BIOTROPIA VOL. 13 NO. 2, 2006 : 111 - 121

ANTAGONISTIC BACTERIA AGAINST *SCHIZOPHYLLUM COMMUNE* FR. IN PENINSULAR MALAYSIA

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ABSTRACT

Schizophyllum commune Fr., is one of the important fungi, causes brown germ and seed rot of oil palm. Biodiversity of antagonistic bacteria from oil palm plantations in Peninsular Malaysia is expected to support in development of biopesticide. Isolation with liquid assay and screening antagonistic bacteria using dual culture assay were carried out in the bioexploration. A total of 265 bacterial isolates from plant parts of oil palm screened 52 antagonistic bacterial isolates against 5. *commune.* Bacterial isolates were identified by using Biolog* Identification System i.e. *Bacillus macroccanus, B. thermoglucosidasius, Burkholderia cepacia, B. gladioli, B. multivorans, B pyrrocinia, B. spinosa, Corynebacterium agropyri, C. misitidis, Enterobacter aerogenes, Microbacterium testaceum, Pseudomonas aeruginosa, P. citronellolis, Rhodococcus rhodochrous, Serratia ficaria, Serratia* sp., *S. marcescens, Staphylococcus sciuri, Sternotrophomonas maltophilia.*

Key words : *Schizophyllum commune,* biodiversity, antagonistic bacteria

INTRODUCTION

Schizophyllum commune Fr. causes brown germ and seed rot. Heavy infection decreased seed germination of oil palm about 60 percent (Dikin *et al.* 2003). Proper seed treatments are required for the control of *S. commune* in the oil palm seeds. Some synthetic fungicides were applied to reduce the loss of germination due to this pathogen, but the negative impact from toxic chemicals to the environment was difficult to avoid. The utilization of bacteria as biological control agents successfully controlled plant pathogen (Sharga and Lyon 1998; Bapat and Shah 2000).

Many studies in exploration of beneficial organisms have been carried out such as *Pseudomonas fluorescent* for the control of *Fusarium* wilt of tomato (Dekkers *et al.* 1998). *Streptomyces halstedii* (K122) and *S. coelicolor* (K139) to inhibit the fungi belonging to Oomycetes, Zygomycetes, Deuteromycetes, Ascomycetes and Basidiomycetes (Frandberg and Schnurer 1998). *Bacillus subtilis* suppressed phytopathogenic microorganism (Phae *et al.* 1990).

The isolation of antagonistic bacteria was early stage for development of biopesticide such as *Pseudomonas fluorescent* from rhizospheres (Dekkers *et al.* 1998), *Bacillus licheniformis* from leaves of citrus orchard at Letaba Estates,

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Tzaneen (Jager and Kosten 1998), *Streptomyces halstedii* (K122) and *S. coelicolor* (K139) from cereal grains (Frandberg and Schnurer 1998), *Bacillus subtilis* from the composts (Phae *et al.* 1990), and *Burkholderia cepacia* from infected oil palm seeds (Dikin *et al.* 2003). The liquid assay technique was a simple method for isolation of bacteria. Fluorescent *Pseudomonas* from Pythium-diseased tulip roots was isolated by extraction of infected root in sterilized water (Weststeijn 1990).

Malaysia is well known as a mega-biodiversity country with complex microbial association. The exploration of beneficial bacteria from oil palm plantations is expected to utilize the antagonistic bacteria from the same ecology of the oil palm pathogen itself.

The purposes of the study were to isolate and to screen the antagonistic bacteria from different plant parts of oil palm for the control of *Schizophyllum commune.*

MATERIALS AND METHODS

Cultural *Schizophyllum commune* Fr. and plant part of oil palm

The culture of *S. commune* was isolated from heavy infection of oil palm seeds. The fungus was confirmed based on their morphological characteristics and the pathogenic fungus of oil palm (Alexopoulus *et al.* 1996). The fungal isolate was sub-cultured onto PDA medium for further study.

Randomized samples of plant parts such as fruits, seeds, rhizosphere, and plant debris were collected from oil palm fields in Selangor (UPM, Bangi, Kajang and Seri Kembangan), Guthrie, Layang-Layang, Johor in Peninsular region, Malaysia. Plant parts were used for isolation of potential bacteria.

Isolation of Antagonistic Bacteria

Plant parts of oil palm such as fruits, seeds, rhizospheres and plant debris were rinsed with tap water to remove the adhered soil on surface. Mesocarp of fruits, endosperm of seeds, and rhizosphere were sliced into 0.5-1.0 cm^2 and then 50 g sliced plant parts were placed into 250 mL Erlemeyer flask added with 100 mL distilled water. Plant debris with 5. *commune* was collected under oil palm tree. Ten g of plant debris were cut off in size 1 cm and then transferred into a 250 mL Erlemeyer flask added with 100 mL sterilized water. Sample materials in flasks were placed on electric rotator at 100 rpm overnight at 26 ± 2°C. Fold serials ($lO^{-}lO''^{4}$) dilutions of suspension were made, 0.5 mL suspension from each diluted suspension was streaked on King's B (KB) and Nutrient Agar (NA) agar media plates. Plates were incubated at 26-28°C for 48 hours. Bacterial colonies on plate were purified by streaking single bacterial colony onto NA medium plates. Each pure culture of bacteria was screened for the antagonistic bacteria based on dual culture (Dikin *et al.* 2002;Montealegree/a/. 2003).

Screening the antagonistic bacteria

Screening of bacterial antagonist was carried out using dual culture assay. One 6-mm diameter of *S. commune* agar plug was placed at the centre of PDA medium in a Petri dish with 9 cm diameter. Bacterial isolate was streaked on PDA medium with a distance of 2.5 cm between *S. commune* agar plug and bacterial isolate. Plates were incubated for 7 days at 26 ± 2°C. The percentage of radial inhibition growth was measured with the formula:

PIRG (%) = (1 - (fungal growth near to bacterial isolate /fungal growth other side at the same plate as control)) x 100%.

Each treatment was replicated 3 times. Receded data were analyzed using SAS® Software. Treatment effect was tested by ANOVA and the means compared using Least Significant Different Test at 5% probability level (Okamoto *et al.* 1998; Anonymous 1999; Montealegreefa/. 2003).

Identification of antagonistic bacteria

Potential antagonistic bacterial isolates were identified by Biolog® identification system which followed the Biolog's procedures. Bacterial suspension was inoculated into GN or GP micro plates depending on gram reaction cluster, 145 uL per well using the 8-channeI repeating pipette. Microplate was covered with its lid and incubated at 28-30°C for 24 hours to allow the utilization of carbon sources. Reading result was directly done after inserting the incubated microplate into the Biolog's reader apparatus and its installed micro soft ware of Biolog® identification system for identifying bacteria up to the species level (Anonymous 2001).

RESULTS AND DISCUSSION

Isolation of antagonistic bacteria

The number of bacterial isolates was extracted from samples of seeds, fruits, rhizospheres and plant debris of oil palm which grew on KB and NA media. A total of 265 bacterial isolates from plant parts of oil palm were found from different locations, Peninsular region, Malaysia. Separation of bacterial isolates was based on the morphological colony performance such as colony colour, elevation, the margin of colony and colony surface (Hayward 1983).

Isolation of potential bacteria from plant parts of oil palm using liquid assay was effective and simple technique. The liquid assay and the agar plate media are commonly used for isolation of pathogenic bacteria from infected plant parts. Bacterial isolates from different plant parts of oil palm on NA and KB media grew well on the cultural plates.

Dual culture assay screened 52 out of 265 bacterial isolates against *S. commune*. The number of antagonistic bacteria from each location isolated from different plant parts is presented in Table 1.

Table 1. Number of bacterial isolates and antagonistic bacteria from plant parts of oil palm were obtained from different locations of Peninsular Region, Malaysia

Locality	Bacterial Code	Obtained from infected	Number of bacterial isolates	Number of antagonists
UPM Selangor	1	Rhizosphere	10	1
UPM Selangor	6, 7	Rhizosphere	8	2
UPM Selangor	17	Rhizosphere	11	1
UPM Selangor	20, 21	Rhizosphere	12	2
UPM Selangor	23, 24	Rhizosphere	9	2
UPM Selangor	31	Rhizosphere	8	1
UPM Selangor	50	Rhizosphere	10	1
Bangi, Selangor	47, 48, 49	Rhizosphere	12	3
Seri Kembangan, Selangor	51	Rhizosphere	7	1
Kajang, Selangor	52	Rhizosphere	9	1
Sub total of rhizosphere (1):			96	15
Layang – Layang, Johor	3, 5	Rotten fruit	8	2
Layang - Layang, Johor	9	Rotten fruit	6	1
UPM Selangor	25, 34	Rotten fruit	6	2
Bangi, Selangor	35, 36	Rotten fruit	5	2
Kajang, Selangor	38	Rotten fruit	7	1
Seri Kembangan, Selangor	40	Rotten fruit	6	1
Seri Kembangan, Selangor	41	Rotten fruit	5	1
Sub-total of fruit (2):			43	10
Layang - Layang, Johor	4	Seed	4	1
Layang – Layang, Johor	12,13	Seed	5	2
UPM Selangor	18, 22	Seed	7	2
UPM Selangor	26	Seed	5	1
Seri Kembangan, Selangor	37	Seed	6	1
Bangi, Selangor	39	Seed	5	1
Kajang, Selangor	46	Seed	5	1
Sub-total of seed (3):			37	9
UPM Selangor	2	Plant debris	6	1
UPM Selangor	8	Plant debris	8	1
UPM Selangor	10, 11	Plant debris	9	2
UPM Selangor	14, 15	Plant debris	8	2
Seri Kembangan, Selangor	16	Plant debris	6	1
Seri Kembangan, Selangor	19	Plant debris	6	1
UPM Selangor	27, 28	Plant debris	7	2
UPM Selangor	29, 30	Plant debris	8	2
Seri Kembangan, Selangor	32	Plant debris	5	1
Seri Kembangan, Selangor	33	Plant debris	6	1
Bangi, Selangor	42	Plant debris	7	1
Kajang, Selangor	43,44	Plant debris	8	2
Kajang, Selangor	45	Plant debris	5	1
Sub-total of plant debris (4):			89	18
Total (1+ 2+ 3+ 4):			265	52

Based on Table 1, 96 bacterial isolates as the highest number were obtained from the rhizosphere followed by plant debris, 89 isolates. The average number of bacterial isolates from rhizosphere was 9.6 followed by 6.8 from plant debris, 6.1 from fruits, and 5.2 from seeds. The bacterial isolates obtained from plant debris were more diverse with the number of bacterial isolates higher than other plant parts such as rhizophere, fruit, and seed.

Eighteen out of 89 antagonistic bacterial isolates were obtained from plant debris, followed by 15 out of 96 isolates from rhizosphere, 10 out of 43 isolates from fruit, and 9 out of 37 isolates from seed. The probability for isolation of antagonistic bacteria from each plant part was 20.2 percent, 15.6 percent, 23.2 percent, and 24.3 percent, respectively.

More dominant bacteria in the rhizosphere and plant debris than seeds and fruits were due to the different available nutrition and the requirement for bacterial growth. Dominance of bacteria in the rhizospheres and plant debris was due to complex interaction between microorganisms and plant parts. Plant debris such as decayed empty bunch, fronds, and rachis were good media for the fungal growth. Blotching symptom with water soak and brown colour in the fruiting bodies of *S. commune* was the indication of interaction between fungus and bacteria.

Dual culture assay of *S. commune* against antagonistic bacteria is presented in Figure 1.

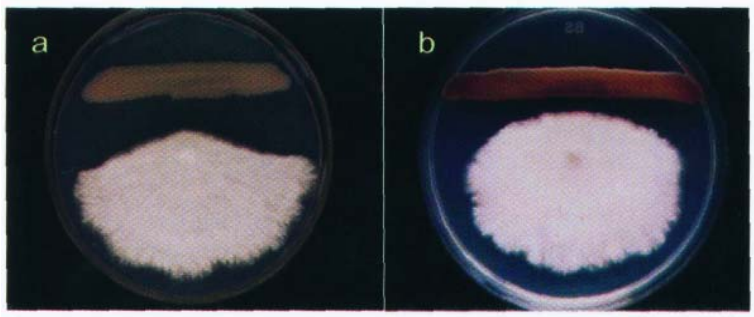

Figure 1. a. *Burkhoderia multivorans* (bacterial code-50) inhibits the growth of *S. commune* on dual culture of PDA medium at 7-day after incubation at 26 ± 2°C
b. *Burkholderia cepacia* inhibits the growth of *S. commune* on dual culture of PDA medium at 7-day after incubation at 26 ± 2°C

Among 52 isolates from different plant parts and different sampling locations inhibited the mycelial growth of *S. commune* with various percentages of radial inhibition. Each bacterial isolate with radial growth inhibition of *S. commune* is presented in Table 2.

The range of radial growth inhibition of antagonistic bacteria was 3.3 percent up to 95.2 percent from the bacterial code 29 and 10, respectively. In Table 2, there are 7 bacterial isolates with highest mean percentage of radial growth inhibition with the bacterial code 10, 8, 9, 14, 50, 7, and 2 with the percentage of inhibition of 95.2,

Table 2. Mean percentage of radial growth inhibition of *S. commune* against antagonistic bacteria

Bacterial code	Radial growth inhibition (% ± standard deviation)#	Bacterial code	Radial growth inhibition (% ± standard deviation) #
1	68.2 ± 0.5 i	27	37.5 ± 0 p
2	81.5 ± 0.3 bcd	28	75.4 ± 0.8 efg
3	68.8 ± 1.1 hi	29	3.3 ± 0.4 v
4	40.3 ± 0.4 p	30	62.5 ± 3.8 jk
5	58.6 ± 2.7 kl	31	46 ± 1.6 o
6	74.9 ± 1.2 fg	32	51 ± 1.4 mn
7	81.8 ± 0.3 bcd	33	78.7 ± 3.2 bcdef
8	90.6 ± 0.9 a	34	55.7 ± 4.2 lm
9	83.2 ± 1.4 b	35	13.9 ± 0.7st
10	95.2 ± 0.2 a	36	24.3 ± 1.8 q
11	68.9 ± 3.7 hi	37	19 ± 0.8 r
12	17.2 ± 3.2 rs	38	80.3 ± 1.2 bcd
13	12.5 ± 1.9 tu	39	8.8 ± 0.7 u
14	83.1 ± 0.2 b	40	79.4 ± 5.7 bcdef
15	48.8 ± 1.6 no	41	20.3 ± 0.4 qr
16	62.6 ± 1.8 jk	42	75.5 ± 5.3 efg
17	78.8 ± 1.6 bcdef	43	80.6 ± 0.9 bcd
18	78.3 ± 1.2 cd	44	17.5 ± 0.8 rs
19	51.1 ± 4.4 mn	45	20 ± 0.8 qr
20	40 ± 1.6 p	46	19.3 ± 0.5 r
21	39.7 ± 2.9 p	47	60 ± 0 kl
22	65.5 ± 4.1 ji	48	20 ± 0 qr
23	73.3 ± 4.8 hg	49	58.3 ± 0 kl
24	65 ± 5.9 ji	50	83 ± 0.8 bc
25	77.4 ± 1.8 defg	51	80 ± 1.6 bcde
26	58.7 ± 1.0 kl	52	62.4 ± 1.6 jk

Average of three replications. Means with standard deviation followed by different letters within the column are significantly different ($LSD_{0.05}$= 4.6 mm).

90.6, 83.2, 83.1, 83, 81.8, and 81.5, respectively. A number of antagonistic bacteria were isolated from plant parts with varied mean percentages of radial growth inhibition against *S. commune*. The bacterial isolates had high radial growth inhibition which were obtained from plant debris, rhizospheres, and fruit.

Many authors have reported that certain antagonistic bacteria suppressed the growth of pathogenic fungus. In *vitro* study showed that *Burkholderia cepacia* from tomato's rhizospheres suppressed the growth of *Fusarium oxysporum* f.sp. *lycopersicae* stronger than *S. commune.* In contrast, *B. cepacia* from rhizhosperes of oil palm suppressed *S. commune* stronger than *F. oxysporum* f.sp. *lycopersicae* (Kamaruzaman and Dikin 2005). In this case , targeted potential antagonistic bacteria against *S. commune* should be isolated from the area of oil palm plantation.

Identification of antagonistic bacteria

Identification of antagonistic bacteria against *S. commune* based on Biolog® Identification system is presented in Table 3.

Table 3. Antagonistic bacteria based on Biolog* Identification System

Plant Part	Bacterial code	Bacteria
Rhizosphere		
	1	*Burkholderia cepacia*
	6	*Corynebacterium agropyri*
	7	*Microbacterium testaceum*
	17	*Pseudomonas citronellolis*
	20	*Bacillus macroccanus*
	23	*B. cepacia*
	24	*Burkholderia spinosa*
	47	*B. cepacia*
	49	*Staphylococcus sciuri*
	50	*Burkholderia multivorans*
	51	*Burkholderia pyrrocinia*
	52	*B. cepacia*
Fruit		
	3	*B. cepacia*
	5	*Serratia marcescens*
	9	*C. agropyri*
	25	*Pseudomonas aeruginosa*
	34	*P. aeruginosa*
	38	*P. aeruginosa*
	40	*P. aeruginosa*
Seed		
	4	*Serratia* sp.
	12	*Bacillus thermoglucosidasius*
	18	*B. pyrrocinia*
	22	*B. cepacia*
	26	*P. aeruginosa*
	37	*Sternotrophomonas maltophilia*
Plant debris		
	2	*S. mallophilia*
	8	*Burkholderia gladioli*
	10	*B. gladioli*
	11	*B. cepacia*
	14	*C. agropyri*
	16	*Serratia ficaria*
	19	*Enterobacter aerogenes*
	28	*B. gladioli*
	30	*P. aeruginosa*
	33	*Corynebacterium masitidis*
	42	*M. testaceum*
	43	*Rhodococcus rhodochrous*

Table 3 presents the identified bacteria and non-identified bacteria by using Biolog Identification system. In the rhizosphere 12 identified species and 3 non-identified species were found. The identified species from rhizosphere were *B. cepacia, C. agropyri, M. testaceum, P. citronellolis, B. macroccanus, B. spinosa, S. sciuri, B. multivorans,* and *B. pyrrocinia.* Among 9 species, the dominant identified species in the rhizosphere was *B. cepacia.*

Seven identified species and 3 non-identified species of antagonistic bacteria were found in fruits. The identified species from fruits were *B. cepacia, S. marcescens, C. agropyri,* and *P. aeruginosa.* Among the 4 species, the dominant identified species in the fruit was *P. aeruginosa.*

P. aeruginosa was isolated from the rhizospheres and plant debris. This bacteria was recognized as the supplier of mineral which was required for metabolism process of plant from the access of bacterial metabolites (Hofte *et al.* 1993; Abdullah *et al.* 2003). There were complex microorganisms around rhizospheres to compete with each other for survival which showed the synergism and antagonism interaction. Infected plant debris with *S. commune* around the rhizosphere was to bait the potential antagonistic bacteria.

Six identified species and 3 non-identified species of antagonistic bacteria in seeds were found. The identified species from fruits were *Serratia* sp., *B. thermoglucosidasius, B. pyrrocinia, B. cepacia, P. aeruginosa,* and *S. maltophilia.* In the plant debris 12 identified species and 6 non-identified were found. The identified species were *S. maltophilia, B. gladioli, B. cepacia, C. agropyri, S. ficaria, E. aeogenes, P. aeruginosa, C. masitidis, M. testaceum,* and *R. rhodochrous.* The dominant species from plant debris was *B. gladioli.*

B. cepacia and *B. gladioli* were dominantly found in the rhizospheres and plant debris, respectively. The presence *of B. cepacia* in the rhizospheres of oil palm was the same evident with the presence of *B. cepacia* in the rhizospheres of banana to protect plant infection caused by *Fusarium oxysporum* f. sp *cubense. B. cepacia* colonizes the surface of hyphae and fungal macrospores (Pan *et al.* 1997). *B. gladioli* was found in the rhizospheres and potential antagonistic bacteria against *S. commune,* however the implication for biological control was less recognized.

The identified species of antagonistic bacteria from different plant parts were *Bacillus macmccanus, B. thermoglucosidasius, Burkholderia cepacia, B. gladioli, B. multivorans, B pyrrocinia, B. spinosa, Corynebacterium agropyri, C. misitidis, Enterobacter aerogenes, Microbacterium testaceum, Pseudomonas aeruginosa, P. citronellolis, Rhodococcus rhodochrous, Serratia ficaria, Serratia* sp., *S. marcescens, Staphylococcus sciuri, and Sternotrophomonas maltophilia.* Out of these species were new recorded species of antagonistic bacteria i.e. *B. thermoglucosidasius, B. multivorans, B. spinosa, C. agropyri, C. misitidis, Enterobacter aerogenes, P. citronellolis, Rhodococcus rhodochrous, Serratia ficaria,* and *Staphylococcus sciuri.* However, *B. cepacia, B pyrrocinia P. aeruginosa, S. marcescens* and *S. maltophilia* were reported as biocontrol agents (Burkhead *et al.* 1994; Kobayashi *et al.* 1995; Suparman *et al.* 2002; Szczech and Shoda 2004). Avirulent isolate of *B. gladioli* strain 1064A is used for suppressing the incidence of bacterial seedling blight of rice caused by *B. plantarii* (Miyagawa 2000).

P. aeruginosa is grouped as fluorescent Pseudomonads based on the production of a fluorescens pigment on KB medium (Sand *et al.* 1980). The bacterium produces siderophores as plant growth promoter (Hofte *et al.* 1993) and broad spectrum antagonistic bacteria against pathogenic fungi (Haas *et al.* 1991).

Several species of fluorescent Pseudomonads were known to be antagonistic bacteria and used as biological control agents. *P. aeruginosa* 7NSSK2 was able to suppress *Pythium splendens,* the causal pre and post-emergence damping-off and root rot of many crops such as tomato (Tambong *et al.* 1998). *P. aeruginosa* and *S. marcescens* were isolated from plant part of oil palm, these isolates were confirmed as biocontrol agent for suppressing *Sclerotium rolfsii* and *Rhizoctonia solani* (Ordentliche/a/. 1987).

CONCLUSIONS

A number of bacterial isolates from plant part such as seeds, fruits, rhizospheres, and plant debris under oil palm trees were potential antagonistic bacteria against *S. commune.* A total of 52 out of 265 bacterial isolates were identified as the antagonistic bacteria against *S. commune.* The identified antagonistic bacteria using Biolog® Identification System were as follows : *Agrobacterium agropyri, Bacillus macroccanus, B. thermoglucosidasius, Burkholderia cepacia, B. gladioli, B. multivorans, B pyrrocinia, B. spinosa, Corynebacterium agropyri, C. misitidis, Enterobacter aerogenes, Microbacterium testaceum, Pseudomonas aeruginosa, P. citronellolis, Rhodococcus rhodochrous, Serratia ficaria, Serratia* sp., *S. marcescens, Staphylococcus sciuri,* and *Sternotrophomonas maltophilia.* Some of bacterial isolates were recognized as biocontrol agents of plant pathogenic fungi and the rest of isolates have yet to be studied for their status. All of these species are required for further studies on the production of their secondary metabolites which might be potential substances to inhibit the growth of *S. commune.*

ACKNOWLEGDMENT

The study is partially supported by IRPA project, Malaysian Government (Vote No. 54400) as part of the Ph.D. thesis of the first author.

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