
Efficiency of *Bacillus Subtilis* EPB14 as Biocontrol to Control Bacterial Leaf Blight of Anthurium

Kumsingkaew, S.* and Akarapisan, A.

Department of Entomology and Plant Pathology, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand.

Kumsingkaew, S. and Akarapisan, A. (2014). Efficiency of *Bacillus subtilis* EPB14 as biocontrol to control bacterial leaf blight of anthurium. International Journal of Agricultural Technology 10(3):755-766.

Abstract Anthurium crops in northern Thailand were damaged by *Xanthomonas axonopodis* pv. *dieffenbachiae*, a casual agent of bacterial leaf blight of anthurium. *X. axonopodis* pv. *dieffenbachiae* TP04 from the affected anthurium leaf var Tropical showed bacterial leaf blight on anthurium and brown spots with yellow halo on spathiphyllum but could not exhibit the symptoms on dieffenbachia. Antagonistic bacterium EPB14 identified as *Bacillus subtilis*, showed the highest efficiency in antibiosis against the pathogenic bacterium. It could exhibit 10 mm radius of a clear inhibiting zone to suppress the growth of *X. axonopodis* pv. *dieffenbachiae* by using cell culture and revealed 6.25 mm radius of a clear inhibiting zone by using filtrated culture medium of the antagonistic bacterium. This strain EPB14 was able to produce activities by enzymes such as amylase (159.45×10^{-3} U/ml/minute, protease (54.18×10^{-3} U/ml/minute), and chitinase (36.57×10^{-3} U/ml/minute). Furthermore, the antagonistic bacterium EPB14 significantly reduced the disease severity of bacterial leaf blight on anthurium 50% in the greenhouse activity test.

Keywords: antagonistic bacterium, *Bacillus subtilis*, *Xanthomonas axonopodis* pv. *dieffenbachiae*, anthurium

Introduction

Anthuriums belong to the family Araceae, which has many genera, including anthurium, dieffenbachiae, aglaonema, epipremnum, xanthosoma, spathiphyllum, and philodendron (Higaki *et al.*, 1983 and Kamemoto *et al.*, 1988). The most well-known species is *Anthurium andraeanum* which is commercially important as cut flowers and which are available in the Hawaiian Islands, the Netherlands and the Philippine Islands. A serious disease problem affecting this species is bacterial leaf blight caused by *Xanthomonas axonopodis* pv. *dieffenbachiae* (previously, *Xanthomonas campestris* pv. *dieffenbachiae*). The disease has two stages: leaf infection and systemic

* **Corresponding author:** Kumsingkaew, S.; **E-mail:** vic_ms7@hotmail.com

infection on the anthurium. The symptoms of this disease caused by *X. axonopodis* pv. *dieffenbachiae* manifest themselves close to the leaf margin on the underside of the leaf as small star-shaped water-soaked spots, eventually causing some yellowing around the spots. Infection is usually through hydrathodes or wounds and occasionally through stomata (European and Mediterranean Plant Protection Organization, 2009). Biological control of bacterial pathogens has increased over the past decade, especially because of the importance attached to using environmentally friendly alternatives to the extensive use of chemical bacteriocides. Most of the bacterial strains that are used as biopesticides belong to the genera *Agrobacterium*, *Pseudomonas* and *Bacillus* (Fravel, 2005). Several species and strains of the genus *Bacillus* have been found to show antibacterial or antifungal activity against different phytopathogens (Katz and Demain, 1997; Yu *et al.*, 2002) They grow fast in culture and form resistant spores with high thermal tolerance, which makes these bacteria suitable candidates for use as biocontrol agents (Tanja *et al.* 2012). The chemical control of bacterial blight on anthurium is still not successful because of the pathogenic bacteria's serious resistance to antibiotics.

There are few publications that report biocontrol studies on the ability of antagonistic bacteria strains to inhibit the pathogens of anthurium blight. The purpose of this study is to isolate antagonistic bacteria from Araceae healthy plants and to characterize their efficiency for biological control on anthurium.

Materials and methods

Pathogen and pathogenicity test

Xanthomonas axonopodis pv. *dieffenbachiae* used in this study were isolated from five varieties of affected anthurium leaves in the laboratory of Plant Pathology, Faculty of Agriculture, Chiang Mai University. *X. axonopodis* pv. *dieffenbachiae* strain TP04 was the representative of four pathogenic bacteria that showed the highest virulence on anthurium and were identified by biochemical examination and characterized by following the guidelines provided by the European and Mediterranean Plant Protection Organization (2009), Lelliott and Stead (1987) and Chase *et al.* (1992). The pathogenicity of *X. axonopodis* pv. *dieffenbachiae* TP04 on anthurium var Tropical, spathiphyllum, and *dieffenbachiae* was observed by using 200 microliters of bacterial suspension of 1×10^8 concentration growth in distilled water injected near the leaf vein of the anthurium. Distilled water was used as the control. Inoculated plants were observed for the development of the symptoms.

Antagonistic bacteria and their activity in laboratory

Thirty three antagonistic bacteria were isolated from the root and leaf samples from healthy Aracea plants family (Anthurium, Dieffenbachiae, Philodendron and Pothos) from the Queen Sirikit Botanic Garden in Chiang Mai, Thailand. All the sterilized samples were cut into small pieces of area 5 mm² and put in 100 ml of distilled water in a flask. Thereafter, the flasks were shaken for 60 minutes and diluted to 10⁻¹–10⁻³ concentration with 9 ml distilled water in tubes before plating on sterile NA plates by spreading method.

After that, all the plates were incubated at 30 °C until the appearance of growth of colonies after incubation for 3 days. Antagonistic bacteria colonies were identified on the basis of morphological characteristics and biochemical tests, [as described in Bergey's Manual of Systemic Bacteriology by Sneath *et al.*, (1986), Tanja *et al.* (2012) and Gordon (1989). Subsequently, the antagonistic bacteria were tested for their inhibition and the ability to control *X. axonopodis* pv. *dieffenbachiae* TP04, using a dual culture method. The cell culture and the filtrated culture medium of the antagonistic bacteria were dipped into 5 mm diameter of autoclaved filter paper disk and added on the surface of the NA plates pre-inoculated with *X. axonopodis* pv. *dieffenbachiae* TP04 by spreading 200 microliters of the pathogen cell suspension, and the distilled water dipped the autoclaved filter paper without the addition of the antagonistic bacteria was used as the control. The final Petri dishes were incubated at 30 °C for 72 hours. Four replicate plates were tested in all the methods. The maximum efficiency of the antagonistic bacteria was evaluated by measuring radius of their clear inhibition zone against *X. axonopodis* pv. *dieffenbachiae* TP04.

Assay of extracellular enzymes activity

Amylase activity

Amylase activity was measured with one milliliter of inoculums grown for 48 hours and cultivated in 250 ml Erlenmeyer flasks containing 100 ml of amylase production medium using the method propounded by Asgher *et al.* (2007) and then shaken at 150 rpm in a shaker incubator at 37 °C for 72 hours. After the incubation, the fermented broth was centrifuged in a refrigerated centrifuge machine at 8000 rpm for 15 minutes at 4 °C.

The amylase activities was determined by using soluble starch 1% (w/v) as substrate in 0.05 M sodium phosphate buffer pH 6.5. The reaction mixture containing one milliliter of substrate solution and one milliliter of enzyme solution was incubated at 40 °C for 10 minutes. The reaction was stopped by

adding three milliliters of dinitrosalicylic acid solution. The reducing sugar was determined by the method given by Miller (1959). The absorbance was measured at 540 nm with a spectrophotometer. One unit (U) of amylase activity is defined as the amount of enzyme releasing one microgram of reducing sugar as glucose per minute, under the standard assay conditions.

Protease activity

Protease activity was measured with one milliliter of antagonistic grown for 48 hours in nutrient broths and cultivated in 250 ml Erlenmeyer flasks containing 100 ml of protease production medium by using the method suggested by Usharani and Muthuraj (2010), and then shaken at 150 rpm in a shaker incubator at 37 °C for 72 hours. After the incubation, the fermented broth was centrifuged in a refrigerated centrifuge machine at 8000 rpm for 20 minutes at 4 °C.

The protease activity was determined by using one milliliter of 2% casein (w/v) as substrate in one milliliter of 0.05 M NaOH buffer (pH 7.5). The reaction mixture containing two milliliters of the substrate solution and one milliliter of the enzyme solution was incubated at 50 °C for 30 minutes. The reaction was stopped by adding one milliliter of 10% trichloroacetic acid (TCA) and then centrifuged at 8000 rpm for 10 minutes. The absorbance was measured at 660 nm with a spectrophotometer using the Lowry method as modified by Peterson (1997). One protease unit is defined as the amount of enzyme that releases one microgram of tyrosine per ml per minute under the standard assay conditions.

Chitinase activity

Chitinase activity was measured by using one milliliter of antagonistic grown for 48 hours in nutrient broths and cultivated in 250 ml Erlenmeyer flasks containing 100 ml of chitinase production medium using the method given by Naradorn (2011) and were shaken at 150 rpm in a shaker incubator at 37 °C for 72 hours. After the incubation, the fermented broth was centrifuged in a refrigerated centrifuge machine at 8000 rpm for 20 minutes at 4 °C.

The chitinase activity was determined by using colloidal chitin 1% (w/v) one milliliter as substrate in one milliliter of 0.05 M NaOH buffer (pH 7). The reaction mixture containing two milliliters of the substrate solution and one milliliter of the enzyme solution was incubated at 50 °C for 30 minutes, and the reaction was terminated by adding one milliliter of dinitrosalicylic acid (DNS) solution and then incubated in a boiling water bath for 10 minutes until the development of the color of the end product. The reducing sugar released was

determined by using the method propounded by Miller (1959). The absorbance was measured at 540 nm with a spectrophotometer. The chitinase activity was determined using N-acetylglucosamine as the standard. One unit (U) of chitinase activity is defined as the amount of enzyme releasing one micromole of N-acetylglucosamine per ml per minute under the standard assay conditions.

Antagonistic activity in greenhouse

The effect of the antagonistic bacteria was tested by spraying cell suspension onto the leaves of anthurium plants infected with *X. axonopodis* pv. *dieffenbachiae* TP04. Antagonistic bacteria isolates B6, EPB13, and EPB14 were sprayed uniformly onto every leaf, three milliliters per leaf. After 3 days, the treated anthurium were inoculated with *X. axonopodis* pv. *dieffenbachiae* TP04. Two hundred microliters of cell suspension of bacterial pathogen of approximately 10^8 cfu/ml was dropped onto 3 mm diameter wounds by puncturing with a sterile needle. The inoculated anthuriums were incubated for 3 days. After 3 days of incubation, the anthuriums were inoculated only with the same density of cell suspension of *X. axonopodis* pv. *dieffenbachiae* TP04 as the positive control. Four treatments were conducted: inoculating control with *X. axonopodis* pv. *dieffenbachiae* TP04 as pathogen strain, spraying anthurium leaves with B6 (antagonistic bacterium in a laboratory culture), *Bacillus subtilis* EPB13 and *B. subtilis* EPB14 [Each treatment was observed for the development of symptoms after the inoculation of the bacterial leaf blight pathogen for 6 weeks]. All the experiments were conducted in sixteen replicates.

Statistical analysis

All the data were analyzed with the least significant difference values, at $P \leq 0.05$, by using the statistix 8.0 software.

Results

The main symptom of bacterial leaf blight on anthurium is the appearance of irregular, water-soaked spots. After that, spots become bright yellow, and then become brown or black spots with a yellow halo. In this study, the anthurium leaves inoculated with *X. axonopodis* pv. *dieffenbachiae* TP04 showed the typical symptoms of bacterial blight with a halo approximately 4–5 days after inoculation, and 21 days after inoculated it was found that the pathogen had brought about a development in the symptom, to leaf blight on anthurium leaves (Figure 1A). The pathogen could develop typical symptoms on spathiphyllum (another species of host family Araceae) with a brown or

black spot, with a yellow halo, approximately 4–5 days after inoculating with the pathogen for 21 days (Figure 1B); however, the pathogen could not show the typical symptoms on dieffenbachia (Figure 1C).

A total of 33 antagonistic bacteria were screened against *X. axonopodis* pv. *dieffenbachiae* isolate TP04. The cell culture of the antagonistic bacteria isolates EPB13 and EPB14 showed the efficiencies that ranged within 7.50–10.00 mm radius of the clear inhibiting zone not different from the efficiency obtained with the antagonistic B6 of the bacteria in a laboratory culture, Department of Entomology and Plant Pathology, Faculty of Agriculture, Chiang Mai University.

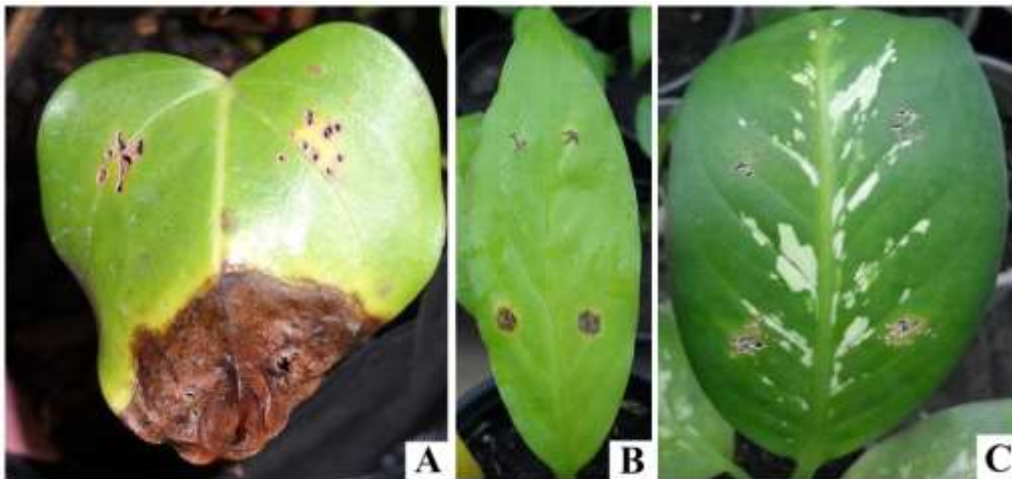


Fig. 1. The symptoms produced on Araceae plants by the *Xanthomonas axonopodis* pv. *dieffenbachiae* strain TP04: (A) Leaf blight on anthurium leaf 21 days after inoculation; (B) Brown spot and yellow halo on spathiphyllum 21 days after inoculation; (C) Lesions on dieffenbachiae 21 days after inoculation.

The antagonistic bacterium EPB14 showed the highest inhibiting activity which was produced 10 mm in radius of a clear inhibiting zone to suppress *X. axonopodis* pv. *dieffenbachiae* TP04 growth. The antagonistic bacterium EPB13 and the antagonistic bacterium EPB14 were identified as *Bacillus subtilis* according to the description provided in Bergey's Manual of Systematic Bacteriology (Sneath *et al.*, 1986). Ten microliters of the filtrated culture media of *Bacillus subtilis* EPB13 and EPB14 could produce 5.50–6.25 mm in radius of a clear inhibiting zone on *X. axonopodis* pv. *dieffenbachiae* TP04 growth. Paper disk dipped in distilled water as the control did not show a clear inhibiting zone with the same assay. *Bacillus subtilis* EPB13 and EPB014 were found to be the most effective in exhibiting a clear inhibiting zone to

suppress the *X. axonopodis* pv. *dieffenbachiae* isolate TP04 growth detected during the period of 2-6 days (Table 1).

The production of extracellular enzymes from *Bacillus subtilis* EPB13 and EPB14 was carried out in order to compare with the antagonistic B6 in a laboratory culture, at the Faculty of Agriculture, Chiang Mai University. There was no significant difference in the product of amylase and protease enzymes between the three isolates. Amylase production from the isolates EPB14, B6, and EPB13 were 159.45×10^{-3} U/ml/minute, 146.77×10^{-3} U/ml/minute, and 145.23×10^{-3} U/ml/minute, respectively. Protease production from the isolates EPB13, EPB14, and B6 were 55.41×10^{-3} U/ml/minute, 54.18×10^{-3} U/ml/minute, and 49.26×10^{-3} U/ml/minute, respectively. But chitinase production from the isolate EPB13 demonstrated the highest activity when compared with those from the isolates EPB14 and B6. The chitinase production from the isolates EPB13, EPB14, and B6 were 158.09×10^{-3} U/ml/minute, 36.57×10^{-3} U/ml/minute, and 27.89×10^{-3} U/ml/minute, respectively (Figure 2).

Table 1. Growth inhibition of *Xanthomonas axonopodis* pv. *dieffenbachiae* isolate TP04 by cell culture and filtrated culture medium of antagonistic bacteria *Bacillus subtilis* B6, EPB13 and EPB14 using paper disk method

| Treatment | Inhibition zone (mm) ¹ | | | | | | |
|-----------------------|-----------------------------------|---------------------------|---------|---------|-----------|-----------|---------|
| | Cell culture | Filtreated culture medium | | | | | |
| | | 2 days | 4 days | 6 days | 8 days | 10 days | 12 days |
| B6 | 10.75 A ² | 5.50 abc ³ | 6.00 ab | 6.13 a | 4.25 cd | 4.13 d | 4.00 d |
| EPB13 | 7.50 A | 6.13 a | 6.25 a | 6.25 a | 5.13 abcd | 4.75 bcd | 3.88 d |
| EPB14 | 10.00 A | 5.63 ab | 5.88 ab | 6.00 ab | 5.00 abcd | 5.00 abcd | 4.25 cd |
| CV (%) | 34.82 | 16.50 | | | | | |
| LSD _(0.05) | 5.2446 | 1.3567 | | | | | |

¹Results are the mean of four replicates.

²Mean values with the same letters in the columns are not significantly different ($P < 0.05$ LSD).

³Mean values with the same letters in the columns and rows are not significantly different ($P < 0.05$ LSD).

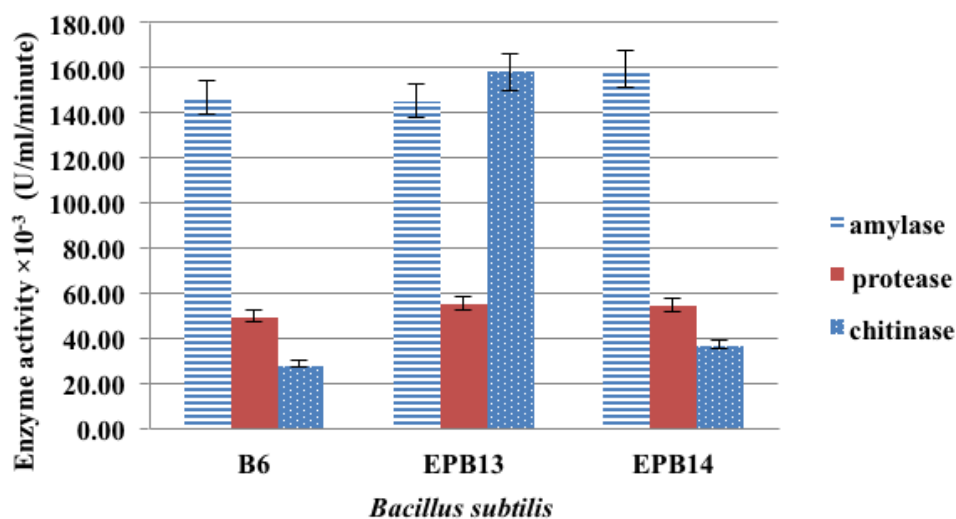


Fig. 2. The comparison of extracellular enzyme productions of *Bacillus subtilis*. The average activities of the three enzymes per minute are represented.

The results of the greenhouse experiment indicated and found out the significant differences between the three antagonistic bacteria isolates. *Bacillus subtilis* EPB14 showed the highest efficiency of inhibition against the bacteria leaf blight pathogen, thus reducing its percentage of disease severity to 50%, whereas the reduced percentages of disease severity of the isolate EPB13 and the isolate B6 were 71.25% and 75%, respectively. The percentages of reduction of the disease severity of the isolates EPB13 and B6 indicates that there were no significant differences between their efficiencies (Table 2).

Table 2. Effect of antagonistic bacteria on reducing the disease severity of bacterial leaf blight on anturium under greenhouse conditions

| Bacterial strain | Disease severity (%) ¹ |
|-----------------------|-----------------------------------|
| Inoculated control | 100.00 a ² |
| B6 | 75.00 b |
| EPB13 | 71.25 bc |
| EPB14 | 50.00 c |
| CV (%) | 43.67 |
| LSD _(0.05) | 22.875 |

¹Means of sixteen replications.

²Means in the same column followed by the letter did not differ significantly at $P \leq 0.05$.

Discussion

Xanthomonas axonopodis pv. *dieffenbachiae* causes the bacterial leaf blight infection to affect the various plant species from the Araceae family (Schoolenberger, 2005). In the present research, it was demonstrated that *X. axonopodis* pv. *dieffenbachiae* TP04 could infect Anthurium and Spathiphyllum (Figure 1).

The results from our investigation reveal the efficiency of the antagonistic bacteria in the thirty three species that were isolated from healthy araceae plants against *X. axonopodis* pv. *dieffenbachiae* TP04. The results of the cell culture from the isolates EPB14 and EPB13 on the suppression of *X. axonopodis* pv. *dieffenbachiae* TP04 were 10.00 mm and 7.50 mm radii of clear inhibiting zones, respectively, and those of the filtrated culture medium on the suppression of the pathogen from the isolates EPB14 and EPB13 were 6.25 mm and 5.63 mm radii of clear inhibiting zones. The antibacterial activity of each isolate is shown in Table 1. According to Moyne *et al.* (2001), it was found that the *B. subtilis* strain AU195 could produce antifungal peptides showing similarity with bacillomycin (group iturin A); also, Lipopeptides such as Kurstakin from *B. thuringiensis kurstaki* HD-1 was found to inhibit the fungal growth (Hathout *et al.*, 2000). Arias *et al.* (2009) found that *B. amyloliquefaciens* GA1 produced antibiotics and other secondary metabolites for the biocontrol of plant pathogens such as surfactin, fengycin, iturin A, macrolactin, difficidin, Bacillaene, and Chlorotetaine. *Bacillus* had mechanisms for the suppression of pathogens, including mycoparasitism, competition, and antibiosis by producing lipopeptides from the surfactin, iturin, and fengycin (Bonmatin *et al.*, 2003). Ongena *et al.* (2007) found that the surfactin and fengycin lipopeptides from the *B. subtilis* strain 168 was associated with inducing systemic resistance in tomato cell and protective effect in bean plants as signals for initiating defense mechanism.

The antagonistic activity described by Lee *et al.* (2008) that *B. subtilis* isolates R33 from rhizosphere soil was found to have exhibiting effect in controlling *Phytophthora capsici* in laboratory and under greenhouse conditions. It showed good antagonistic activity in inhibiting *P. capsici* growth with an inhibition zone radius of 12 mm in laboratory upon the use of the dual culture method. It exhibited high reduction in disease severity under greenhouse conditions and also produced the siderophores HCN, IAA, phosphatase, and ACC-deaminase. According to Wang and Liang (2014), it was found that the endophytic bacteria *B. amyloliquefaciens* strain BZ6-1 isolated from healthy peanut plants demonstrated the highest antagonistic effects against *Ralstonia solanacearum*, a causal agent of bacterial peanut wilt. It generated an inhibiting zone of 34.2 mm diameter in the dual culture plates assay and a reduction in the

percentage of disease severity of 12.1% from the control. The analyzed antimicrobial substances from the *B. amyloliquefaciens* strain BZ6-1 using high performance liquid chromatography showed that surfactin and fengycin were the main substances.

The results obtained in the greenhouse activity test showed that significant differences were observed between the studied isolates of *B. subtilis* in their efficiency to suppress *X. axonopodis* pv. *dieffenbachiae* TP04 growth in laboratory and to reduce disease severity under greenhouse conditions. *B. subtilis* EPB14 showed the highest efficiency in reducing the disease severity of bacterial leaf blight, at 50% (Table 2). According to Salerno and Sagardoy (2003), it was found that *B. subtilis* 210 was resistant to rifampicin at 20 µg ml⁻¹ and showed the highest degree of antibiosis against *Xanthomonas campestris* pv. *glycines* under greenhouse conditions. Soybean leaf surfaces treated with *B. subtilis* stain 210 before inoculating with the pathogenic bacteria reduced the lesion of bacterial pustules by *X. campestris* pv. *glycines*. Monterio *et al.* (2013) demonstrated the efficiency of the *B. subtilis* strain metabolites by performing antibiosis test to prove its efficiency in the inhibition against *Sclerotinia sclerotiorum* on lettuce. *B. subtilis* showed a decrease in the disease severity by 16.33% in the antibiosis test and an 83.33% reduction in the *Sclerotinia* mycelial growth.

Bacillus species produce extracellular enzymes (Kumar *et al.*, 2011), ribosomally synthesized peptides, and other unusual antibiotic peptides such as the rhizoctin, L-amino acid ligase from *B. subtilis* NBRC3134 (Kino *et al.*, 2009). The extracellular cell wall degrades the enzymes excreted by the many strains of *Bacillus*, due to their physical interactions (Yu *et al.*, 2002). Kumar *et al.* (2012) found the cell wall degrading enzymes as chitinase, β-1, 3-glucanase, protease and cellulase from the rhizospheric *Bacillus* spp. and Praveen *et al.* (2010) found both of the antibiotic and lytic enzymes from the *Bacillus* species suppressing the growth of soil borne plant pathogens. The results of the enzyme production activity showed that *B. subtilis* EPB13 and EPB14 also produce amylase, protease and chitinase. (Figure 2).

In conclusion, it can be stated that the results of this study show that *B. subtilis* EPB14 demonstrates the best antimicrobial activity against the pathogen as a biological agent in controlling this disease.

Acknowledgements

This research was financially supported by the Graduate School, Chiang Mai University, Chiang Mai, Thailand.

References

- Asgher, M., Asad, M. J., Rahman, S. U., Legge, R. L. (2007). A thermostable α -amylase from a moderately thermophilic *Bacillus subtilis* for starch processing. *Journal of Food Engineering* 79:950-955.
- Ashwini, N. and Srividya, S. (2013). Potentiality of *Bacillus subtilis* as biocontrol agent for management of antracnose disease of chilli caused by *Colletotrichum gloeosporioides* OGC1. *Biotechnology*. Retrieved from <http://link.springer.com/article/10.1007%2Fs13205-013-0134-4>
- Bonmatin, J. M., Laprevote, O. and Peypoux, F. (2003). Diversity among microbial cyclic lipopeptides: iturins and surfactins. Activity–structure relationships to design new bioactive agents. *Combinatorial Chemistry and High Throughput Screening* 6:541–556.
- Chase, A. R., Stall, R. E., Hodge, N. C. and Jones, J. B. (1992). Characterization of *Xanthomonas campestris* strains from aroids using physiological, pathological and fatty acid analysis. *Phytopathology* 82:754-759.
- European and Mediterranean Plant Protection Organization (2009). *Xanthomonas axonopodis* pv. *Dieffenbachiae*. *Eppo bulletin* 39:393-402. Retrieved from <http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2338.2009.02327.x/pdf>
- Fravel, D. R. (2005). Commercialization and implementation of biocontrol. *Annual Review of Phytopathology* 43:337-359.
- Gordon, R. E. (1989). The genus *Bacillus*; in practical handbook of microbiology. In O’Leary, W.M. CRC Press Inc. pp. 109-126.
- Hathout, Y., Ho, Y. P., Ryzhov, V., Demirev P., and Fenselau, C. (2000). Kurstakins: a new class of lipopeptides isolated from *Bacillus thuringiensis*. *Journal of Natural Product* 6:1492-1496.
- Higaki, T., Watson, D. P., and Leonhardt, K. W. (1983). Anthurium culture in Hawaii. Cooperative Extension Service circular 420, College of Tropical Agriculture and Human Resources, University of Hawaii, Manoa.
- Kamemoto, H. (1988). History and development of anthuriums in Hawaii. Proceedings of the Anthurium Blight Conference, Hawaii Institute of Tropical Agriculture and Human Resources, University of Hawaii, Honolulu. pp. 4-5.
- Katz, E., Demain, A. L. (1977). The peptide antibiotics of *Bacillus*: Chemistry, biogenesis, and possible functions, *Bacteriological Reviews* 41:449-474.
- Kino, K., Kotanaka, Y., Arai, T. and Yagasaki, M. (2009). A novel l-amino acid ligase from *Bacillus subtilis* NBRC3134, a microorganism producing peptide-antibiotic rhizocin. *Bioscience biotechnology and biochemistry* 73:901-907.
- Kumar, A., Prakash, A. and Johri, B. N. (2011). *Bacillus* as PGPR in crop ecosystem. *Crop Ecosystems*. 434 pp.
- Kumar, P., Dubey, R. C. and Maheshwari, D. K. (2012). *Bacillus* strains isolated from rhizosphere showed plant growth promoting and antagonistic activity against phytopathogens. *Microbiological Research* 167:493-499.
- Lee, J. K., Kannan, S. K., Sub, H. S., Seong, C. K. and Lee, G. W. (2008). Biological control of phytophthora blight in red pepper (*Capsicum annuum* L.) Using *Bacillus subtilis*. *World Journal of Microbiology and Biotechnology* 24:1139-1145.
- Lelliott, R. A. and Stead, D. (1987). *Methods for the diagnosis of bacterial diseases of plants*. Blackwell, Oxford.

- Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry* 31:426-428.
- Moyne, A. L., Shelby, R., Cleveland, T. E. and Tuzun, S. (2001). Bacillomycin D: an iturin with antifungal activity against *aspergillus flavus*. *Journal of Applied Microbiology* 90:622–629.
- Monterio, F. P., Ferreira, L. C., Pacheco, L. P. and Souza, P. E. (2013). Antagonism of *bacillus subtilis* against *sclerotinia sclerotiorum* on *lactuca sativa*. *Journal of Agricultural Science* 5:214-223.
- Naradorn, C. (2011). Screening and isolation of chitinase gene from entomopathogenic fungi for enhancing the infection of diamondback moth larvae. Graduate school, Chiang Mai University. 140 pp.
- Ongena, M., Jourdan, E., Adam, A., Paquot, M., Brans, A., Joris, B., Arpigny, J. L. and Thonart, P. (2007). Surfactin and fengycin lipopeptides of *bacillus subtilis* as elicitors of induced systemic resistance in plants *environmental microbiology* 9:1084-1090.
- Peterson, G. L. (1977). A simplification of the protein assay method of lowry *et al.* Which is more generally applicable. *Analytical Biochemistry* 83:346-356.
- Praveen, K. D., Singh R. K., Kumar, S., Srivastava, A. K. and Arora, D. K. (2010). Isolation of chitinolytic bacteria from different agro climatic regions of india and characterization of their PGPR activity, potential in antifungal biocontrol. *Plant growth promotion by rhizobacteria by rhizobacteria for sustainable agriculture*.
- Scollenberger, M. (2005). Bacterial leaf spot of philodendron. *Polish Phytopathological Society* 35:103–108.
- Sneath, P. H. A., Mair, N. S., Sharpe, M. E. and Holt, J. G. (1986). *Bergey's manual of systematic bacteriology*. 2 pp.
- Salerno, C. M. and Sagador, M. A. (2003). Short communication: antagonistic activity by *bacillus subtilis* against *xanthomonas campestris* pv. *glycines* under controlled condition. *Spanish Journal of Agricultural research* 1:55-58.
- Tanja, B., Milan, K., Slavisa, S., Ljubisa, T., Giuliano, D., Micheal, M., Vittorio, V. and Djordje, F. (2012). Antimicrobial activity of *bacillus* sp. natural isolates and their potential use in the biocontrol of phytopathogenic bacteria. *Food Technology and Biotechnology* 50:25-31.
- Usharani, B. and Muthuraj, M. 2010. Production and characterization of protease enzyme from *bacillus laterosporus*. *African Journal of Microbiology Research* 4:1057-1063.
- Wang, X. B. and Liang, G. B. (2014). Control efficacy of an endophytic *bacillus amyloliquefaciens* strain bz6-1 against peanut bacterial wilt, *ralstonia solanacearum*. *Journal of Biomedicine and Biotechnology*. Retrieved from <http://dx.doi.org/10.1155/2014/465435>
- Yu, G. Y., Sinclair, J. B., Hartman, G. L. and Bertagnolli, B. L. (2002). Production of iturin a by *bacillus amyloliquefaciens* suppressing *rhizoctonia solani*, *soil biology and biochemistry* 34:955-963.

(Received 12 March 2014; accepted 30 April 2014)