The Potential of Endophytic Bacteria as a Biological Control Agent for *Ganoderma* Disease in Oil Palm
(Potensi Bakteria Endofit sebagai Agen Kawalan Biologi untuk Penyakit *Ganoderma* pada Pokok Sawit)

NUR RASHYEDA RAMLI*, MAIZATUL SURIZA MOHAMED, IDRIS ABU SEMAN,
MADIHAH AHMAD ZAIRUN & NASYARUDDIN MOHAMAD

ABSTRACT

This study was conducted to screen the endophytic bacteria as a biological control agent (BCA) against Ganoderma boninense. *A total of 581 endophytic bacteria were successfully isolated from symptomless oil palm root tissues at Teluk Intan, Perak, Malaysia. Three endophytic bacteria,* Pseudomonas aeruginosa *GanoEB1,* Burkholderia cepacia *GanoEB2, and* Pseudomonas syringae *GanoEB3 were found to have a potential as BCA based on their percentage inhibition of radial growth (PIRG) in dual culture and culture filtrate tests. Two nursery trials were conducted to evaluate the capability of these bacteria to suppress* Ganoderma *disease in oil palm seedlings that were artificially infected with* G. boninense *using rubber wood block (RWB) sitting technique. The percentage of disease incidence (DI), severity of foliar symptoms (SFS) and dead seedlings were used as the assessment tools. As a result, DI and SFS have developed much slower in the seedlings that were pre-treated with bacteria compared to untreated seedlings. After 6 months of inoculation,* Ganoderma *disease incidence was reduced from 62-75% in the seedlings treated with* P. aeruginosa *GanoEB1, followed by* B. cepacia *GanoEB2 (31-59%) and* P. syringae *GanoEB3 (30-31%). Among these three endophytic bacteria,* P. aeruginosa *GanoEB1 was the most effective in controlling* Ganoderma *disease and the dead seedlings were in the range of 13.3-26.7%, followed by* B. cepacia *GanoEB2 (33.3% for both trials) and* P. syringae *GanoEB3 (33.3-40.0%) compared to untreated seedlings at 60% for both trials. A field study needs to be conducted to verify their effectiveness in controlling* Ganoderma *in oil palm.*

Keywords: Burkholderia*; endophytic bacteria;* Ganoderma boninense*;* Ganoderma *disease;* Pseudomonas

ABSTRAK

Suatu kajian telah dijalankan untuk menyaring bakteria endofit sebagai agen kawalan biologi terhadap kulat Ganoderma boninense. *Sebanyak 581 bakteria endofit telah berjaya diasingkan daripada tisu akar pokok sawit sihat di Teluk Intan, Perak, Malaysia. Tiga bakteria endofit telah dikenal pasti sebagai* Pseudomonas aeruginosa *GanoEB1,* Burkholderia cepacia *GanoEB2 dan* Pseudomonas syringae *GanoEB3 menunjukkan potensi sebagai agen kawalan biologi berdasarkan peratus rencatan pertumbuhan miselium* G. boninense *yang tinggi dan signifikan. Dua ujian nurseri telah dijalankan untuk menilai keberkesanan bakteria ini untuk mengawal penyakit* Ganoderma *pada anak sawit. Anak sawit telah dirawat dengan bakteria endofit dan seterusnya dijangkitkan dengan* G. boninense *menggunakan teknik blok kayu getah (RWB). Peratusan kejadian penyakit (DI), keterukan simptom daun (SFS) dan kematian anak sawit telah digunakan sebagai alat penilaian. Melalui hasil kajian ini, kadar peratusan DI dan SFS adalah lebih rendah pada anak sawit yang telah dirawat dengan bakteria endofit berbanding dengan anak sawit yang tidak dirawat. Selepas 6 bulan rawatan, kejadian penyakit* Ganoderma *telah dapat dikurangkan pada kadar 62-75% pada anak sawit yang telah dirawat dengan* P. aeruginosa *GanoEB1 diikuti oleh* B. cepacia *GanoEB2 (31-59%) dan* P. syringae *GanoEB3 (30-31%). Antara ketiga-tiga bakteria endofit ini,* P. aeruginosa *GanoEB1 adalah yang paling berkesan dalam mengawal penyakit* Ganoderma *kerana hanya 13.3-26.7% anak sawit telah mati diikuti oleh* B. cepacia *GanoEB2 (33.3% untuk kedua-dua ujian) dan* P. syringae *GanoEB3 (33.3-40.0%) berbanding dengan anak sawit yang tidak dirawat pada 60% untuk kedua-dua ujian. Namun begitu, kajian lapangan perlu dijalankan untuk mengesahkan keberkesanan bakteria endofit ini dalam mengawal penyakit* Ganoderma *pada pokok sawit di ladang.*

Kata kunci: Burkholderia; *bakteria endofit;* Ganoderma boninense*; penyakit* Ganoderma; *Pseudomonas*

INTRODUCTION

Basal stem rot (BSR) caused by the species of *Ganoderma* has been identified as a major disease of oil palm (*Elaeis quineensis Jacq*) in Southeast Asia (SEA), especially in Malaysia and Indonesia (Ariffin et al. 2000; Idris 1999; Susanto 2009; Turner & Gillbanks 2003). Previously,

Ganoderma disease was only found on older palms, however, in 1990s it has been reported that *Ganoderma* has the ability to infect young palms (1-2 years old) and more commonly for oil palms aged 4 - 5 years old particularly in replanted areas (Ariffin et al. 1996; Singh 1991). Since the disease has caused a significant loss in the palm oil

production, control and management strategies for the *Ganoderma* disease are crucial. Several techniques have been suggested to control *Ganoderma* disease including surgery (Turner & Gillbanks 2003), soil mounding (Tuck & Khairudin 1997), sanitation by the removal of diseased oil palms (Idris et al. 2005) and sanitation by destroying old stump, root masses and ploughing along the new planting row before replanting (Idris et al. 2004a). Field trials to control *Ganoderma* disease using systemic and contact fungicides have been performed by various researchers with no conclusive results even with those *in vitro* screened effective against the *Ganoderma* fungus (Idris et al. 2004b, 2002; Khairudin 1990; Loh 1976). The possible approach in controlling the disease caused by *Ganoderma* could be through the manipulation of biological agents such as fungi, mychorhiza, actinomycetes and bacteria.

Biological agents have been considered as an alternative approach for controlling various plant diseases (Nega 2014; Tjamos et al. 2010). Studying endophytic bacteria as a biological control agent that suppresses plant diseases has gained much attention in pathological research (Soylu et al. 2005). Endophytic microbes are microorganisms that live in the intercellular spaces of plant for most if not all of their life cycles with no pathogenic effects on their hosts (Azevedo et al. 2000; Kobayashi & Palumbo 2000). Several bacteria have been reported to support the growth, improve the health of plants (Hallman et al. 1997) and also displayed the ability to inhibit fungal pathogen growth *in vitro* and subsequently suppress plant diseases (Chen et al. 1995; Pan et al. 1997). Endophytic bacteria have been isolated from a wide range of hosts, such as oil palm (Zaiton et al. 2006), banana (Pan et al. 1997), grass (Clay 1998) and eggplant (Ramesh et al. 2009). The capability of colonizing host tissue has made endophytes valuable and effective for agriculture as a tool to improve crop performance compared to other biological agents. As an internal colonizer of the root system, endophytes are able to compete within the vascular system, inhibiting pathogens to obtain both nutrients and space for its proliferation. Rika et al. (2014) have shown that a type of endophytic bacteria, namely *Burkholderia* sp. B212 has the potential to produce secondary metabolites such as pyrrolnitrin. Pyrrolnitrin metabolite of *Pseudomonas* and *Burkholderia* sp. strains were reported to have a strong antifungal activity to control plant diseases against fungal plant pathogens (Hammer et al. 1997). Thus, this study aimed to isolate endophytic bacteria from oil palm roots and to establish their potential as biological control agents against *G. boninense* fungus *in vitro* and *in vivo* at nursery trials.

MATERIALS AND METHODS

ISOLATION AND IDENTIFICATION OF ENDOPHYTIC BACTERIA

The samples of oil palm roots were collected from oil palm plantation in Teluk Intan, Perak, Malaysia. The roots were randomly sampled from 15 palms with no obvious symptoms of *Ganoderma* disease. At each palm, five random roots with 10 cm in length were taken about 1.0 m away from the palm bases at 30-60 cm depth. The root samples were then brought to the laboratory and rinsed under running tap water for 20 min to remove any adhering soil from their surfaces. For the isolation of endophytic bacteria, 0.5 cm from both ends of the roots was discarded and the remaining roots were divided into three sections of three cm in length each (Zaiton 2006). These sections were surface-sterilized by dipping it in 10% sodium hypochlorite and subsequently in 50, 70, 90 and 100% ethanol (modified from Schena et al. 2003). The roots were then rinsed twice with sterilized distilled water and allowed to dry on a sterilized filter paper before transferred onto nutrient agar (NA). The NA plates were then incubated at 28±2ºC for three days and subjected to Biolog® System for identification.

IDENTIFICATION OF ENDOPHYTIC BACTERIA

All isolates were identified using Biolog® System. Pure culture of bacteria from fresh cultures (24 h) growing on the NA were streaked on Biolog® Universal Growth (BUG) medium. The bacteria isolates were initially determined by tetrazolium violet as colorimetric indicator of the gram reaction and oxidation and were categorized as enteric or non-enteric bacteria. The bacteria isolates were then suspended in inoculation fluid (0.40% sodium chloride, NaCl; 0.03% pluronic F-68; 0.01% gellan gum). The bacterial suspension was inoculated in a Biolog 96-well microtiter plate with 150 μL per well. The microtiter plate was incubated at 28-30ºC for 24 h and the resulted pattern of coloured wells was analysed using the Microstation™ system and Biolog MicroLog™ software for bacterial identification based on the database.

IN VITRO SCREENING OF ENDOPHYTIC BACTERIA AGAINST *G. BONINENSE*

All isolates were screened for their antagonistic activity against *G. boninense in vitro* via dual culture and culture filtrate tests. Therefore, the potential isolates were selected based on the percentage inhibition of radial growth (PIRG) (Zaiton 2006). All tests were conducted in three replicates, in a completely randomized design (CRD).

PREPARATION OF *GANODERMA BONINENSE* INOCULUM ON RUBBER WOOD BLOCK

The *Ganoderma* inoculum was inoculated into a rubber wood block (RWB) of 6 cm × 6 cm × 6 cm in size as described by Idris et al. (2006) and Khairudin (1990). The blocks were sterilized and autoclaved at 121ºC for 20 min. Each block was placed in heat-resistant polypropylene bags and 100 mL of molten malt extract agar (MEA) were added as a supplementary nutrient for *G. boninense*. The bags with RWB and molten MEA were autoclaved at 121ºC for 30 min. After sterilization and cooling, the RWB in the bag was rotated to ensure that it was well covered with the agar before solidified. A half plate of the *G. boninense* culture

(isolate code PER 71) was inoculated on each RWB. The inoculated blocks were then incubated in a dark cabinet at 28±2°C until *G. boninense* mycelium was fully colonized.

PREPARATION OF ENDOPHYTIC BACTERIA

The suspension of *P. aeruginosa* GanoEB1, *B. cepacia* GanoEB2 and *P. syringae* GanoEB3 were prepared separately using 48 h culture on nutrient broth (NB). The inoculum suspensions were prepared and adjusted to 10^8 colony forming unit (cfu) mL^{-1}.

DISEASE ASSESSMENT AND DATA ANALYSIS

The assessment of the effect of endophytic bacteria on *Ganoderma* incidence was carried out based on the quantitative assessment measured as percentage of disease incidence (DI), severity of foliar symptom (SFS) and dead seedlings (DS) at two months intervals. The DI (%) represented the number of seedlings assessed as diseased visually (chlorosis and necrosis of leaves, with or without the production of fruiting body) (Idris et al. 2006): (number of seedlings infected/total number of seedlings assessed) ×100. SFS (%) was assessed according to Sariah and Zakaria (2000) as:

$$SFS (\%) = ((a \times 1) + (b \times 0.5))/c \times 100,$$

where *a* is the number of desiccated (browned/wilted) leaves; *b* is the number of yellowing leaves; *c* is the total number of leaves; *1* is the index for desiccated leaves; and *0.5* the index for yellowing leaves.

The area under the disease progress curve (AUDPC) was calculated using the formula (Shaner & Finney 1977):

$$AUDPC = \sum_{i}^{n-1} [(y_i + y_{i+1})/2](t_{i+1} + t_i) ,$$

where *n* is the number of assessment time; *y* is the disease measurement (DI); and *t* is the time (months) after inoculation.

Disease reduction (DR) was calculated based on the value of AUDPC. All percentage data (DI, SFS, and dead seedlings) were transformed by arcsine transformed (Gomez & Gomez 1984) and subjected to ANOVA with the means was compared to the least significant difference (LSD) at $p \leq 0.05$ using SAS® software (SAS Institute Inc. 1995).

RESULTS AND DISCUSSION

ISOLATION, IDENTIFICATION AND *IN VITRO* SCREENING OF ENDOPHYTIC BACTERIA AGAINST *GANODERMA BONINENSE*

After three days of incubation, a total of 581 endophytic bacteria were successfully isolated from oil palm roots. About 78% of the 581 isolates were identified as gram negative bacteria and 22% were gram positive bacteria. Therefore, gram-negative bacteria were more abundant in the oil palm roots compared to the gram-positive bacteria,

where this is in agreement with Bivi et al. (2010) whom also reported that the bacteria isolated from the oil palm xylem tissues were mainly gram negative bacteria. In addition, all isolates were identified and grouped into 12 potential species as follows: *Pseudomonas aeruginosa*, *Burkholderia cepacia*, *Burkholderia vietnamiensis*, *Serratia marcescens*, *Staphylococcus aureus*, *Pseudomonas syringae* pv. helianthi, *Corynebacterium nitriphilus*, *Clavibacter agropyri*, *Pseudomonas putida*, *Pseudomonas syringae* pv. persicea, *Klebsiella pneumoniae* and *Bacillus cereus*. In this study, after screening their antagonistic activity, all the endophytic bacteria tested showed different degrees of inhibition towards the mycelial growth of *G. boninense* (Table 1). Two groups of endophytic bacteria identified as *P. aeruginosa* and *B. cepacia* produced significantly (*p*<0.05) higher PIRG values of 70 and 55.5% based on dual culture, respectively, than other isolates, with respect to the control after seven days of incubation. Culture filtrate test showed a significant difference in the percentage of mycelia growth (*p*<0.05). Endophytic bacteria, *P. aeruginosa*, *B. cepacia* and *P. syringae* suppressed the fungal growth by an average of 80.0, 65 and 46.5%, respectively. *Pseudomonas aeruginosa* displayed the most significant inhibition level against *G. boninense*. The possible explanation would be *P. aeruginosa* has the ability to produce secondary metabolites that have strong antifungal activities against *G. boninense* (Figure 1(a) & 1(b)). Chen et al. (2000) stated that *Pseudomonas* group is known as growth-promoting bacteria and inducer of systemic resistance against fungal, bacterial and viral diseases. Apart from that, *Burkholderia cepacia* has also been reported as a biological control agent for controlling plant diseases such as damping off and blast disease on rice (Homma & Suzui 1989). However, endophytic bacteria identified as *Serratia marcescens*, *Staphylococcus aureus*, *Corynebacterium nitriphilus*, *Clavibacter agrophyri*, *Klebsiella pneumoniae* and *Bacillus cereus* showed less antagonistic activity against *G. boninense* even though some of these endophytic bacteria have been reported as a potent biological control agent on other plant diseases.

NURSERY EVALUATION OF ENDOPHYTIC BACTERIA AS BIOLOGICAL CONTROL OF *GANODERMA BONINENSE* INFECTION IN OIL PALM SEEDLINGS

Two nursery Trials (1 and 2) have been conducted to evaluate the efficacy of three potential endophytic bacteria as a biological control against *G. boninense*, namely *P. aeruginosa* GanoEB1, *B. cepacia* GanoEB2 and *P. syringae* GanoEB3. Each trial was conducted with four treatments (Table 2), replicated thrice with five seedlings per replicate, and arranged in a randomized complete block design (RCBD). Overall, the *Ganoderma* symptoms were progressive, yellowing of the lower leaves of the oil palm seedlings and followed by desiccation from the oldest to the youngest leaf at 2-3 months after artificially infected with *G. boninense*. Leaves desiccation usually starts at the tip of the leaf followed by rapid yellowing and drying of the entire lamina. The development of fruiting body was

TABLE 1. Antagonistic activity of endophytic bacteria in dual culture and
culture filtrate tests against *Ganoderma boninense in vitro*

Endophytic bacteria	No. of isolates	Gram stain	Average of PIRG values (%)*	
			Dual culture	Culture filtrate
Pseudomonas aeruginosa	9	Negative	70.0a	80.0a
Burkholderia cepacia	16	Negative	55.5b	65.0b
Burkholderia vietnamiensis	33	Negative	41.5cd	42.0d
Serratia marcescens	4	Negative	40.0d	40.0d
Staphylococcus aureus	23	Positive	22.5e	0
Pseudomonas syringae pv. helianthi	133	Negative	42.5c	46.5c
Corynebacterium nitriphilus	37	Positive	0	0
Clavibacter agropyri	34	Positive	0	0
Pseudomonas putida	87	Negative	10.0h	0
Pseudomonas syringae pv. persicea	137	Negative	20.0f	0
Klebsiella pneumoniae	31	Negative	10.0h	0
Bacillus cereus	37	Positive	17.5g	0
Total	581			

* Means with the same letter in the same column are not significantly different according to the least significant different (LSD) test at *p*=0.05
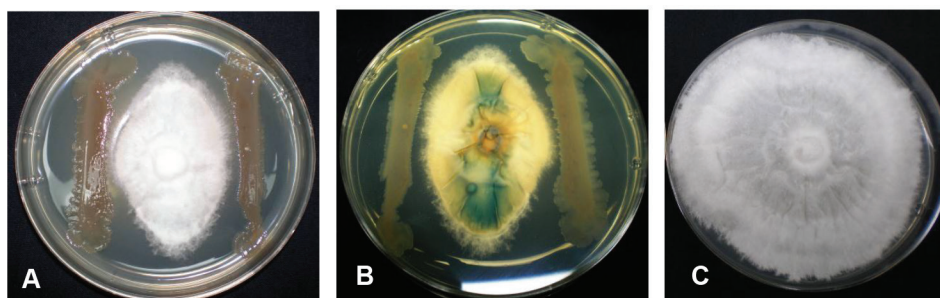

FIGURE 1(a). Effect of *P. aeruginosa* GanoEB1 on the radial growth of *G. boninense* in the dual culture test (after 7 days of incubation) (a) Top, (b) bottom side of of *P. aeruginosa* GanoEB1 and (c) *G. boninense* in control plate
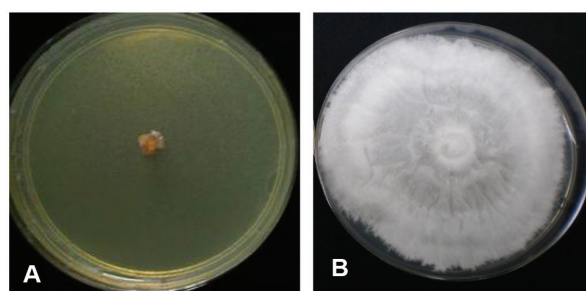

FIGURE 1(b). Effect of (a) *P. aeruginosa* GanoEB1 on the radial growth of *G. boninense* in the culture filtrate test (at 7 days of incubation) and (b) *G. boninense* in control plate

TABLE 2. Treatment of endophytic bacteria applied to oil palm seedlings

Treatment	Seedling description
T1	*P. aeruginosa* GanoEB1 + *G. boninense*
T2	*B. cepacia* GanoEB2 + *G. boninense*
T3	*P. syringae* GanoEB3+ *G. boninense*
T4	Untreated + *G. boninense* (as control)

observed at two months as a white mass of tissues (white mycelium) and followed by the emergence of a button-like fruiting body of *G. boninense*. This was confirmed by planting fruiting body of *G. boninense* on the *Ganoderma* selective medium (GSM) (Ariffin & Idris 1991).

For Trial 1, after 6 months of treatment duration, seedlings that were artificially infected with *G. boninense* treated with *P. aeruginosa* GanoEB1 showed significantly lower percentage of DI (Table 3) and SFS (Figure 2) (33.3 and 24.8%, respectively), followed by *B. cepacia* GanoEB2 (46.7 and 35.8%, respectively), *P. syringae* GanoEB3 (60 and 43.1%, respectively) and untreated (86.7 and 67.8%, respectively). Disease incidence (%) and SFS (%) treatments with both *B. cepacia* GanoEB2 and *P. syringae* GanoEB3 were significantly different compared to control treatment but not statistically significant among the three treatments. The percentage of dead seedlings for treatment with endophytic bacteria was shown to have a significant difference compared to untreated (Figure 3). However, the percentage of dead seedlings was not statistically significant among the three endophytes bacteria tested in this study.

For Trial 2, the percentage of DI (Table 4) and SFS (Figure 4) of seedlings treated with *P. aeruginosa* GanoEB1 (40 and 47.1%, respectively) were significantly lower than *B. cepacia* GanoEB2 (66.7 and 57%, respectively), *P. syringae* GanoEB3 (66.7% and 58.7%, respectively), and untreated control (93.3 and 70.3%, respectively). Treatment with *P. aeruginosa* displayed the lowest percentage of dead seedlings, followed by GanoEB1 (26.7%) and the highest (60%) was displayed by untreated (Figure 5). However, no significant difference was recorded in seedlings treated with *B. cepacia* GanoEB2 and *P. syringae* GanoEB3 when compared to untreated control seedlings.

In general, the disease development was slower in the seedlings pre-treated with endophytes bacteria than in the untreated seedlings. The endophytic bacteria enters oil palm seedling tissues through roots during the booster of bacterial suspension and once inside, the endophytic bacteria may either remain localized at the point of entry or spread throughout the plant (Hallmann et al. 1997; Sapak et al. 2008). These microorganisms can colonize and reside within the cells (Jacobs et al. 1985) in the

TABLE 3. Percentage of disease incidence (DI) in oil palm seedlings after inoculation with G. *boninense* (Trial 1)

Treatment	Disease incidence (%)[#]		
	2 MAI[##]	4 MAI	6 MAI
T1: *P. aeruginosa* GanoEB1 + *G. boninense*	0	6.7c	33.3c
T2: *B. cepacia* GanoEB2 + *G. boninense*	0	13.3c	46.7bc
T3: *P. syringae* GanoEB3 + *G. boninense*	0	33.3b	60b
T4: Untreated + *G. boninense*	0	46.7a	86.7a

[#] Means with the same letter in the same column are not significantly different according to the least significant different (LSD) test at *p*=0.05
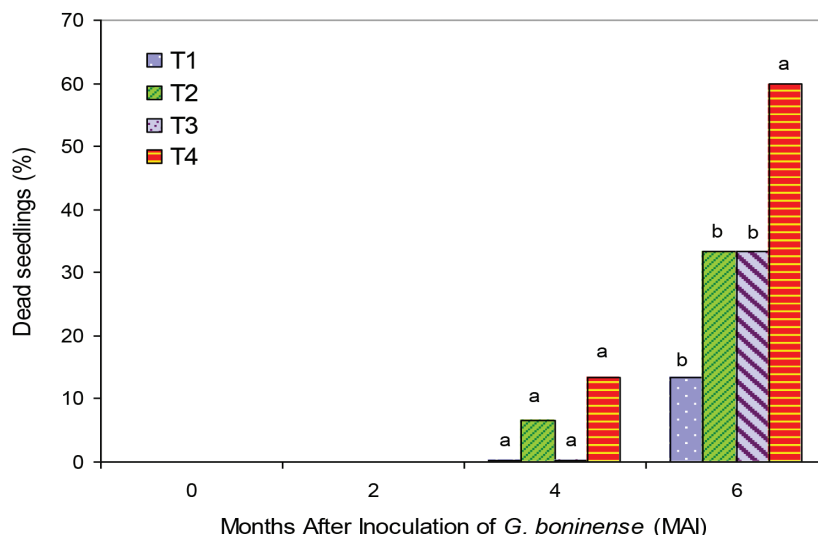[##] MAI = Months after artificially infected with *G. boninense*
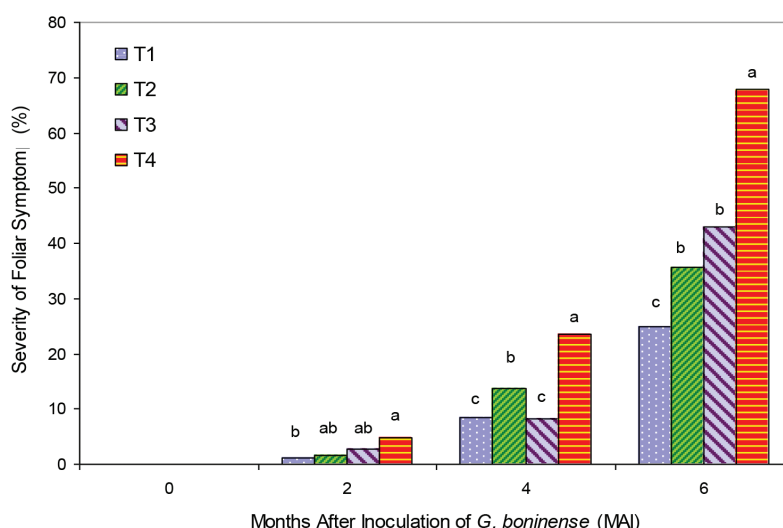
Means with the same letters between treatments are not significantly different according to the least significant different (LSD) test at *p*=0.05
Treatments: T1: Seedlings treated with *P. aeruginosa* GanoEB1 + *G. boninense*; T2: Treated with *B. cepacia* GanoEB2 + *G. boninense*; T3: Treated with *P. syringae* GanoEB3 + *G. boninense*; T4: Seedlings untreated + *G. boninense*

FIGURE 2. Percentage severity of foliar symptoms (SFS) of oil palm seedlings due to *G. boninense* infection (Trial 1)
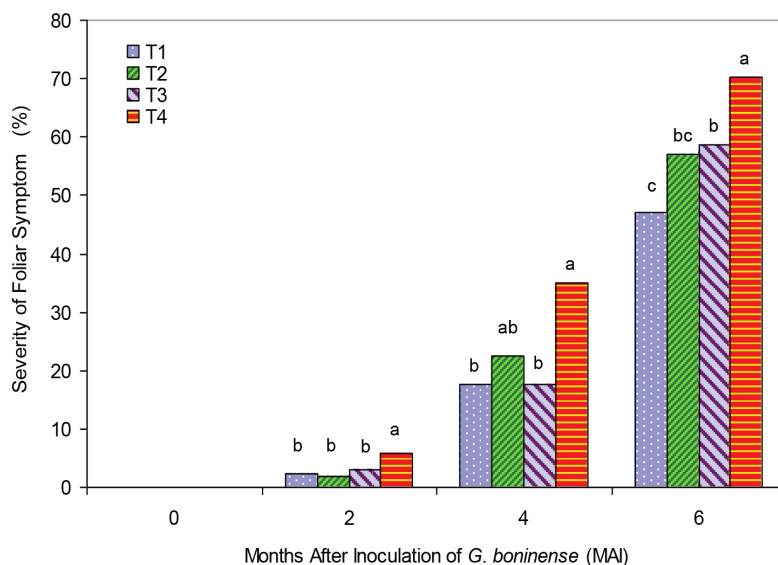
Means with the same letters between treatments are not significantly different according to the least significant different (LSD) test at p=0.05

Treatments: T1: Seedlings treated with *P. aeruginosa* GanoEB1 + *G. boninense*; T2: Treated with *B. cepacia* GanoEB2 + *G. boninense*; T3: Treated with P. syringae GanoEB3 + *G. boninense*; T4: Seedlings untreated + *G. boninense*

FIGURE 3. Dead seedlings (%) due to *G. boninense* infection (Trial 1)

TABLE 4. Percentage of disease incidence (DI) in oil palm seedlings after inoculation with *G. boninenses* (Trial 2)

Treatment	Disease incidence (%)[#]		
	2 MAI[##]	4 MAI	6 MAI
T1: *P. aeruginosa* GanoEB1 + *G. boninense*	0	13.3a	40.0c
T2: *B. cepacia* GanoEB2 + *G. boninense*	0	26.7a	66.7b
T3: *P. syringae* GanoEB3 + *G. boninense*	0	26.7a	66.7b
T4: Untreated + *G. boninense*	0	33.3a	93.3a

[#] Means with the same letter in the same column are not significantly different according to the least significant different (LSD) test at p=0.05
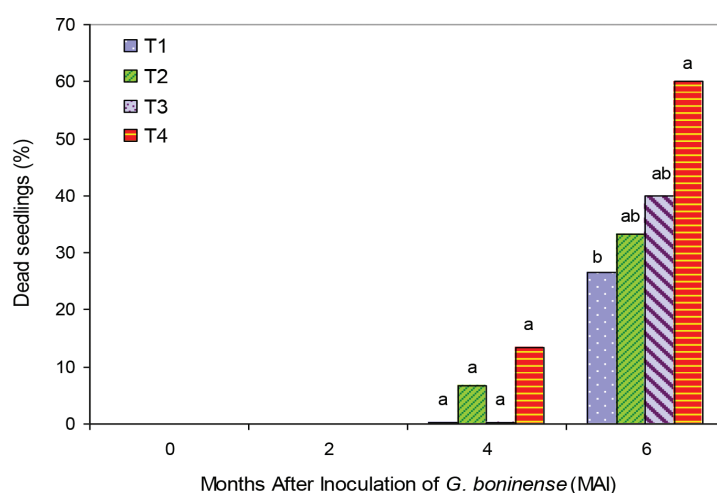[##] MAI = Months after artificially infected with *G. boninense*

Means with the same letters between treatments are not significantly different according to the least significant different (LSD) test at p=0.05

Treatments: T1: Seedlings treated with *P. aeruginosa* GanoEB1 + *G. boninense*; T2: Treated with *B. cepacia* GanoEB2 + *G. boninense*; T3: Treated with P. syringae GanoEB3 + *G. boninense*; T4: Seedlings untreated + *G. boninense*

FIGURE 4. Percentage severity of foliar symptoms (SFS) of oil palm seedlings due to *G. boninense* infection (Trial 2)

intercellular spaces or in the vascular system (Bell et al. 1995). Endophytic bacteria are internal colonizer of root systems; therefore, they are able to compete within the vascular systems, inhibiting pathogens for both nutrient and space for its proliferation. Disease suppression could be due to the induction of the host defence mechanisms, such as the formation of structural barriers like lignified cell walls and production of antifungal metabolites to slow down the infection progress (Hammerschmidt & Kuc 1995).

Seedlings treated with endophytes gave a lower value of DI (%) and SFS (%) throughout the experiment compared to untreated seedlings. This results indicated that some disease suppression by the endophytes resulted in the reduction of DI (%) compared to the control and this would be a degree of effectiveness in suppressing the disease. In both trials (1 and 2), *P. aeruginosa* GanoEB1 displayed a good potential as a biocontrol agent to suppress *Ganoderma* incidence in oil palm seedlings based on the percentage of DI (%) and SFS (%). In addition, the percentage of dead seedlings recorded for treatment with *P. aeruginosa* GanoEB1 was also found to be significantly lower than untreated control treatment, which exhibits the ability of this bacterium to reduce mortality of oil palm seedlings due to *G. boninense* infection. The ability of the bacterial endophytes to reduce *Ganoderma* symptoms was expressed as the percentage of DR derived from the values of AUDPC (Table 5). The AUDPC values suggest the amount of disease developed in each treatment where the treatment with the lowest AUDPC value indicates the effectiveness of the biocontrol in reducing the disease. The pre-inoculated seedlings with *P. aeruginosa* GanoEB1 gave the lowest AUDPC of 46.7 and 66.7 in Trials 1 and 2, respectively, followed by *B. cepacia* GanoEB2 (73.3 and 120, respectively) and *P. syringae* GanoEB3 (126.6 and 120, respectively). In addition, six months after artificially infected with *G. boninense*, *Ganoderma* disease incidence was reduced by 62-74% in seedlings treated with *P. aeruginosa* GanoEB1, followed by *B. cepacia* GanoEB2 (31-59%) and *P. syringae* GanoEB3 (30-31%). Therefore, based on the results obtained from both Trials (1 and 2), *P. aeruginosa* GanoEB1 was identified as the most effective EB in suppressing *Ganoderma* among the other endophytic bacteria tested. A field study is required to verify their effectiveness in controlling *Ganoderma* in oil palm.

Means with the same letters between treatments are not significantly different according to the least significant different (LSD) test at *p*=0.05
Treatments: T1: Seedlings treated with *P. aeruginosa* GanoEB1 + *G. boninense*; T2: Treated with *B. cepacia* GanoEB2 + *G. boninense*; T3: Treated with *P. syringae* GanoEB3 + *G. boninense*; T4: Seedlings untreated + *G. boninense*

FIGURE 5. Dead seedlings (%) due to *G. boninense* infection (Trial 2)

TABLE 5. The effect of endophytes on the development of *Ganoderma* disease in oil palm seedlings after artificially infected with *G. boninense* for 6 months

Treatment	Trial 1		Trial 2	
	AUDPC[1]	DR[2] (%)	AUDPC	DR (%)
T1: *P. aeruginosa* GanoEB1 + *G. boninense*	46.7	74	66.7	62
T2: *B. cepacia* GanoEB2 + *G. boninense*	73.3	59	120.0	31
T3: *P. syringae* GanoEB3 + *G. boninense*	126.7	30	120.0	31
T4: Untreated + *G. boninense*	180.0	-	173.3	-

[1]Area under disease progress curve (AUDPC) [2]Disease reductions (DR)

CONCLUSION

The use of biocontrol agent such as endophytic bacteria as an alternative way to combat *Ganoderma* disease is ideal option, apart from chemical and cultural control methods. Thus this study demonstrated the ability of three endophytic bacteria: *P. aeruginosa, B. cepacia* and *P. syringae,* isolated from symptomless oil palm root tissues to suppress *G. boninense* infection in oil palm seedlings. Biological control agents also offer an alternative tool for controlling *Ganoderma* disease besides cultivating a sustainable agricultural environment.

REFERENCES

Ariffin, D., Idris, A.S. & Singh, G. 2000. Status of *Ganoderma* in oil palm. In *Ganoderma Diseases of Perennial Crops*, edited by Flood, J., Bridge, P.D. & Holderness, M. Wallingford, United Kingdom: CAB International. pp. 49-68.

Ariffin, D., Idris, A.S. & Marzuki, A. 1996. Spread of *Ganoderma boninense* and vegetative compatibility studies of a single field palm isolates. In *Proceedings of the 1996 PORIM International Palm Oil Congress (Agriculture),* September 1996, edited by Ariffin, D., Basri, M.W., Jalani, B.S., Rajanaidu, N., Mohd Tayeb, D., Paranjothy, K., Chang, K.C. & Ravigadevi, S. Selangor, Malaysia: PORIM. pp. 317-329.

Ariffin, D. & Idris, A.S. 1991. A selective medium for the isolation of *Ganoderma* from diseased tissues. In *Proceedings of the 1991 International Palm Oil Conference, Progress, Prospects & Challenges Towards the 21st Century, September 1991,* edited by Basiron, B., Jalani, B.S., Chang, K.W., Cheah, S.C., Henson, I.E., Norman, K., Paranjothy, K., Mohd Tayeb, D. & Ariffin, D. Selangor, Malaysia: PORIM. pp. 517-519.

Azevedo, J.L., Maccheroni, J.W., Pereira, O.J. & Araujo, L.W. 2000. Endophytic microorganisms: A review on insect control and recent advance on tropical plants. *Journal of Biotechnology* 3: 40-65.

Bell, C.R., Dickie, G.A., Harvey, G.L.W. & Chan, F.Y.W.J. 1995. Endophytic bacteria in grapevine. *Canada Journal of Microbiology* 41: 46-53.

Bivi, M.R., Farhana, M.S.N., Khairulmazmi, A. & Idris, A.S. 2010. Control of *Ganoderma boninense*: A causal agent of basal stem rot disease in oil palm with endophyte bacteria *in vitro. International Journal of Agriculture & Biology* 12: 833-839.

Chen, C., Belanger, R.R., Benhamou, N. & Paulitz, T.C. 2000. Defense enzymes induced in cucumber roots by treatment with plant growth promoting rhizobacteria (PGPR) and *Pythium aphanidermatum. Physiological and Molecular Plant Pathology* 56: 13-23.

Chen, C., Bauske, E.M., Musson, G., Rodriquez-Kabana, R. & Kloeppe, J.W. 1995. Biological control of *Fusarium* wilt on cotton by use of endophytic bacteria. *Biological Control* 5: 83-91.

Clay, K. 1998. Fungal endophytes infection and the population biology of grasses. In *The Population Biology of Grasses,* edited by Cheplick, G.P. United Kingdom: Cambridge University Press. pp. 255-285.

Gomez, K.A. & Gomez, A.A. 1984. *Statistical Procedures for Agricultural Research.* 2nd ed. Chapter 2. New York: John Wiley & Sons. pp. 7-83.

Hallmann, J., Quadt-Hallmann, A., Mahaffee, W.G. & Kloepper, J.W. 1997. Bacterial endophytes in agricultural crops. *Canada Journal of Microbiology* 43: 895-914.

Hammer, P.E., Hill, D.S., Lam, S.T., van Pee, K.H. & Ligon, J.M. 1997. Four genes from *Pseudomonas fluorescens* that encode the biosynthesis of pyrrolnitrin. *Applied and Environmental Microbiology* 63(6): 2147-2154.

Hammerschmidt, R. & Kuc, J.A. 1995. *Induced Resistance to Disease in Plants.* Dordrecht, The Netherlands: Kluwer Academic Publishers. p. 182.

Homma, Y. & Suzui, T. 1989. Role of antibiotic production in suppression of radish damping-off by seed bacterization with *Pseudomonas cepacia. Annual Phytopathology Society Japan* 55: 643-652.

Idris, A.S., Kushairi, A., Ariffin, D. & Basri, M.W. 2006. *Techniques for Inoculation of Oil Palm Geminated Seeds with Ganoderma.* MPOB Information Series, MPOB TT No. 314. Selangor, Malaysia: MPOB.

Idris, A.S., Ismail, S. & Ariffin, D. 2005. *Reducing Risk of Ganoderma in Supply Palms.* MPOB Information Series, MPOB TT No. 260. Selangor, Malaysia: MPOB.

Idris, A.S., Ismail, S. & Ariffin, D. 2004a. *Innovative Technique of Sanitation for Controlling Ganoderma at Replanting.* MPOB Information Series, MPOB TT No. 213. Selangor, Malaysia: MPOB.

Idris, A.S., Ismail, S. & Ariffin, D. 2004b. *Prolonging the Productive Life of Ganoderma-Infected Palms with Hexaconazole.* MPOB Information Series, MPOB TT No. 214. Selangor, Malaysia: MPOB.

Idris, A.S., Ismail, S., Ariffin, D. & Ahmad, H. 2002. *Control of Ganoderma-infected Palm-Development of Pressure Injection and Field Applications.* MPOB Information Series No.148, MPOB TT No. 131. Selangor, Malaysia: MPOB.

Idris, A.S. 1999. Basal stem rot (BSR) of oil palm (*Elaeis guineensis* Jacq.) in Malaysia: Factors associated with variation in disease severity. PhD Thesis, Wye College University of London, United Kingdom (Unpublished).

Jacobs, M.J., Bugbee, W.M. & Gabrielson, D.A. 1985. Enumeration, location and characterization of endophytic bacteria within sugar beet roots. *Canadian Journal of Botany* 63: 1262-1265.

Khairudin, H. 1990. Basal stem rot of oil palm: Incidence, etiology and control. MSc Thesis, Universiti Putra Malaysia, Selangor, Malaysia (Unpublished).

Kobayashi, D.Y. & Palumbo, J.D. 2000. Bacterial endophytes and their effect on plant and uses in agriculture. In *Microbial Endophytes*, edited by Bacon, C.W. & White Jr. J.F. New York: Marcel Dekker. pp. 199-233.

Loh, C.F. 1976. Preliminary evaluation of some systemic fungicides for *Ganoderma* control and phytotoxity to oil palm. *Journal of Malaysian Agriculture* 32: 223-230.

Nega, A. 2014. Review on concepts in biological control of plant pathogens. *Journal of Biology, Agriculture and Healthcare* 4(27): 33-54.

Pan, M.J., Rademan, S., Kuner, K. & Hasting, J.W. 1997. Ultrastructural studies on colonization of banana tissues and *Fusarium oxysporum* f. sp. Cubense race 4 by the endophytic bacterium *Burkholderia cepacia. Journal of Phytopathology* 145: 479-489.

Rika Fithri, N.B., Aris Tri, W. & Nurita, T.M. 2014. Control activity of potential antifungal-producing *Burkholderia* sp. in suppressing *Ganoderma boninense* growth in oil palm. *Asian Journal of Agricultural Research* 8: 259-268.

Ramesh, R., Joshi, A.A. & Ghanekar, M.P. 2009. *Pseudomonads*: Major antagonistic endophytic bacteria to suppress bacterial wilt pathogen, *Ralstonia solanacearum* in the eggplant (*Solanum melongena* L.). *World Journal of Microbiology and Biotechnology* 25: 47-55.

Sapak, Z., Sariah, M. & Ahmad, Z.A.M. 2008. Effect of endophytic bacteria on growth and suppression of *Ganoderma boninense* infection in oil palm. *International Journal of Agriculture & Biology* 10: 127-132.

Sariah, M. & Zakaria, K. 2000. The use of soil amendments for the control of basal stem rot of oil palm seedling. In *Ganoderma Diseases of Perennial Crops*, edited by Flood, J., Bridge, P.D. & Holderness, M. Wallingford, United Kingdom: CAB International. pp. 88-99.

SAS. 1995. Guide to the use of PC-SAS Version 6.04 for DOS for Statistical Analysis. SAS Institute, Cary, North Carolina.

Schena, L., Nigro, F., Pentimone, I., Ligorio, A. & Ippolito, A. 2003. Control of postharvest rots of sweet cherries and table grapes with endophytic isolates of *Aureobasidium pullulans*. *Journal of Postharvest Biology and Technology* 30: 209-220.

Shaner, G. & Finney, R.F. 1977. The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. *Phytopathology* 67: 1051-1056.

Singh, G. 1991. *Ganoderma* - The scourge of oil palms in the coastal areas. *The Planter* 67: 421-444.

Soylu, S., Soylu, E.M., Kurt, S. & Ekici, O.K. 2005. Antagonistic potentials of rhizosphere-associated bacterial isolates against soil-borne diseases of tomato and pepper caused by *Sclerotinia sclerotiorum* and *Rhizoctonia solani*. *Pakistan Journal of Biological Sciences* 8: 43-48.

Susanto, A. 2009. Basal stem rot in Indonesia: Biology, economic importance, epidemiology, detection and control. In *Proceedings of the International Workshop on Awareness, Detection and Control of Oil Palm Devastating Diseases*, November 2009, edited by Kushairi, A., Idris, A.S. & Norman, K. Selangor, Malaysia: MPOB. pp. 58-89.

Tjamos, E.C., Tjamos, S.E. & Antoniou, P.P. 2010. Biological management of plant diseases: Highlights on research and application. *Journal of Plant Pathology* 92(4): 17-21.

Tuck, H.C. & Khairudin, H. 1997. Usefulness of soil mounding treatments in prolonging productivity of prime-aged *Ganoderma* infected palms. *The Planter* 73(854): 239-244.

Turner, P.D. & Gillbanks, R.A. 2003. Field diseases and disorders of oil palm. In *Oil Palm Cultivation and Management*. Kuala Lumpur: The Incorporated Society of Planter. pp. 682-700.

Zaiton, S. 2006. Bacterial endophytes from oil palm (*Elaeis guineensis*) and their antagonistic activity against *Ganoderma boninense*. MSc Thesis, Universiti Putra Malaysia, Selangor, Malaysia (Unpublished).

Zaiton, S., Sariah, M. & Zainal Abidin, M.A. 2006. Isolation and characterization of microbial endophytes from oil palm roots: Implication as biocontrol agents against *Ganoderma*. *The Planter* 82: 587-597.

Nur Rashyeda Ramli*, Idris Abu Seman & Madihah Ahmad Zairun
Malaysian Palm Oil Board (MPOB)
No. 6, Persiaran Institusi, Bandar Baru Bangi
43000 Kajang, Selangor Darul Ehsan
Malaysia

Maizatul Suriza Mohamed
University of Nottingham
Loughborough, Leicestershire, LE12 5RD
United Kingdom

Nasyaruddin Mohamad
The University of Sydney
NSW 2006
Australia

*Corresponding author; email: shyeda@mpob.gov.my