1.61	29.	*Methylobacterium mesophilicum*	0.69
4.	*Alteromonas haloplanktis*	0.23	30.	*Ochrobactrum anthropi*	2.76
5.	*Arthrobacter citreus*	0.46	31.	*Paenibacillus pabuli*	0.23
6.	*Aureobacterium barkeri*	1.61	32.	*Paracoccus denitrificans*	0.23
7.	*Bacillus cereus*	1.61	33.	*Peinococcus erythromixa*	0.23
8.	*Bacillus circulans*	1.15	34.	*Phyllobacterium myrcinacearum*	0.23
9.	*Bacillus mycoides*	0.46	35.	*Phyllobacterium rubiacearum*	0.23
10.	*Bacillus megaterium*	14.31	36.	*Pseudomonas aeruginosa*	0.46
11.	*Bacillus pumilus*	0.23	37.	*Pseudomonas chlororaphis*	2.76
12.	*Bacillus sphaericus*	2.54	38.	*Pseudomonas corugata*	0.23
13.	*Bacillus thuringiensis*	1.85	39.	*Pseudomonas fluorescens*	3.70
14.	*Burkholderia cepacia*	2.07	40.	*Pseudomonas flekteus*	0.69
15.	*Burkholderia gladioli*	1.15	41.	*Pseudomonas mendocina*	2.54
16.	*Burkholderia pickettii*	0.46	42.	*Pseudomonas putida*	12.44
17.	*Cellulomonas cellulans*	0.69	43.	*Pseudomonas savastanoi*	0.23
18.	*Chryseobacterium balustinum*	1.15	44.	*Rathayibacter rathayi*	0.23
19.	*Chryseobacterium meningosepticum*	0.23	45.	*Spingobacterium multirorum*	0.23
20.	*Chryseobacterium indologens*	0.69	46.	*Staphylococcus sciari*	0.23
21.	*Curtobacterium flaccumfaciens*	0.23	47.	*Stenotrophomonas maltophilia*	1.15
22.	*Comamonas ocidovorans*	0.23	48.	*Serratia marcescens*	10.85
23.	*Enterobacter tylorae*	1.15	49.	*Weeksella verosa*	0.23
24.	*Erwinia amylovora*	0.23	50.	*Xanthobacter agilis*	1.85
25.	*Gluconobacter oxidans*	0.69	51.	*Xanthomonas campestris*	0.23
26.	*Kluyvera cryocrescens*	2.31	52.	Unidentified	13.86

endophytic bacteria are a prerequisite for research with endophytes. Due to their secluded niche inside plant tissues, endophytic bacteria are most commonly isolated from macerated plant tissue following surface sterilization (Hallmann *et al*. 1997). Disinfectants generally used for isolation of endophytic bacteria include sodium hypochlorite (NaOCl), ethanol, hydrogen peroxide (H_2O_2), mercury chloride (HgCl) or a combination of two or more of these disinfectants followed by several washes in sterile distilled water or buffer solutions (He *et al*. 2009). To reduce surface tension of the solvent and therefore allow the disinfectant to penetrate into micro niches on the root surface, Tween 20 or Tween 80 can be added (Yasuda *et al*. 2009).

In this study, 3% and 6% NaOCl supplemented with 0.01% Tween 20 were used as surface disinfectants. The total number of plants passing the sterility check was higher for 6% NaOCl (85%) than for 3% NaOCl (60%). However, at 6% NaOCl the total population density was lower than that of 3% NaOCl (Table 1). The relatively lower number of bacterial endophytes at 6% NaOCl was due to the fact that surface sterilization was too strong and probably killed some endophytic bacteria within the internal root tissue. Hallmann *et al*. (1997) reported that slight variations in the concentration and incubation time of the surface disinfestation process greatly affects the number and type of bacteria isolated.

Population densities of bacterial endophytes have been shown to be greatest in plant roots. In this experiment, the population densities of endophytic bacteria isolated from tomato roots were in the range from 1.0 to 3.0 (in $\log_{10}$ scale) CFU g^{-1} fresh weight

Table 3 *In vitro* antagonism of endophytic bacteria from tomato roots towards *Rhizoctonia solani*, *Fusarium oxysporum* subsp. *radicis-lycopersici,* and *F. oxysporum* subsp. *lycopersici* five days after inoculation on PDA media

Species	Number of strains tested	Number of strains with antibiosis activity towards		
		Rs [1)]	Forl [2)]	Fol [3)]
Agroba cterium radiobacter	4	0 [4)]	0	0
Aureobacterium esteroaromaticum	2	0	0	0
Bacillus cereus	3	0	0	0
Bacillus circulans	2	0	0	0
Bacillus megaterium	28	3	3	3
Bacillus sphaericus	3	0	0	0
Bacillus thuringiensis	3	0	0	0
Burkholderia cepacia	3	2	1	0
Comamonas oxidovorans	3	1	0	0
Comamonas testoteroni	2	0	0	0
Chryseobacterium meningsepticum	2	0	0	0
Chryseobacterium indologens	4	0	0	0
Curtobacterium flaccumfaciens	2	0	0	0
Chryseobacterium balustinum	6	0	0	0
Enterobacter tylorae	2	0	0	0
Gluconobacter oxidans	3	0	0	0
Kluyvera cryocrescens	7	0	0	0
Micrococcus varians	2	0	0	0
Ochrobactrum anthropi	4	0	0	0
Paracoccus denitrificans	2	0	0	0
Phyllobacterium myrcinacearum	1	0	0	0
Pseudomonas aeruginosa	3	0	0	0
Pseudomonas chlororaphis	9	1	1	1
Pseudomonas corrugata	2	0	0	0
Pseudomonas fluorescens	8	0	0	0
Pseudomonas mendocina	3	1	1	0
Pseudomonas putida	27	3	1	2
Pseudomonas syringae	2	1	1	0
Pseudomonas stutzeri	3	1	1	0
Serratia marcescens	14	2	1	1
Xanthobacter agilis	4	0	0	0
Unidentified	8	0	0	0
Total	181	14	11	7

[1)] *Rhizoctonia solani*
[2)] *Fusarium oxysporum* f.sp. *radicis0lycopersici*
[3)] *Fusarium oxysporum* f.sp. *lycopersici*
[4)] 0: No antibiosis effect on PDA medium

(when surface sterilized with 6% NaOCl) and from 3.0 to 4.4 (in $\log_{10}$ scale) CFU g^{-1} fresh weight (when surface sterilized with 3% NaOCl). McInroy and Kloepper (1995) reported common population sizes of indigenous bacterial endophytes found for roots and stems from cotton and sweet corn ranging between 4.0 to 6.0 (in $\log_{10}$ scale) CFU g^{-1} fresh weight. Similar results for the population densities of bacterial

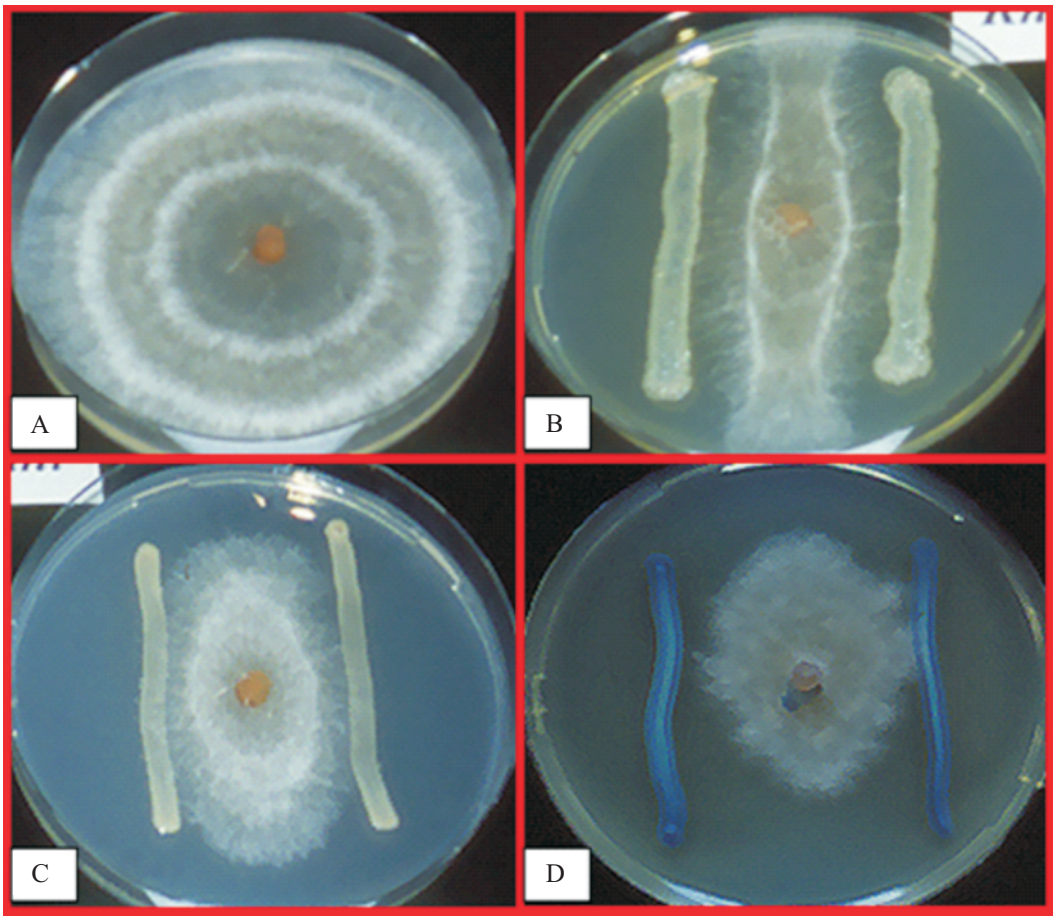

Fig 1 *In vitro* antagonisms of 3 endophytic bacteria isolated from tomato root towards *Rhizoctonia solani*. The figures show growth of *R. solani* on PDA alone (A), and in the presence of endophytic bacteria showing various degrees of inhibition: strong inhibition by *Pseudomonas* spp. MT004 (B), moderate inhibition by *P. putida* MT019 (C), *P. savastanoi* f.sp. *fraxinus* (D). Images were taken 5 d after fungal inoculation.

endophytes were obtained from different plant roots including alfalfa, citrus twigs, sugar beet, cotton, grapevine, pine seedlings, red clover and potato of 2.0 to 6.0 (in $\log_{10}$ scale) CFU g^{-1} fresh root (Misaghi and Donndelinger 1990; Bell *et al*. 1995; Hallmann *et al*. 1997; Sturz *et al*. 1997). Population densities of bacterial endophytes differ depending on the types of plant tissue studied and seem to be highest in the root and lower in the stem and decreases acropetally. The root is thought to be the preferred site of bacterial entrance into the plant, which would explain the high bacterial numbers in the root at early growth stages. The root system seems to provide a more buffered habitat with regards to water availability and temperature changes (Sessitsch *et al*. 2004; He *et al*. 2009; Sarr *et al*. 2010).

The present experiments showed that tomato plants were found to host a diverse spectrum of endophytic bacteria. The spectrum of endophytic bacteria isolated was similar to those found for other locations and/or other host plants. McInroy and Kloepper (1995) reported 46 bacterial species in 31 genera in roots and stems of sweet corn and 45 species in 32 genera in roots and stems of cotton. In grapevine 24 bacterial species in 13 genera were found (Bell *et al*. 1995), in clover roots 31 species in 14 genera (Sturz *et al*. 1997), in cotton roots 43 bacterial species of 28 genera and in potato tubers 24 bacterial species of 13 genera (Hallmann *et al*. 1997). It should be mentioned that, these are only the culturable bacteria. Almost nothing is known about the non-culturable bacteria inside root tissue.

The diversity of endophytic bacteria reported in this study has many similarities with the endophytic spectrum detected in other crops. Some bacterial genera, namely, *Agrobacterium, Arthrobacter, Bacillus, Burkholderia, Chryseobacterium, Enterobacter, Pseudomonas*, and *Stenotrophomonas* have been commonly recovered from roots of cucumber, sugar beet, corn, and lemon (McInroy and Kloepper 1995; Mahaffee and Kloepper 1997; Hallmann *et al.* 1997). Factors like crop rotation, organic matter, temperature,

rainfall, soil physical, and chemical properties affect the bacterial spectrum and these factors were the source of endophytic colonizers in these tests (Mahaffee and Kloepper 1997; Sessitsch *et al*. 2004).

In the present study, *in vitro* antibiosis was tested on potato dextrose agar (PDA). The choice of the growth medium can affect the degree of inhibition as demonstrated in these experiments (Yang *et al*. 2011). Strains of endophytic bacteria were screened for *in vitro* inhibition of *R. solani, F. oxysporum* f.sp. *radicis-lycopersici*, and *F. oxysporum* subsp. *lycopersici* on PDA. Testing *in vitro* antagonism of bacteria against fungal pathogens provides a rapid method to pre-select biological control candidates based on antibiosis. Results showed that some bacteria inhibited the growth *in vitro* of *R. solani* (8.3%), *F. oxysporum* subsp. *radicis-lycopersici* (6.6%), and *F. oxysporum* subsp. *lycopersici* (3.9%). Yang *et al*. (2011) reported that the inhibitory rates of endophytic bacteria in dual test assay against *Botrytis cinerea* were varied and less than 40%. This result might be affected by medium used for antibiosis *in vitro* assay. Fuchs and Defago (1990) studied the *in vitro* antagonism of bacteria towards fungal disease *Phomopsis sclerotioides* on PDA, Malt Agar (MA), and King's B Medium Agar (KBA). Some bacteria, especially *Pseudomonas fluorescent*, inhibited fungal pathogens on KBA, but not on PDA or MA and vice versa, whereas other bacteria showed antibiosis on all three media.

In conclusion, endophytic bacteria can be isolated with surface-sterilized method. More than fifty one species of endophytic bacteria were recovered from tomato roots and three most abundant species, *B. megaterium*, *P. putida*, and *S. marcescens,* were frequently found in association with tomato roots from West Java, Indonesia and showed antibiosis activity against fungal diseases. The close relationship between endophytic bacteria and their hosts make them ideal candidates for biological control and plant growth promotion.

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