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BIODIVERSITY OF ENDOPHYTIC BACTERIA AND THEIR ANTAGONISTIC ACTIVITY TO *RHIZOCTONIA SOLANI*AND *FUSARIUM OXYSPORIUM*

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ABSTRACT

Understanding plant microflora interaction and their diversity as phyloplant and rhizoplant bacteria, 153 endophytic bacteria had been isolated from 67 plant species. The plant samples gathered from agriculture area and next to riparian tropical forest in slope of Salak mount area, West Java, Indonesia. Three bacterial strains (ES05, ES36, and ES78) showed the highest suppression to *Rhizoctonia solani* J.G. Kühn 1858, and their suppressive ability was about 69% higher than that of control. The bacteria were isolated from part plant of *Ageratum conyzoides*, *Camellia sinensis* and *Ficus benyamina*, respectively. In the second step of exertion, the screened strains were showed their ability to suppress *R. solani* growth in range of 16-60% in potatoes dextrose agar (PDA) medium, and 5-70% in nutrient agar (NA) medium. Five strains (ES50, ES69, ES79, ES120, and ES145) have negative effect to restrain *R. solani* growth in NA medium. Nine of the selected strains inhibited *Fusarium oxysporum* Schlecht growth in the range of 10-47% in PDA medium, and 12 of them inhibited *F. oxysporum* growth in range 5-35% in NA medium. Five strains (ES05, ES79, ES83, ES91, and ES145) did not restrain *F. oxysporum* in PDA medium, while two others strain (ES50 and ES145) did not either in NA medium. Twenty-one bacterial strains gained from nineteen plant species were tested qualitatively for antibiotics vocation, and only 7 strains (ES42, ES50, ES78, ES81, ES82, ES83, and ES91) produced iturin, one strain (ES79) produced surfactin, while other three strains (ES17, ES81 and ES145) produced chitinase. Thirty-three isolates were successfully identified based on 16S rDNA sequences which had high homology examination refer to DNA Data Bank of Japan.

Key words: endophyte bacteria, biocontrol agents, iturin, surfactin, chitinase.

INTRODUCTION

Research on endophytic bacteria as biocontrol agents against fungal pathogen is incomplete. Otherwise, endophytic bacteria provide many opportunities to become various biological agents to produce new biopesticide compounds. That bioactive compound might be applicable in agriculture management work. To get a reliable biocontrol technology it is necessary to do various stages of basic research especially detecting the ability of endophytic bacteria to produce antibiotics. Bacterial endophytic as the genetic resources are continuously explored for various purposes such as in the field of environment, agricultural, medicine, and health care. The bacteria can be further developed as biocontrol agent because of producing valuable antibiotics for replacing chemical fungicides purposes which are harmful to environment and human health.

In some research activities, endophytic bacteria had been recognized in the plant parts such as leave, flower, stem, fruit, and seed of various plant species (Ferreira *et al*., 2008; Mano and Morisaki, 2008). Many authors have well documented the important roles of the endophytes, such as in reducing disease severity (Sturz and Nowak, 2000; Kloepper *et al*., 2004), inducing plant defense mechanisms (Bakker *et al*., 2007), increasing plant mineral uptake (Malinowski *et al*., 2000), promoting plant growth (Kang *et al*., 2007), and biologically fixing nitrogen (Martinez *et al*., 2003). One of the secondary metabolites of bacterial endophytic that rarely explore was called polypeptide antibiotics production like iturin and surfactin. In the previous work by using a bacterial isolated from compost, *Bacillus subtilis* strain RB-14CS, iturin was able to reach up to 3300 mg per liter production (Yuliar, 2002). Some other of bacterial genus which had been successfully detected as biocontrol agents are *Agrobacterium*, *Pseudomonas*, *Bacillus*, *Alcaligenes*, and *Streptomyces* (Shoda, 2000).

Biological control of soil-borne diseases by using bacteria were become necessary since the diseases recognize more limiting factors in production of many crops, and negligence in chemical pesticide practice also might make environmental hazardous. Amongst of the soil–borne pathogens are called *R. solani* and *F. oxysporum.* Soil microflora of *R. solani* were well known to be an important cosmopolitan necrotophic soil-borne fungus, caused to plant damping-off diseases and making yield losses in more than 200 crops globally (Lee *et al*., 2008); while *F. oxysporum* able to survives in soil for long periods and thus plant susceptible genotypes cannot be grown in an infested field for up to 30 years (Ploetz, 2000). Although many studies had been published on "bacterial biocontrol agents (BCAs)" isolated from various soils and composts, but there is still limited information on BCAs belongs to endophytic bacteria. As due to that challenge, the recent study had been carried out to examine diversity of bacterial isolates from various plants to evaluate their potential as biocontrol agent, and to determine their ability to suppress against pathogenic fungus of *R. solani* and *F. oxysporum*.

MATERIALS AND METHODS

Isolation and identification of endophytic bacteria

Several agriculture plants and a number of riparian forest vegetation in the slope of Salak mount (800 m above sea level), West Java, Indonesia, were collected and kept in ice-box to bring into laboratory. Plant part samples such as stems, leaves, root, and tubers were washed in running tap water; continued to surface sterilance by using 70% ethanol for 1-2 minutes, two minutes in 5.3% of sodium hypochlorite solution, finally rinsed three times with sterile distilled water. Plant parts then were dried for 3-4 hours in a clean bench, cut them into pieces to 5-10 mm long, and about six of plant pieces of each sample were put into a half strength (soft) nutrient agar (Eiken Chemical Co., Tokyo, Japan: NA) media supplemented with 100 ug ml^{-1} cyclohexamide inside the plates and incubated in room condition for about 10-14 days. Each colony that grew on the NA plates was purified by repeated transfers of culture into new NA media.
For identification work, endophytic bacterial strains were cultured in 1/10 strength nutrient broth (NB: Eiken Chemical Co, Tokyo, Japan) overnight and their DNA were extracted. The primers 27f (5'AGAGTTTGATCCTGGCTCAG-3') and 1378r (5'-CGG TGT GTA CAA GGC CCG GGA-ACG-3') were used to amplify the segment of bacterial 16S rDNA from nucleotides 27 to 1378 (*Escherichia coli* numbering). Each 25 µl PCR reaction contained 1 µl DNA template plus 24 µl of amplification mixture ; and to make the amplification mixture are as follows: 7.5 µl sterilized distilled water, 2 µl BSA (5 mg ml^{-1}), 1 µl 27f (10 pmol μl^{-1}), 1 µl 1378r (10 pmol μl^{-1}), and 12.5 µl GoTaq (Promega KK, Tokyo, Japan). The thermocycling consisted of an initial denaturing step at 94^0C for 3 min, 30 amplification cycles of 94^0C for 1 min, 55^0C for 1 min, 72^0C for 3 min, and final step at 72^0C for 7 min with a GeneAmp PCR System (PCR Thermal Cycles, PERSONAL, Takara Bio Inc., Otsu, Japan). PCR products were confirmed in electrophoresis using agarose gel with 0.5 µg mL^{-1} of ethidium bromide. PCR samples were purified with Suprec^{TM}-PCR (Takara Bio Inc.) and sent for sequencing at Takara Bio Inc. The resulting 16S rDNA sequences were examined using the DDBJ homology search system BLAST (http://blast.ddbj.nig.ac.jp).

Cultivation of endophytic bacteria

Precultivation of the endophytic bacteria strains were performed as follows: 5 ml sterilized LB (Luria-Bertani) medium in a test tube was inoculated with one loop of the strain. LB medium was used as seed medium and it had the following composition (per liter of distilled water): 10 g polypepton, 5 g yeast extract, and 5 g NaCl. Then, it was incubated and shakes at 30^0C temperature, 124 rpm, for 16 hours. Forty ml of a sterilized media No.3 (prepared as follow: 10 g polypepton, 10 g glucose, 1 g KH_2PO_4, and 0.5 g $MgSO_{4.7}H_2O$, and dissolved into 1000 ml aquadest and finally adjusted to pH 6.8) placed in 200ml-Erlenmeyer flask and inoculated with 400 µl of 16 hours as pre-cultivated strain. The Erlenmeyer then keep in incubator at 30^0C with 124 rpm for five days (Asaka and Shoda, 1996).

Growth inhibition

R. solani was inoculated onto the center of a plate containing potatoes dextrose agar (PDA) medium, and also performed to NA media, too. After that, four holes were made surround the center by using cork borer, and all of the holes have same distance to the center. One hundred µl of seven day cultivated endophytic bacterial strain in No.3 medium was put into each hole. For the negative control, the holes were dropped with distilled water. With the same method was done to *F. oxysporum* pathogen. Finally, the plates were incubated for 5 days, and growth inhibition area was observed and calculated by the following formula:

$$\text{Percentage of Inhibition Growth} = \frac{\text{(diameter of fungal pathogen mycelium in control) be diminished by (diameter of fungal pathogen mycelium in treatments)}}{\text{diameter of mycelium pathogen in control}} \times 100\ \%$$

Qualitative test of antifungal iturin

Iturin detection method was described by Hiraoka *et al*. (1992). Suspension of *Fusarium oxysporum* culture were mixed with PDA media and poured it into the petridish. Make a hole in the plate center and filled with 70 ul endophytic bacteria (which has cultivated in No.3 medium), and incubated for 1 to 2 days period. Iturin producing bacteria was representing by clear zone around the hole to inhibit the *F. oxysporum* mycelia growth.

Detection of surfactin activity

Surfactin antibiotic test was followed to Huang *et al*. (1993) method. Twenty µl of try-butyrin was spread onto LB agar plate by a sterilized glass rod after those of endophytic bacterial strains were spotted onto the plates. When a clear zone was observed around the colony, it means that surfactin was produced.

Chitinase test

Chitin media preparation were made by dissolving 0.7 g K_2HPO_4, 0.3 g KH2PO4, 0.5 g $MgSO_4.5H_2O$, 0.01 g $FeSO_4.7H_2O$, 0.001 g $ZnSO_4$, 0.001 g $MnCl_2$, 8 g of colloidal chitin, and 22 grams agar in order to 1000 ml of distilled water and mixed by using a magnetic stirrer, and finally the media sterilized in the autoclave. The media poured into petridish inside laminar air flow hood. Chitin medium inside petridish was inoculated with a pure bacterial isolates, and incubated for 2-3 days. Chitinase-producing bacteria are characterized by the holozone formation surround bacterial colonies growing.

RESULTS AND DISCUSSION

Endophytic bacterial isolates

Healthy plant part were being chosen from cultivated vegetation and shrub which were grown surround the agriculture area to riparian of tropical forest, in the altitude of 400 to 800 m asl (above sea level), to have well

endophytic bacteria controlling pathogenic fungi. Those plant part samples collections keep in fresh condition and straightly bring into laboratory for isolation work. One hundred and fifty three strains of endophytic bacteria successfully isolated from various parts of sixty-seven plants species. Isolate selection was based on their ability to suppressed *Rhizoctonia solani* growth in the laboratory test. Whole of them were isolated from leaf, stalk, stem, flower, fruit, pod, and tuber belongs to plant species collected from the field, and isolate purity of strain number determine based on colonies morphology differences and as well as of the growth performance of the isolates, although it is gathered from the same or even difference plants part but at the same plant species (**Table-1**).

Tabel-1. List of endophytic bacteria hostplant on their origin to agriculture (A) and riparian (B) area

No	Host plants			Part of the plant to be selected for samples	Isolates acquiesce number
	Scientific name and its origin		Common Name		
1.	*Acacia mangium* Wild.	B	Black Wattle	leaf and stalk	2
2.	*Agave sisalana* Perr.	B	Sisal	leaf and stalk	2
3.	*Ageratum conyzoides* Linn	B	Billy Goat Weed	leaf and stalk	2
4.	*Allium fistulosum* L.	A	Green Onion	leaf and stalk	4
5.	*Alternanthera amoena* Voss.	A	Red spinach	leaf	1
6.	*Ananas comosus* L. Merr	A	Pineapple	leaf	1
7.	*Arachis hypogaea* L.	A	Peanut	leaf	3
8.	*Araucaria* sp.	B	(araucaria)	leaf	1
9.	*Artocarpus heterophyllus* Lamk	B	Jack Fruit	leaf and stalk	4
10.	*Asparagus officinalis* L.	A	Asparagus	leaf and stalk	2
11.	*Averrhoa carambola* L.	B	Starfruit	leaf and stalk	1
12.	*Brassica* sp.	A	Cabbage	stalk	3
13.	*Brassica chinensis* L.	A	Chinese Cabbage	leaf and stalk	6
14.	*Brassica juncea* (L) Czern.	A	Indian Mustard	leaf	2
15.	*Calliandra calothyrsus* Meissner	B	Caliandra	leaf and stalk	1
16.	*Camellia sinensis* (L.) Kuntze	B	Green Tea	leaf, stalk, flower, fruit	6
17.	*Capsicum* sp.	A	Chili	stem and fruit	2
18.	*Capsicum frutescens* L.	A	Red Pepper	leaf and stalk	2
19.	*Carica papaya* L.	A	Papaya	Leaf/leaf	4
20.	*Cinchona succirubra* Pav.	B	Cinchona	leaf	1
21.	*Citrus aurantiifolia* (Christ. Et panz.) Swingle	B	West Indian lime	leaf, stalk, fruit	3
22.	*Citrus grandis* (L.) Osbeck	B	Pomelo	leaf, stalk, flower, fruit	3
23.	*Citrus reticula* Blanco	B	Mandarin Orange	leaf and stalk	2
24.	*Citrus* sp.	B	(orange)	leaf and stalk	1
25.	*Clitoria ternatea* L.	A	Cordovan pea	leaf and pod	2
26.	*Coffea* sp.	B	Coffee	leaf, stalk, flower	3
27.	*Colocasia* sp.	A	Black Radish	tuber	1
28.	*Crotalaria incana* L.	A	Rattlepods	leaf and stalk	2
29.	*Cucumis sativus* L.	A	Cucumber	leaf and stalk	2
30.	*Daucus carota* L.	A	Carrots	tuber and stalk	3
31.	*Eugenia jambos*	B	Plum rose	leaf and stalk	2
32.	*Eupatorium odoratum* L.	B	(expansive weed)	leaf and stalk	3
33.	*Ficus benyamina* L.	B	Weeping Pig	leaf and stalk	1
34.	*Fragaria vesca* L.	A	Strawberry	leaf and stalk	2
35.	*Glycine max* (L.) Merr.	A	Soybean	leave, stalk, flower, pod	6
36.	*Grevillea robusta* A. Cunn. Ex.R.Br.	B	Mahogany	leaf and stalk	1
37.	*Hevea brasiliensis* (Wild. Ex A.Juss.) Muell.Arg.	B	Rubber tree	leaf and stalk	1
38.	*Hibiscus rosa-sinensis* L.	B	Rose Mallow	leaf and stalk	2
39.	*Hyptis capitata* Jack	B	Knob weed	leaf,stalk, and flower	3
40.	*Hyptis pectinata* (L) Poir	B	Wild Mint	stalk and flower	3
41.	*Ipomoea aquatica* Forsk.	A	Water Spinach	leaf, stalk, stem	3
42.	*Ipomea batatas* (L.) Lamk.	A	Sweet Potato	leaf and stalk	3
43.	*Lactuca sativa* L.	A	Lettuce	leaf	3
44.	*Lannea coromandelica* (Houtt.) Merr.	B	Kamila	leaf and stalk	2
45.	*Lantana camara* L.	A	Spanish Flag	stalk	1
46.	*Limnocharis flava* (L.) Buchenou	A	(yellow velvet-leaf)	stem	1
47.	*Lycopersicon lycopersicum* L. Kaersten	A	Tomato	leaf , flower, fruit	3
48.	*Manihot esculenta* Crantz	A	Cassava	leaf and stalk	3
49.	*Melastoma* sp.	B	(meadow-beauty family)	leaf and stalk	1
50.	*Morindra citrifolia* L.	B	Great Morinda	leaf, stalk, fruit	3
51.	*Oryza sativa* L.	A	Rice	Leaf and stem	4
52.	*Piper nigrum* L.	A	Black Pepper	leaf and stalk	1
53.	*Pisum sativum* L.	A	Pea	leaf and stalk	3
54.	*Pyrrosia nummularifolia* (Swartz) Ching	B	Creeping Button Fern	leaf, stem, root	2

55.	*Raphanus sativus* L.	A	Radish	leaf	1
56.	*Ricinus communis* Linn.	B	Jatropa	leaf and stalk	1
57.	*Schyzostachyum* sp.	B	Murray Island Bamboo	leaf and stalk	1
58.	*Selaginella plana* (Desv. ex Poir.) Hieron.	B	Asian spike-mosses	leaf and stalk	3
59.	*Solanum mammosum* L.	B	Nipplefruit	leaf, stalk, fruit	3
60.	*Solanum torvum* Sw.	B	Turkey Berry	leaf	1
61.	*Switenia mahagonia* Jack	B	Mahogany	leaf and stalk	1
62.	*Syzygium aqueum* (Burm.f.) Alston	B	Water Apple	stalk	2
63.	*Tephrosia vogelii* J.D. Hooker	B	Fish Poison Bean	stalk and pod	2
64.	*Toona sinensis* Roem	B	Chinese Mahogany	leaf and stalk	3
65.	*Toona sureni* Merr.	B	Red Cedar	leaf and stalk	1
66.	*Vigna unguiculata* (L.) Walp.	A	Cowpea	leaf	2
67.	*Zea mays* L.	A	Corn	stem	3
Total isolates					153

According to some recent study, the presence of various bacteria species in plant tissues have been reported, such as *Bacillus pumilus* was found as dominant endophytes in citrus plants (Araujo *et al*., 2002), *Pseudomonas putida* in carrot (Surette, 2003), *Seratia marcescens* was isolated from rice (Gyaneshwar *et al*., 2001). Bacteria of *Stenotrophomonas* sp. were isolated as endophytes from sweet potato plant (Khan and Dothy, 2009) and in the coffee seed (Vega *et al*., 2005). In the result study exploring endophytic bacteria in the plant part samples, highest number to reach six strain varieties was produced by plant part samples of *Brassica chinensis*, *Camellia sinensis* and *Glycine max*; while other group strain which has four varieties attained from *Allium fistulosum*, *Artocarpus heterophyllus*, *Carica papaya*, and *Oryza sativa*. The isolates were coded and started with ES01, and for the last number was ES153. Those endophytic bacterial isolates became working collection and prepared for continuous accession.

Suppression to fungal pathogen

The highest suppression through fungal pathogen were observed in three bacterial isolates (ES05, ES36,and ES78), their suppressiveness was about 69% higher than that of control. Those isolates were obtained from *Ageratum conyzoides*, *Camellia sinensis* and *Ficus benyamina*, respectively. In the second screening, 14 bacterial strains possessing high potential antagonistic activity to *R. solani* were selected for further antagonistic test through their inhibiton ability to both of *R. solani* and *F. oxysporum* growth in PDA and NA media, respectively. The selected strains showed that thier ability to suppress of *R. solani* growth were in range of 16-60% in PDA media, and 5-70% in NA media. Five strains (ES50, ES69, ES79, ES120, and ES145) did not suppress *R.solani* growth in NA media. Nine of the selected strains inhibited *F. oxysporum* growth in the range of 10-47% in PDA media and 12 of them inhibited *F. oxysporum* growth in range 5-35% in NA media. Five strains (ES05, ES79, ES83, ES91, and ES145) did not suppress *F. oxysporum* in PDA media, and 2 strains (ES50 and ES145) did not inhibit *F. oxysporum* in NA media (**Table-2**).

Tabel-2. Some of endophytic bacteria were tested *invitro* against fungal pathogen in NA and PDA media.

No.	Bacterial Isolate Codes	Host plants	Growth restrain (%) to *R.solani* tested in the media: NA	Growth restrain (%) to *R.solani* tested in the media: PDA	Growth restrain (%) to *F. oxysporum* tested in the media: NA	Growth restrain (%) to *F. oxysporum* tested in the media: PDA
			0 35 70	0 35 70	0 35 70	0 35 70
1.	ES05	*Ageratum conyzoides* L.			NEGATIVE	
2.	ES17	*Artocarpus heterophyllus* Lamk.				
3.	ES36	*Camellia sinensis* (L.) Kuntze				
4.	ES42	*Carica papaya* L.				
5.	ES46	*Capsicum frutescens* L.				
6.	ES50	*Cinchona succirubra* Pav.		NEGATIVE		NEGATIVE
7.	ES69	*Cucumis sativus* L.		NEGATIVE		
8.	ES79	*Fragaria vesca* L.		NEGATIVE	NEGATIVE	
9.	ES81	*Glycine max* (L.) Merr.				
10.	ES82	*Glycine max* (L.) Merr.				
11.	ES83	*Glycine max* (L.) Merr.			NEGATIVE	
12.	ES91	*Hyptis capitata* Jack.			NEGATIVE	
13.	ES120	*Oryza sativa* L.		NEGATIVE		
14.	ES145	*Toona sinensis* Roem.		NEGATIVE	NEGATIVE	NEGATIVE

Some research work had been done by Wang *et al*. (2009) on bacterial biocontrol agents (BCAs) collected from rice plant. In the *invitro* observation, *Bacillus* sp. strain CHM1 as endophytic bacteria, tested against infection by *Rhizoctonia solani* on horse bean (*Vicia faba*). In the other work, Naik *et al*. (2009) isolated BCAs gained from rice actually able to inhibit the growth of *R.solani*, *Nigospora oryzae*, *Macrophomina phaseolina*, *Phoma sorghin*a, and *Alternaria alternate* in the *invitro* test. For the future vocation, it shall be necessary to determine those of isolates of endophytic bacteria available from this work were tested against some other microbial pathogenic resources.

Antibiosis and chitinase test

Antibiosis mechanism could due to their extracellular metabolites production excreted by endophytic bacteria which were acted to fungal cell membrane damage. Dissimilarity result of antifungal acted through PDA and NA media might have special reason of physical and chemical filtration function in diffusion processes in the media. The range of inhibition ability in different media showed different effect to inhibition percentage (**Table-3**). Qualitative test to iturin producing bacteria clearly showed that seven strains (ES42, ES50, ES78, ES81, ES82, ES83, and ES91) produce iturin in No.3 medium, and strain ES79 produced surfactin, while three strain (ES17, ES81, and ES145) showed chitinolytic activity. The result of the study confirms previous reports of antibiotic polypeptide production by biocontrol investigation had been worked by Shoda (2000), Akpa *et al*., (2001), and Raaijmaker (2002). The suppressive affect against fungal and bacterial plant pathogens in soil by these BCAs to be triggered by polypeptide antibiotic in their culture broth.

Tabel-3. Selected endophytic bacteria isolates which have antagonism to *R. solani* growth, and tested for antibiosis and chitinolytic activities

No	Isolate Codes	Host Plants	Percentage Inhibition	Antibiosis		Chitinase
				Iturin	Surfactin	
1.	ES05	*Ageratum conyzoides* L.	69	-	-	-
2.	ES17	*Artocarpus heterophyllus* Lamk.	64	-	-	+
3.	ES36	*Camellia sinensis* (L.) Kuntze	69	-	-	-
4.	ES42	*Carica papaya* L.	52	+	-	-
5.	ES50	*Cinchona succirubra* Pav.	53	+	-	-
6.	ES65	*Colocasia* sp.	45	-	-	-
7.	ES68	*Cucumis sativus* L.	48	-	-	-
8.	ES73	*Eugenia jambos* L.	46	-	-	-
9.	ES78	*Ficus benyamina* L.	78	+	-	-
10.	ES79	*Fragaria vesca* L.	40	-	+	-
11.	ES81	*Glycine max* (L.) Merr.	61	+	-	+
	ES82		58	+	-	-
	ES83		53	+	-	-
12.	ES91	*Hyptis capitata* Jack.	53	+	-	-
13.	ES108	*Lantana camara* L.	58	-	-	-
14.	ES117	*Morindra citrifolia* L.	58	-	-	-
15.	ES120	*Oryza sativa* L.	58	-	-	-
16.	ES125	*Pisum sativum* L.	58	-	-	-
17.	ES135	*Solanum mammosum* L.	47	-	-	-
18.	ES145	*Toona sinensis* Roem.	53	-	-	+
19.	ES151	*Zea mays* L.	58	-	-	-

Some possibilities of fungal pathogens suppression mechanisms by biocontrol agent had been investigated by some author within series of action including antibiosis work (Souto *et al*.,2004; Yuliar *et al*., 2011), as due to lytic enzymes activities such as chitinases and proteases production (Huang et al.,2005; Yuliar, 2008), competition of iron through the production of siderophore and caused the induction of systematic resistance (De Boer *et al*., 2003), parasitism processes (Cortest *et al*., 1998), and production of a plant hormone promoting growth (Compant *et al*., 2005). Accordingly, the bacteria had been reported that are able to control *R. solani* growth there were *Trichoderma* spp. and *Pseudomonas fluorescens* (Rini and Sulochona, 2007), *Bacillus amyloliquefaciens* (Yu *et al*., 2002), *Streptomyces* sp. Di-944 (Sabaratnam and Traquair, 2002), *B. subtilis* RB14-C (Kondoh *et al*., 2001), *B. brevis*, *B. pantotheinticus*, and *Bacillus* sp. (Yuliar, 2008). Whereas bacterial control of *F. oxysporum* was *Aureobacterium sapardae*, *B. pumilus*, *Phyllobacterium rubiacerum*, *Pseudomonas putida*, and *Burkholderia solanacearum* (Chen *et al*., 1995), while Chen *et al*. (2009) reporting on *Bacillus amyloliquefaciens* strain FZB42.

Endophytic bacteria obtained from various samples in the investigation showed that some of the collected bacteria could be stimulated by certain selected media inducer to show ability producing antibiosis of iturin and surfactin, as well as due to enzymatic chitinase action (**Figure-1**). Some of isolates were succesfully identified with 16S rDNA sequences and continued to examine by using the DDBJ homology search system BLAST (**Table-4**).

Table-4. Based on 16S rDNA sequences and its examination by using DNA Data Bank of Japan (DDBJ) homology search system BLAST, the isolates identified as follow.

No.	Microbial isolates which has a hundred percent similarity to taxonomic character of:	Host plants	Isolates Code
	Achromobacter xylosoxidans Yabuuchi and Yano 1981	*Oryza sativa* L.	ES 120
	Acinetobacter schindleri Nemec et al. 2001	*Pyrrosia nummularifolia* (Swatz)	ES 128
	Alcaligenes sp.	*Glycine max* (L.) Merr.	ES 81
	Bacillus amyloliquefaciens Priest et al 1987	*Brassica chinensis* L.	ES 29
	Bacillus cereus Frankland & Frankland 1887	*Ageratum conyzoides* L.	ES 05
	Bacillus cereus Frankland & Frankland 1887	*Ananas comosus* L.	ES 12
	Bacillus cereus Frankland & Frankland 1887	*Capsicum frutescens* L.	ES 46
	Bacillus cereus Frankland & Frankland 1887	*Ficus benyamina* L.	ES 78
	Bacillus cereus Frankland & Frankland 1887	*Grevillea robusta* A. Cunn. Ex.R.Br.	ES 87
	Bacillus cereus Frankland & Frankland 1887	*Hibiscus rosasinensis* L.	ES 89
	Bacillus cereus Frankland & Frankland 1887	*Morindra citrifolia* L.	ES 117
	Bacillus cereus Frankland & Frankland 1887	*Syzygium aqueum* (Burm.f.) Alston	ES 141
	Bacillus pumilus Meyer and Gottheil 1901	*Calliandra calothyrsus* Miessner	ES 35
	Bacillus pumilus Meyer and Gottheil 1901	*Camellia sinensis* L. Kuntze	ES 36
	Bacillus pumilus Meyer and Gottheil 1901	*Oryza sativa* L.	ES 121
	Bacillus thuringiensis Berliner 1915	*Camellia sinensis* L. Kuntze	ES 37
	Bacillus thuringiensis Berliner 1915	*Colocasia* sp.	ES 65
	Bacillus thuringiensis Berliner 1915	*Ipomea batatas* (L.) Lamk.	ES 100
	Brachybacterium sp.	*Pisum sativum* L.	ES 125
	Brevibacterium sp.	*Citrus grandis* L. Osbeck	ES 54
	Brevibacterium sp.*	*Manihot esculenta* Crantz	ES 113
	Enterobacter sp.	*Glycine max* (L.) Merr.	ES 82
	Leucobacter tardus Behrendt et al. 2008	*Lycopersicon lycopersicum* L. Kaersten	ES 109
	Microbacterium foliorum Behrendt et al. 2001*	*Capsicum frutescens* L.	ES 48
	Microbacterium sp.*	*Melastoma* sp.	ES 116
	Ochrobactrum intermedium Velasco et al 1998	*Melastoma* sp.	ES 116
	Ochrobactrum intermedium Velasco et al 1998	*Allium fistulosum* L.	ES 08
	Ochrobactrum oryzae Tripathi et al. 2006	*Agave sisalana* Perr.	ES 03
	Paenibacillus favisporus Velázquez et al. 2004*	*Asparagus officinalis* L.	ES 21
	Pseudomonas plecoglossicida Nishimori et al. 2000	*Toona sinensis* Roem	ES 145
	Pseudomonas putida Trevisan 1889	*Lycopersicon lycopersicum* L. Kaersten	ES 110
	Rhodobacter sphaeroides (van Niel 1944) Imhoff et al.1984	*Oryza sativa* L.	ES 122
	Stenotrophomonas maltophilia Palleroni & Bradbury 1993	*Oryza sativa* L.	ES 123

*****99 percent similarities

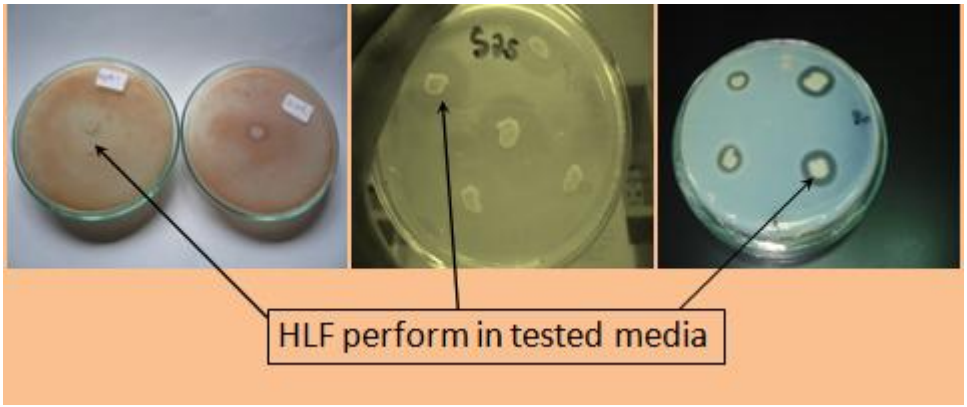

Figure-1. Holozone-formation (HLF) surround isolate growth indicate strain producing antibiosis of iturin (left) and surfactin (middle), and as well as chitinase (right) enzyme activity as due to strain of isolates number ES50, ES79, and ES17, correspondingly

CONCLUTION

Biodiversity of endophytic bacteria including their characteristic had been open mind and give much more challenge to be developed through many executions for two reasons following. We conclude here that form of mechanism involved in the suppression of fungal pathogen *R. solani* and *F. oxysporum* by some certain endophytic bacterial strains are because of antibiosis production of bioactive iturin and surfactin, and also caused by chitinase enzyme production. In the laboratory test for antagonism through this observation, media used causing dissimilarity of positive and negative result of antagonism affect for some certain endophytic strain, while the quantity result differences also occurrence within the same strain.

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REFERENCES

Akpa E., P. Jacques, B. Wathelet, M. Paquot, R. Fuchs, H. Budzikiewicz, and P. Thonart. (2001). Influence of culture conditions on lipopeptide production by *Bacillus subtilis*. *Applied Biochemical & Biotechnology*. 91-93:551-561.

Araujo W.L., J. Marcon., W. Maccheroni,Jr., J.D. van Elsas., J.W.L. van Vuurde., and J.L Azevedo.(2002). Diversity of endophytic bacterial populations and their interaction with *Xylella fastidiosa* in citrus plants. *Applied and Environmental Microbiology*. 68(10):4906-4914.

Asaka O. and M. Shoda. (1996). Biocontrol of *Rhizoctonia solani* damping-off of tomato with *Bacillus subtilis* RB14. *Applied and Environment Microbiology*. 62(11):4081-4085.

Bakker P.A.H.M., C.M.J. Pieterse, and L.C.Van Loon. (2007). Induced systemic resistance by fluorescent *Pseudomonas* spp. *Phytopathology*. 97:239-243.

Chen XH, A. Koumoutsi, R. Scholz, K. Schneider, J. Vater, R. Sussmuth, J. Piel and R. Borriss. (2009). Genome analysis of *Bacillus amyloliquefaciens* FZB42 reveals its potential for biocontrol agent of plant pathogen. *Journal Biotechnolology.* 140:27-37.

Chen C., E.M. Baueke, G. Muson, R. Rodrguezkabana and J.W. Kloepper. 1995. Biological control of *Fusarium* wilt on cotton by use of endophytic bacteria. Biological Control. 5(1):83-91.

Compant S., B. Duffy, J. Nowak, C. Clement and E.A. Barka. (2005). Minireview ;Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. *Appl. and Environ. Microbiol.* 71(9):4951-4959.

Cortes C., A. Gutierrez, V. Olmedo, J.I. Chet, A. Herrera-Estrella. (1998). The expression of genus involved in arasitism by *Trichoderma harzianum* is triggered by a diffusible factor. *Mol.Gen. Genet.* 260:218-225.

de Boer M., P. Boom, F. Kindt, Joost, J.B Keurentjes, I.van der Sluis, L.C.van Loon and P.H.A.M. Baker. (2003). Control Fusarium wilt of radish by combining of *Pseudomonas putida* strains that have different diseases mechanisms. *Phytopatology*. 93(5):626-632.

Ferreira A., M.C. Quecine, P.T. Lacava, S.Oda, J.L. Azevedo and W.L. Araujo. (2008). Diversity of endophytic bacteria from *Eucalyptus* species seed and colonization of seedling by *Pantoea agglomerans*. *FEMS Microbiology Letter.* 287:8-14.

Gyaneshwar P., E.K. James, N. Mathan, P.M. Reddy, B. Reihhold-Hurek and J.K. Ladha. (2001). Endophytic colonization of rice by a diazotrophic strain of *Serratia marcescens*. *Journal of Bacteriology*.183(8):2634-2645.

Hiraoka H., O. Asaka, T. Ano and M. Soda.(1992). Characterization of *Bacillus subtilis* RB14 cooproducer of peptida antibiotics iturin A and surfactin. *Journal Genetic &.Applied Microbiology.* 38: 635-640.

Huang C.J., T.K. Wang, S.C. Chung and C.Y. Chen. (2005). Identification of an antifungal chitinase from a potential biocontrol agent, *Bacillus cereus* 28-9. *Journal of. Biochemistry and Molecular Biology*. 38(1):82-88.

Huang C-C., T Ano and M. Shoda.(1993). Nucleotide sequence and characteristics of the gene, *lpa-14*, responsible for biosynthesis of the lipopeptide antibiotics iturin A and surfactin from *Bacillus subtilis* RB 14. *Journal of Fermentation & .Bioenginering*. 76:445-450.

Kang S. Hoon, H-S. Cho, H. Cheong, C-M. Ryu, J.F. Kim and S-H. Park. (2007). Two bacterial endophytes eliciting both plant growth promotion and plant defense on pepper (*Capsicum annuum* L.). *Journal of Microbiology and Biotechnology.* 17(1): 96–103.

Khan Z. and S.L. Doty. (2009). Characterization of bacterial endophytes of sweet potato plants. *Plant Soil*. 322:197-207.

Kloepper J.W., C.M. Ryu and S. Zhang. (2004). Induced systemic resistance and promotion plant growth by *Bacillus* spp. *Phytopathology.* 94:1259-1266.

Kondoh M., M. Hirai and M. Shoda. (2001). Integrated biological and chemical control of damping-off caused by *Rhizoctonia solani* using *Bacillus subtilis* RB14-C and Flutolanil. *Journal of Bioscience and Bioenginering*. 91(2):173-177.

Lee T.O., Z. Khan, S.G. Kim and Y.H. Kim. (2008). Amendment with peony root bark improves the biocontrol efficacy of *Trichoderma harzianum* against *Rhizoctonia solani*. *Journal of Microbiology and Biotechnology*. 18: 1537–1543.

Malinowski D.P., G.A. Alloush and D.P. Belesky. (2000). Leaf endophyte *Newtyphodium coenophialum* modifies mineral up take in tall fescue. *Plant Soil.* 227:115-126.

Mano H. and H. Morisaki. (2008). Endophytic bacteria in the rice plant. *Microbes and Environment.* 23(2):109-117.

Martinez L., C. Mellado, J. Orezco and M. Romeiro. (2003). Diazotrophic bacteria associated with banana (*Musa* spp). *Plant and Soil*. 257:35-47.

Naik, B.S., J. Shashikala and Y.L. Krishnamurthy. (2009). Study on the diversity of endophytic communities from rice (*Oryza sativa* L.) and their antagonistic activities in vitro. *Microbiological Research*. 164:290-296.

Ploetz, R.C. (2000). Panama disease: a classic and destructive disease of banana plant. *Helath Progress*. 10:1-7.

Raaijmakers, J. M., M. Vlami, and J. T. de Souza. (2002). Antibiotic production by bacterial biocontrol agents. *Antonie van Leeuwenhoek*. 81:537-547.

Rini C.R. and K.K. Sulochana. (2007). Usefulness of *Trichoderma* and *Pseudomonas* against *Rhizoctonia solani* and *Fusarium oxysporum* infecting tomato. *Journal of Tropical Agriculture*. 45(1-2):21-28.

Sabaratnam S. and J.A. Traquair. (2002). Formulation of a *Streptomyces* Biocontrol Agent for suppression of *Rhizoctonia solani* Dampingg off in tomato transplants. *Biological Control*. 23:245-253.

Shoda, M. (2000). Bacterial control of plant disease. *Journal of Bioscience and Bioengineering*. 89(6): 515—521.

Souto G.I., O.S. Correa, M.S. Montecchia, N.L. Kerber, N.L. Pucheu, M. Bachur and A.F. Garcıa. (2004). Genetic and functional characterization of *Bacillus* sp., strain excreting surfactin and antifungal metabolites partially identified as iturin-like compounds. *Journal of Apllied Microbiology*. 97:1247-1256.

Sturz A.V. and Nowak. (2000). Endophytic communities of *Rhizobacteria* and the strategies required to create yield enhancing associated with crops. *Applied Soil Ecology*. 13:183-190.

Surette M.A., A.V. Sturz, R.R. Lada and J. Nowak. (2003). Bacterial endophytes in processing carrots (*Daucus carota* L.var.sativus): their localization, population density, biodiversity and their effects on plant growth. *Plant and soil*. 253:381- 390.

Vega F.E., P.R. Monica, P. Francisco and S.B. Jefry. (2005). Endophytic bacteria in *Coffea arabica* L. *Journal Basic Microbiology*. 45:371-380.

Wang H., K.Wen, X. Zhao, X. Wang, A. Li and H. Hong. (2009). The inhibitory activity of endophytic *Bacillus* sp. Strain CHM1 against plant pathogenic fungi and its plant growth-promoting effect. *Crop Protection*. 28:634-639.

Yu G.Y., J.B. Sinclair, G.L. Hartman and B.L. Bertagnolli. (2002). Production of iturin A by *Bacillus amyloliquefaciens* suppressing *Rhizoctonia solani*. *Soil Biology & Biochemistry*. (34): 955—963.

Yuliar, Z. Abidin and W. Mangunwardoyo. (2011). Potency of Biocontrol Agents isolated from compost and peat soil of tropical swamp forest in Kalampangan zone, Central kalimantan. *Journal of Forestry Research*. 8(2): 144-157

Yuliar. (2002). Study on medium compositions to enhance iturin A productivity by *Bacillus subtilis* RB14-CS. A Master Thesis. The Graduate School of Tokyo Institute of Technology, Tokyo: 59 p.

Yuliar. 2008. Screening of bioantagonistic bacteria for biocontrol agent of *Rhizoctonia solani* and surfactin producer. *Journal of Biological Diversity*. 9(2): 83-86.