International Food Research Journal 23(6): 2616-2622 (December 2016)

Journal homepage: *http://www.ifrj.upm.edu.my*

Screening for antifungal-producing bacteria from *Piper nigrum* plant against *Phytophthora capsici*

Toh, S. C., *Samuel, L. and Awang, A. S. A. H.

Department of Molecular Biology, Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300 Kota Samarahan Sarawak, Malaysia

Article history

Received: 20 December 2015
Received in revised form: 10 March 2016
Accepted: 16 March 2016

Keywords

Phytophthora capsici
Endophytic bacteria
Antagonism assays
16S rDNA sequencing

Abstract

Piper nigrum, commonly known as black pepper, is one of the most important spice crops with high demand by the world market. However, diseases like foot rot and stem blight cause by *Phytophthora capsici* have become the important production constraints in black pepper industry. The frequent application of toxic fungicides to counter the diseases in pepper plantations has raised certain environmental issues. In order to mitigate the use of fungicides, biological approach to control *P. capsici* has been suggested. In this study, endophytic bacteria were isolated from six *P. nigrum* roots and screened for *in vitro* antagonistic activity against *P. capsici* through dual culture, mycelial growth, spore germination and double plate assay. The antagonism testing involved the secretion of volatile and diffusible bioactive compounds by the endophytic bacteria. Out of 19 isolates tested, two isolates DB(2)7 and SB(2)6 produced volatile bioactive compounds and these two isolates showed highest antagonism against *P. capsici* mycelia with the percentage of inhibition up to 47.63% and 43.33%, respectively. Diffusible compounds from isolates DB(2)7, DB(2)9 and SB(2)6 produced clear zones in spore germination test with radii measurements of 10.0-17.0 mm. Three isolates with promising antifungal activity were further characterised through 16S rDNA sequencing. The analysis of their sequences via National Center for Biotechnology Information (NCBI) suggests close identity towards *Enterobacter cancerogenus, Enterobacter cloacae* and *Enterobacter asburiae*. This research study demonstrated that these endophytic bacteria isolates are potentially to be used as biocontrol agent in pepper cultivation.

Introduction

Black pepper (*Piper nigrum* L.), synonym with the title "king of spices", is a type of flowering vine belong to the family Piperaceae which originate from Malabar's coast of Southern India (Ravindran, 2000; Augstburger *et al*., 2001; Nazeem *et al.*, 2008). It was spread by the Hindus trader and travellers to Malaysia and Indonesia. Nowadays, it is commercially cultivated in tropical areas including Malabar, Thailand, Malaysia, Indonesia, Brazil, Sri Lanka, Vietnam and People's Republic of China to support the world populations' demand for black pepper (Sivaraman *et al*., 1999; Augstburger *et al.*, 2001; Farhana *et al*., 2013). In Malaysia, pepper cultivation is concentrated in Sarawak (90%) with the remaining grown in Peninsular Malaysia (Johor and Melaka) and Sabah (Anita *et al.*, 2013). According to the report published by State Planning Unit, Chief Minister's Department of Sarawak in 2013, a total of 26,000 tonnes of white and black pepper were produced from 14,900 hectares of planted area in Sarawak (Farith *et al*., 2015). However, foot rot disease is still the main constraint in the black pepper production and it has been one of the main disease which cause an estimated about five to six million ringgit losses in export or 5-10% of annual crop loss in Sarawak alone (Kueh, 1990).

Phytophthora capsici is an oomycete plant pathogen that commonly regarded as causal agent of foot rot and stem blight in black pepper (Anandaraj and Sarma, 1995a; Babadoost, 2005; Nguyen, 2015). The foliage, leaves and fruits are susceptible to the *Phytophthora* infection. It is known as a type of virulent, hemi-biotrophic and wet weather pathogen that inflicted significant loses reported from western hemisphere, Asia and Europe (Anandaraj and Sarma, 1995a; Gevens *et al*., 2008; Lamour *et al*., 2012a, 2012b; Fisher et al., 2012). Relative humidity which is more than 79% with intermittent showers and low temperature are conducive for the initiation and spreading of *P. capsici*, thus *Phytophthora* diseases are prevalent during wet period of the year which may varies from countries to countries (Babadoost, 2005; Sarma *et al*., 2013).

The infection associated with *P. capsici* was first reported in Lampung, Indonesia in 1885 and then identified by Muller in 1936 (Drenth and Guest,

*Corresponding author.
Email: *lsamuel@frst.unimas.my*

2004; Sarma *et al*., 2013). Between November 2010 and January 2011, thirteen major pepper growing orchards located in four divisions of Sarawak state of Malaysia were surveyed for *Phytophthora* foot rot disease and the severity of infection incidence during an outbreak (Farhana *et al*., 2013). Ulu Sarikei in Sarikei had the highest disease incidence reported (75%) followed by Pasai Siong in Sibu (70%) and the lowest at Tatau in Bintulu (5%) (Farhana *et al*., 2013). Meanwhile, the highest disease severity was recorded at Ulu Sarikei (70%) followed by Pasai Siong (62%) and the lowest at Tatau (4%) (Farhana *et al*., 2013). The causal agent of foot rot disease of black pepper was confirmed as *P. capsici* Leonian.

Increased awareness of public on the deleterious effect of chemical fungicides to human health, environment and ecosystem had promoted the development of several safe management alternatives to control *Phytophthora* diseases (Anandaraj and Sarma, 1995b; Marín *et al*., 2014). In order to mitigate the application of fungicides, biological approach has been suggested. The objective of this study was to screen potential endophytic bacteria from *Piper nigrum* plant that can be used to inhibit the growth of *P. capsici.*

Materials and Methods

Root sample collection

Roots of *Piper nigrum* L. were collected from different *Piper nigrum* farms with *P. capsici* infection history within Kota Samarahan District, Sarawak, Malaysia in December 2013.

Isolation of endophytic bacteria

Approximately 100 g of root samples were weighted, washed with sterile distilled water, surface disinfected by soaking in 2% (v/v) sodium hypochlorite for 10 min, followed by washing with 70% (v/v) ethanol for one min and later rinsed five times with sterile distilled water. The samples were then grinded aseptically in phosphate buffered saline (PBS) solution (gL^{-1} NaCl 8, KCl 0.2, Na_2HPO_4 1.44 and KH_2PO_4 0.24, pH 7.4) and centrifuged at 60 x g at 4˚C for 1 min. The supernatants were serially diluted up to 10^{-5}, spread plated on tryptic soy agar (TSA) and then incubated at 28 ˚C for 4 days. The population of bacteria in the grinded root samples was expressed as colony forming units (CFU) of tissue. Five individual bacterial colonies from each agar plate were randomly selected and purified on TSA plate. A set of the pure culture was kept in slant agar stored at 4°C and another set was stocked in glycerol kept at -20°C.

Dual culture assay

Dual culture assay was performed as described by Živković *et al*. (2010) and Jeyaseelan *et al.* (2012) with slight modification. A total of 129 isolates of endophytic bacteria were streaked at approximately 1 cm from the edges of potato dextrose agar (PDA) plates, meanwhile a *P. capsici* culture on agar plug with the size of 1 cm^2 was placed at the opposite of the bacteria streaks. Plate containing the fungus without any bacteria inoculation was used as negative control. The cultures were incubated at 30°C for six days and the percentage inhibition of radial growth (PIRG) was calculated as follows:

$$PIRG\ (\%) = [(R1-R2) / R1] \times 100\% \ldots\ldots (1)$$

whereby, R1: The radial growth of *P. capsici* in control plate; R2: The radial growth of *P. capsici* towards the antagonist endophytic bacteria.

The percentages inhibition of radial growth were categorized from low to very high antifungal activity; <30% = low antifungal activity, 30 - <50% = moderate antifungal activity, 50 - <70% = high antifungal activity, ≥70% = very high antifungal activity. Those bacteria isolates with more than 30% of inhibition from this dual culture assay were selected and kept for further studies.

Mycelial growth test

Plugs of mycelium with approximately 1 cm^2 were taken from the edge of six-days-old culture of *P. capsici* and dipped into broth cultures of the selected bacteria isolates for 30 min (Edward *et al.*, 2013). The plugs were air dried in transparent chamber for 30 min. Each of the mycelia plugs was then transferred to the centre of PDA plates. *P. capsici* plug dipped in sterile distilled water served as negative control. The PDA plates were then incubated at 30°C for 4 days and the diameter of colony was measured after the incubation. Percentage inhibition of diameter growth (PIDG) was calculated as follows:

$$PIDG\ (\%) = [(D1-D2) / D1] \times 100\% \ldots\ldots (2)$$

Whereby, D1: Diameter growth of *P. capsici i*n control plate. D2: Diameter growth of *P. capsici* treated with endophytic bacteria.

Spore germination test

P. capsici spores from 2 days old potato dextrose broth (PDB) culture was spread over other potato dextrose agar (PDA) plates by swabbing and then allowed to dry at room temperature for 30 min. The

bacteria isolates were sub-cultured into nutrient broths and allowed to grow for 24 h. Double layers of sterile filter paper discs (0.6 cm in diameter) were inoculated with 65, 55 and 50 µl of bacterial cultures in a sterile petri discs. The discs were then transferred onto the PDA plates after being air dried for 30 min. Discs loaded with 65, 55 and 50 µl of un-inoculated nutrient broth (NB) served as controls. The radii of clearing zones around the discs were measured after 24, 48 and 72 hours after incubation at 30°C.

Double plate assay

Bacteria and *P. capsici* cultures were grown on NA and PDA plates for 1 and 3 days, respectively. NA plate without inoculation served as the control. The PDA plate was inverted over corresponding NA plate without cover lids, and the two plates were sealed together with a parafilm strip. The diameter growth of fungus on PDA plates were first measured and then incubated at 30°C for another 4 days. The growth inhibition of *P. capsici* by endophytic bacteria was assessed based on PIDG values shown in equation (2) by calculating the difference in diameter of fungus culture before and after sealing together the two plates.

Analysis

The bacteria isolates that fulfilled most of the antagonistic characteristics towards *P. capsici* from in vitro antagonistic screening test (>40% of PIRG value from dual culture assay; >40% of PIDG value from double plate assay; > 10% of PIDG value from mycelial growth test; >10 mm of radius forming clear inhibition zone) were chosen for further characterisation.

DNA extraction and PCR amplification

Bacterial DNA was extracted by boiled cell method as described by Freschi *et al*. (2005). The PCR was carried out using universal primer sets 27F and 519R in 50 µl of PCR reaction Master Mix containing 10 µl of 5× Green GoTaq ® Flexi buffer, 6 µl 25 mM MgCl2, 3 µl 10 mM dNTPs, 8 µl sterile distilled water, 20 µl sample of 20-40 ng DNA, 1 µl GoTaq® Flexi DNA polymerase, 1 µl each forward primer and reverse primer. The thermocycling conditions consist of an initial denaturation at 95°C for 10 min, 26 amplification cycles of 94°C for 30 s, 55°C for 1 min, 72°C for 1 min 30 s and a final polymerization step of 72°C for 10 min. Each reaction was analysed by agarose gel electrophoresis in 1% (w/v) agarose gel and ethidium bromide staining. The agarose gel was electrophoresed at 90 V with 200 mA for 30 min and then visualised under UV transilluminator.

16S rDNA gene sequencing and identification

DNA fragments in gel were purified using Qiagen QIAquick® Gel Extraction Kit (Kathleen *et al*., 2014). The purified DNA fragments were sent to 1st Base Laboratory Sdn. Bhd., Malaysia for sequencing. The sequences obtained were subjected to BLAST analysis and nucleotide sequence similarities were determined by comparing with National Center for Biotechnology Information (NCBI) databases.

Results and Discussion

Endophytic bacteria found in *Piper nigrum* are ubiquitous in most of the plant species (Hallmann *et al.*, 1997; Aravind *et al*., 2009). It can latently reside or actively colonise the plant tissue locally as well as systematically without showing external symptom or bad effect on the plant host (Carroll, 1988; Wilson, 1995; Ryan *et al.*, 2007).

The study on endophytic bacteria has shown that endophytes were able to prevent the disease development through endophyte-mediated de novo synthesis of certain compounds or metabolites with antimicrobial activity (Ryan *et al*., 2007; Samuel *et al*., 2014). Besides, some of the endophytic bacteria are considered mutualistic by stimulating the growth and development of hosts through synthesis of plant-growth-promoting hormone (Ahmad *et al*., 2008). Hence, further study on endophytic bacteria is needed to search for new bioactive compounds that can be used to fight against *Phytophthora* foot rot diseases in *P. nigrum* and at the same time can promote the growth and development of plants.

In this research study, a total number of 129 endophytic bacteria were isolated from the root of *P. nigrum* and all of the isolates were screened for their *in vitro* antagonistic activity against *P. capsici* through dual culture, mycelial growth, spore germination and double plate assay.

Dual culture assay

Dual culture assay was carried out as preliminary screening test to select the endophytic bacteria with high antagonistic effects toward *P. capsici* (Oldenburg *et al*., 1996). The information regarding the bioactive compound produced, such as specificity, potency or stability were obtained through this preliminary test (Oldenburg *et al*., 1996). The radial growth of *P. capsici* toward the endophytic bacteria is shown in Figure 1. From dual culture assay, only 19 bacteria isolates showing more than 30% of inhibition were chosen for further analysis. Bacterial isolates KDKS5-49, KRBR-15 and BR(1)6 were observed to have significantly higher antagonistic effect towards

Table 1. The calculated percentage of inhibition of radial growth of isolated bacteria

Culture label	Radial Growth (cm)	Inhibition length (cm)	PIRG value (%)
Control	6.2	-	-
KDKS5-49	3.20	3.00	48.39
KRBR-15	3.50	2.70	43.55
BR(1)6	3.70	2.50	40.32
HB(2)7	3.70	2.50	40.32
KRS6-84	3.70	2.50	40.32
DB(2)7	3.80	2.40	38.71
KRS6-24	3.80	2.40	38.71
SB(1)1	4.00	2.20	35.48
DB(2)8	4.00	2.20	35.48
DB(2)9	4.00	2.20	35.48
HR(2)6	4.10	2.10	33.87
SB(1)10	4.10	2.10	33.87
SB(2)6	4.10	2.10	33.87
HB(2)6	4.10	2.10	33.87
KDKBB-83	4.10	2.10	33.87
HR(1)8	4.20	2.00	32.26
BR(1)10	4.30	1.90	30.65
HR(1)9	4.30	1.90	30.65
HB(1)9	4.30	1.90	30.65

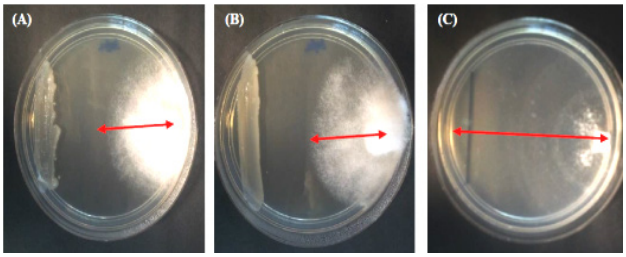

Figure 1. The inhibitory effects of endophytic bacteria isolates on *P. capsici* demonstrated by the clear zones between the bacteria and the fungal cultures. The red arrow shows the radial growth of the *P. capsici* after 7 days of incubation period. The PIRG values (%) were calculated by subtracting the radial growth in control plate with the bacteria treated plate to study the percentage of inhibition of the radial growth. Plate A, KDKS5-49; plate B, KRS6-84 and plate C, negative control.

P. capsici (48.39%, 43.55% and 40.32%) compared with the other 16 bacteria isolates as listed in Table 1. The result of the antagonistic effect of the bacteria against black pepper fungal disease in this study is comparable with study reported by Edward *et al.* (2013). In their study, mycelia growth inhibition value associated with *B. megaterium* and *B. cereus* against *Fusarium* wilt ranged from 41.0 - 42.6%.

Mycelia growth test

The mycelial growth test was carried out to study non-volatile bioactive compound produce by the bacteria isolates in broth media (Grover and Moore, 1962; Edward *et al*., 2013). Mycelial plugs were cut form the edge of the fungus by sterile forceps and directly dipped into the broth solution. This allowed non-volatile bioactive compound produced by the bacteria isolates to react antagonist directly on the mycelium plugs of *P. capsici* (Edward *et al*., 2013). Figure 2 shows the histogram of percentage of the inhibition by the bacterial isolates in the mycelial growth test after 4 days of incubation at 30°C. From the histogram, the highest PIDG value was produced by KDKBB-83 which is 30.19%, while the lowest was produced by HB(2)6 which is only 2.05%. This result indicates the presence of non-volatile bioactive compound in some of the inoculated broth solution which were able to inhibit mycelial growth of *P. capsici*. For isolate KDKS5-49, although this isolate showed the highest PIRG value under the dual culture assay, it produces less non-volatile bioactive compound which specifically target by the mycelial growth test. Hence, the results obtained were paradoxically from the dual culture assay. In a study to investigate potential biocontrol for *Verticillium* wilt fungal disease, Lin *et al.* (2013) found the maximal zone of fungistasis of 29 mm and 26 mm by two bacterial strains KDRE25 and KDRE01, respectively.

Spore germination test

The results of the spore germination test was analysed by observing the inhibition zones formed by endophytic bacteria towards *P. capsici*. The radii of the inihibition zones were measured from the centre of discs to the edge of the inhibition zone. The result was considered positive if inhibition zone is formed while the results was negative if the bacteria neither overgrown or could not produce inhibition zones. Figure 3 shows the radii of inhibition zone obtained in the spore germination test. The largest radii of inhibition zone obatined was 17 mm as shown by DB(2)7 and HB(2)6, while the shortest radii was recorded as 4 mm exhibited by isolate DB(2)8. The results were compared among the different bacterial

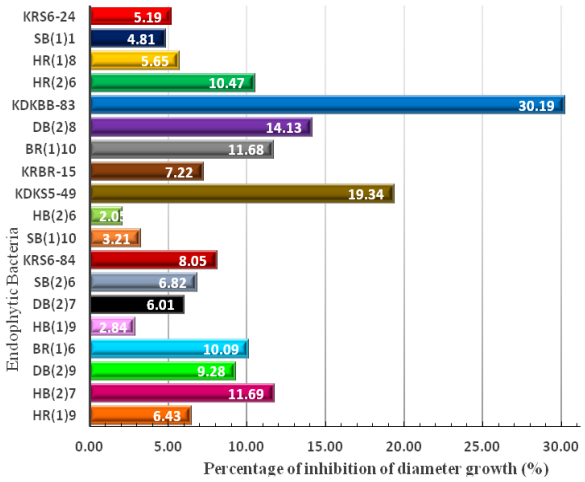

Figure 2. Histogram showing the isolated endophytic bacteria against percentage of inhibition of diameter growth (PIDG) value

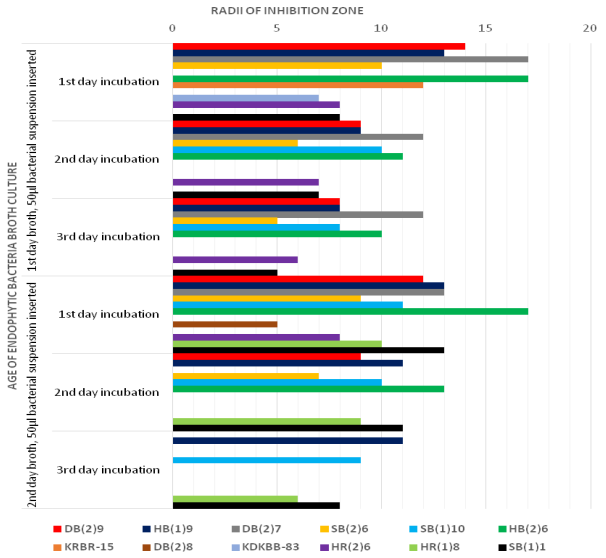

Figure 3. Histogram showing radii of inhibition zone in spore germination test. Majority of the endophytic bacteria cultures for example DB(2)9, HB(2)6 and DB(2)7 produced larger inhibition zone with lower bacteria cell concentration (1st day broth cultures) while minority of the endophytic bacteria for example SB(1)1, HR(1)8 and HB(2)6 produced larger inhibition zone with higher bacteria cell concentration (second day broth culture).

cultures age inoculated, incubationn period and volume of bacteria culture inoculated.

The spore germination test revealed strong inhibition ability of *P. capsici* by isolates DB(2)7, HB(2)6 and DB(2)9 with the radii of inhibition measured 17 mm, 17 mm and 14 mm, respectively. The result of the inhibition of the fungus in the spore germination test in this study is higher that study reported by Edward *et al.* (2013), where they reported inhibition zone ranged from 12.5 to 15 mm. Despite achieving higher PIRG value in dual culture assay, the bacteria isolates KDKS5-49, KRBR-15, BR(1)6, HB(2)7 and KRS6-84 showed limited or no suppression on the spore germination of the *P. capsici*. This contradictory results obtained might be attributed to the nature or chemical properties of bioactive compound produced by the bacteria isolates which might have low potency, stability and diffusible ability. Besides, the spore germination test was tested on the different volume and days of incubation of the bacterial inoculated onto the disk. It shows that the filter paper disk inoculated with higher volume of bacteria broth culture caused bacteria to overgrow and less positive results were obtained from the test. By reducing the volume inoculated into the filter paper disk, there were more positive results obtained from the test. On the other hand, the inhibition zone obtained by majority of the bacteria isolates were larger in size in the first day broth (lower bacteria concentration) compare to second day broth (higher bacteria concentration) as shown in Figure 3.

Double plate assay

The result of the double plate assay is displayed in Figure 4. By calculating the percentage of inhibition of diameter growth (PIDG) of the *P. capsici* after 4 days of incubation period at 30°C, DB(2)7 showed the highest PIDG value (47.63%) followed by SB(2)6 (43.33%) and KDKS5-49 (38.29%). This result indicates the ability of the isolates to synthesise and release volatile gas which aids in the inhibition of the growth of *P. capsici.* On the other hand, the negative result obtained by DB(2)8 (-20.58%), BR(1)6 (-8.58%) and HR(2)6 (-7.54%) from the double plate assay indicated that the bacteria isolates might produce the volatile gases that stimulate the growth of *P. capsici* (Figure 4).

The double plate assay was designed mainly to target and detect the volatile gas produced by the bacteria isolates. The antimicrobial compound in the form of volatile gas can move faster to far distance due to their physical properties compared to diffusible compound (Edward *et al.,* 2013). Besides, it has also been reported that the volatile compound were able to exhibit stronger antagonistic effects toward rhizospheric strains of *B. subtilis* and *Pseudomonas corrugate* compared with the diffusible compound (Sood *et al*., 2007).

16S rDNA gene sequencing and identification

Three endophytic bacteria isolates DB(2)7, SB(2)6 and DB(2)9 were chosen for the DNA sequencing. These bacteria isolates have moderately high PIRG value (more than 40%) from dual culture assay test, large radii of inhibition zone in spore germination test and large PIDG value from both the mycelial growth

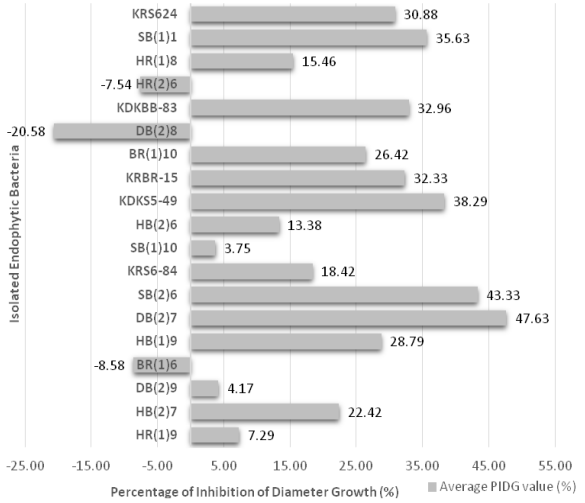

Figure 4. Histogram showing endophytic bacteria and their percentage of inhibition of diameter growth (PIDG) value

test and double plate assay. From NCBI database, the endophytic bacteria that have high similarity with the bacteria isolates from root samples were identified as *Enterobacter cancerogenus, Enterobacter cloacae* and *Enterobacter asburiae*. Nelson and Maloney (1992) reported that *Enterobacter cloacae* can inhibit the growth of seed rotting fungus *Pythium ultimum*. Pal and Gardener (2006) discovered volatile bioactive compound like ammonia produced by *Enterobacter cloacae* can suppressed *Pythium ultimum* that induced the damping-off of cotton. Besides, Rani *et al*. (2011) tested the activity of *Enterobacter cancerogenus* against pathogenic fungi *Macrophomina phaseolina* and *Fusarium udum* and they found positive antagonistic effects.

Conclusion

This research reported antifungal producing bacteria found living within the root samples of *P. nigrum* plant. A total of 19 endophytic bacteria isolated from the root samples of *P. nigrum* was tested positive in primary screening which can inhibit the growth of *Phytophthora capsici*. Mycelial growth test, spore germination test and double plate assay which specifically test the bacteria isolates on the direct antagonism of bioactive compound on the fungus, production of diffusible antifungal compound and production of volatile antifungal compound. Three bacteria were sequenced and identified as *Enterobacter cancerogenus, Enterobacter cloacae* and *Enterobacter asburiae*. These bacteria were able to produce various types of metabolites in liquid or volatile gaseous, therefore these bacteria isolates are potentially used as biocontrol agent in pepper cultivation.

Acknowledgement

This research project was funded by the Ministry of Education Malaysia through the Exploratory Research Grant Scheme (ERGS) vot no. ERGS/01(02)/852/2014(4).

References

Ahmad, F., Ahmad, I. and Khan, M.S. 2008. Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. Microbiological Research 163: 173-181.

Anandaraj, M. and Sarma, Y.R. 1995a. Diseases of black pepper (*Piper nigrum* L.) and their management. Journal of Spices and Aromatic Crops 4(1): 17-23.

Anandaraj, M. and Sarma, Y.R. 1995b. *Phytophthora* foot rot of black pepper. New Delhi, India: Indian Institute of Spices Research.

Anita, R., Alias, R. and Khalid, A.R. 2013. Technology adoption in pepper farming: a case study in Sarawak, Malaysia. The International Journal of Social Sciences 11(1): 16-22.

Aravind, R., Kumar, A., Eapen, S.J. and Ramana, K.V. 2009. Endophytic bacterial flora in root and stem tissues of black pepper (*Piper nigrum* L.) genotype: Isolation, identification and evaluation against *Phytophthora* capsici. Letters in Applied Microbiology 48: 58–64.

Augstburger, F., Berger, J., Censkowsky, U., Heid, P., Milz, J. and Streit, C. 2001. Organic farming in the tropics and subtropics: Pepper (2nd ed.). Germany: Naturlande. V.

Babadoost, M. 2005. *Phytophthora* blight of cucurbits. The Plant Health Instructor, DOI: 10.1094/PHI-I-2005-0429-01.

Carroll, G. 1988. Fungal endophytes in stems and leaves: From latent pathogen to mutualistic symbiont. Ecology 69(1): 2-9.

Drenth, A. and Guest, D.I. (Eds.). 2004. Diversity and management of *Phytophthora* in Southeast Asia. Australian Centre for International Agricultural Research. Monograph no. 114, 238p.

Edward, E.J., King, W.S., Teck, S.L.C., Jiwan, M., Aziz, Z.F.A., Kundat, F.R., Ahmed, O.H. and Majid, N.M.A. 2013. Antagonistic activities of endophytic bacteria against Fusarium wilt of black pepper *(Piper nigrum)*. International Journal of Agricultural and Biological 15: 291-296.

Farhana, S.N.M.D., Bivi, M.R., Khairulmazmi, A., Wong, S.K. and Sariah, M. 2013. Morphological and molecular characterization of *Phytophthora capsici*, the causal agent of foot rot disease of black pepper in Sarawak, Malaysia. International Journal of Agriculture and Biology 15: 1083-1090.

Farith, K., Awang, A.S.A.H, Samuel, L., Hasnain, M.H. and Hairul, A.R. 2015. *In vitro* antagonism of *Phytophthora capsici* and *Fusarium solani* by bacterial isolates from Sarawak. Malaysian Journal of Microbiology 11(2): 137-143.

Fisher, M.C., Henk, D.A., Briggs, C.J., Brownstein, J.S., Madoff, L.C., McCraw, S.L., Gurr, S.J. 2012. Emerging fungal threats to animal, plant and ecosystem health. Nature 484(7393): 186-194.

Freschi, C.R., Carvalho, L.F.O.S. and Oliveira, C.J.B. 2005. Comparison of DNA-extraction methods and selective enrichment broths on the detection of *Salmonella* Typhimurium in swine feces by polymerase chain reaction (PCR). Brazilian Journal of Microbiology 36: 363-367.

Gevens, A.J., Donahoo, R.S., Lamour, K.H. and Hausbeck, M.K. 2008. Characterization of *Phytophthora capsici* causing foliar and pod blight of snap bean in Michigan. Plant Disease 92(2): 201-209.

Grover, R.K. and Moore, J.D. 1962. Toximetric studies of fungicides against the brown rot organisms, *Sclerotinia fructicola* and *S. laxa*. Phytopathology Journal 52: 876-879.

Hallmann, J., Quadt-Hallmann, A., Mahaffee, W.F. and Kloepper, J.W. 1997. Bacterial endophytes in agricultural crops. Canadian Journal of Microbiology 43(10): 895-914.

Jeyaseelan, E.C., Tharmila, S. and Niranjan, K. 2012. Antagonistic activity of *Trichoderma* spp. and *Bacillus* spp. against Pythium aphanidermatum isolated from tomato damping off. Archives of Applied Science Research 4(4): 1623-1627.

Kathleen, M.M., Samuel, L., Felecia, C., Ng K.H., Lesley, M.B. and Kasing, A. 2014. (GTG)5-PCR analysis and 16S rRNA sequencing of bacteria from Sarawak aquaculture environment. International Food Research Journal 21(3): 915-920.

Kueh, T.K. 1990. Major diseases of black pepper and their management. The Planter 66(767): 59-69.

Lamour, K.H., Mudge, J., Gobena, D., Hurtado-Gonzales, O.P., Schmutz, J., Kuo, A. Kingsmore, S.F. 2012a. Molecular Plant-Microbe Interactions 25(10): 1350-1360.

Lamour, K.H., Stam, R., Jupe, J. and Huitema, E. 2012b. The oomycete broad-host-range pathogen *Phytophthora* capsici. Molecular Plant Pathology 13(4): 329-337.

Lin, T. X., Zhao, L.F, Yang, Y., Guan, Q. and Gong, M. 2013. Potential of endophytic bacteria isolated from *Sophora alopecuroides* nodule in biological control against Verticillium wilt disease. Australian Journal of Crop Science 7(1): 139-146.

Marín, F., Diánez, F., Santos, M., Carretero, F., Gea, F.J., Castañeda, C., Navarro, M.J. and Yau, J.A. 2014. Control of *Phytophthora* capsici and *Phytophthora* parasitica on pepper (*Capsicum annuum* L.) with compost teas from different sources, and their effects on plant growth promotion. Phytopathologia Mediterranea 53(2): 216−228.

Nazeem, P.A., Achuthan, C.R., Babu, T.D., Parab, G.V., Girija, D., Keshavachandran, R. and Samiyappan, R. 2008. Expression of pathogenesis related proteins in black pepper (*Piper nigrum* L.) in relation to *Phytophthora* foot rot disease. Journal of Tropical Agriculture 46 (1-2): 45–51.

Nelson, E.B. and Maloney, A.P. 1992. Molecular approaches for understanding biological control mechanisms in bacteria: Studies of the interaction of Enterobacter cloacae with *Pythium ultimum*. Canadian Journal of Plant Pathology 14(1): 106-114.

Nguyen, V.L. 2015. Spread of *Phytophthora* capsici in Black Pepper *(Piper nigrum)* in Vietnam. Engineering 7: 506-513.

Oldenburg, K.R., Vo, K.T., Ruhland, B., Schatz, P.J. and Yuan, Z. 1996. A dual culture assay for detection of antimicrobial activity. Journal of Biomolecular Screening 1(3): 123-130.

Pal, K.K. and Gardener, B.M. 2006. Biological Control of Plant Pathogens. The Plant Health Instructor. DOI: 10.1094/PHI-A-2006-1117-02.

Rani, M.U., Arundhathi and Reddy, G. 2011. Bacillus cereus and Enterobacter cancerogenus screened for their efficient plant growth promoting traits rhizobacteria (PGPR) and antagonistic traits among sixteen bacterial isolates from rhizospheric soils of Pigeon Pea. African Journal of Microbiology Research 5(15): 2090-2094.

Ravindran, P.N. 2000. Introduction. In P.N. Ravindran (Ed.). Black Pepper, Harwood Academy, pp. 1-23.

Ryan, R.P., Germaine, K., Franks, A., Ryan, D.J. and Dowling, D.N. 2007. Bacterial endophytes: Recent developments and applications. FEMS Microbiology Letters 278(1): 1-9.

Samuel, L., Chai, S. L., Ismail, A., Fazia, M. S., Ng, K. H. and Awang, A. S.H. 2014. Antimicrobial producing microbes isolated from soil samples collected from Nanga Merit Forest in Sarawak, Malaysian Borneo. European Journal of Experimental Biology 4(1): 494-501.

Sarma, Y.R., Manohara, D., Premkumar, T. and Eapen, S.J. (Eds.). 2013. Diseases and insect pests of black pepper (*Piper nigrum* L.). Jakarta, Indonesia: International Pepper Community (IPC).

Sivaraman, K., Kandiannan, K., Peter, K.V. and Thankamani, C.K. 1999. Agronomy of black pepper (*Piper nigrum* L.) - A review. Journal of Spices and Aromatic Crops 8(1): 1-18.

Sood, A., Sharma, S. and Kumar, V. 2007. Comparative efficacy of diffusible and volatile compounds of tea rhizospheric isolates and their use in biocontrol. International Journal of Biological and Chemical Sciences 1(1): 28-34.

Wilson, D. 1995. Endophyte - The evolution of a term, and clarification of its use and definition. Oikos 73(2): 274-276.

Živković, S., Stojanović, S., Ivanović, Ž., Gavrilović, V., Popović, T. and Balaž, J. 2010. Screening of antagonistic activity of microorganisms against *Colletotrichum acutatum* and *Colletotrichum gloeosporioides*. Archives of Biological Sciences, Belgrade 62(3): 611-623.