Journal of Agricultural Technology 2012 Vol. 8(2): 725-737
Available online http://www.ijat-aatsea.com
ISSN 1686-9141

Antifungal activity of soil actinomycetes to control chilli anthracnose caused by *Colletotrichum gloeosporioides*

Nisakorn Suwan[1,2], Wassamon Boonying[1,2] and Sarunya Nalumpang[1,2*]

[1]Department of Entomology and Plant Pathology, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand, [2]Center of Excellence on Agricultural Biotechnology (Ag-BIO/PERDO-CHE), Bangkok 10900, Thailand

Nisakorn Suwan, Wassamon Boonying and Sarunya Nalumpang (2012) Antifungal activity of soil actinomycetes to control chilli anthracnose caused by *Colletotrichum gloeosporioides*. Journal of Agricultural Technology 8(2): 725-737.

One-hundred and ninety one isolates of actinomycetes were screened for antifungal activity against anthracnose of long cayenne chilli pepper causing *C. gloeosporioides*. With this, six effective isolates of soil actinomycetes shwoed their potent antagonistic efficiency against *C. gloeosporioides*. Those isolates were belong to genus *Streptomyces* based on morphology and chemotaxonomy and 16S rDNA sequences. The non filtrates of isolates NSP1 and NSP2 were significantly highest to inhibit the colony growth of *C. gloeosporioides* and followed by isolate NSP 3. The filtrates of isolate NSP 1 gave significantly highest colony inhibition and followed by isolate NPS2. The application of NF-NSP1 before inoculation of *C. gloeosporioides* was significantly lowest number of anthracnose lesions and highest in disease reduction of 81.66 %, followed by NF-NSP2, F-NSP1, F-NSP3, NF-NSP3 and F-NSP2, respectively. Moreover, the application of NF-NSP1 after inoculation of *C. gloeosporioides* was significantly lowest number of anthracnose lesions and highest in disease reduction of 66.66 % when compared to the inoculated control. These effective antagonistic actinomycetes are identified as candidate to be a new *Streptomyces* spp and new candidate as biological control agents against chilli anthracnose.

Key words: *Colletotrichum gloeosporioides*, soil actinomycetes, antifungal activity

Introduction

Long cayenne chilli pepper (*C. annuum* var *acuminatum*) is one of the economically crop in Thailand and severely infected by *Colletotrichum* spp. (*C. capsici* and *C. gloeosporioides*) causing anthracnose disease (Than *et al*., 2008, Waller *et al*., 2002) due to yield loss and reduced marketability (Mananadhar *et al*., 1995). Anthracnose disease can infect on every part of chilli and typical symptoms on stem such as leaf tip die-back, stem die-back, foliar blight, leaf

* Corresponding author: Sarunya Nalumpang; e-mail: Sarunya@Chaingmai.ac.th

spot, leaf lesion and fruit also include sunken necrotic tissues, with concentric rings of acervuli that are often wet (Mananadhar *et al*., 1995; Than *et al*., 2008).

The management and control of anthracnose disease are still under extensive research. In some case eradicative sanitation methods are not feasible, systemic fungicides were substituted. Due to the reliance on fungicides, pathogen resistance to some systemic fungicides repeatedly used throughout the year, and the deleterious to the environment and consumer were concerned. The benzimidazole fungicides, particularly carbendazim, have been reported to be effective against higher fungi. However, continuous using showed the frequency of resistant isolates in some regions of China increased gradually (Zhang and Huang, 2007). Antimicrobial organisms are interested over the decade and has been extensively studied (Shimizu *et al*., 2009), the actinomycetes are important producers of bioactive compounds such as chitinase and β-1, 3-glucanase (El-Tarabily *et al*., 2000) and constitute a potential as biological control agents because of its intense antagonistic activity via the production of various antifungal substances (El-Tarabily and Sivasithamparam, 2006; Rothrock and Gottlieb, 1984; Xiao *et al*., 2002). The aims of this study were to isolate, identify and investigate antimicrobial activities of soil actinomycetes to control *C. gloeosporioides* causing chilli anthracnose disease.

Materials and methods

Isolation soil actinomycetes

Soil samples were collected from Suthep-Pui National Park and soil planted to chilli pepper brought to laboratory. Soil samples were air dried at room temperature for 7 days and to isolate thermotolerant actinomycetes, air dried soil samples were ground and pretreated at 120^{o}C for 1 h (Xu *et al*., 1996) become fine soil particles ready to use for isolation of actinomycetes. Soil extract agar (SEA) medium was used for isolation, modified from Takefumi *et al*. (2005) was prepared as soil solution by using the other organic soil from Suthep-Pui Nattional Park. It consisted of 500 g of soil sample mixed to 50 mM NaOH/L and incubated overnight at room temperature, then the soil solution was filtrated and the soil filtrate was then centrifuged for 60 min at 15,000 rpm. The 500 ml of supernatant was sterilized under 121°C at 15 psi for 15 min, then mixed into agar 20 g/L of water. The soil particles were taken in random and spread over SEA medium and incubated at room temperature. The colonies of actinomycetes were observed and transferred to yeast extract-malt extract (YEME) agar plates which consisted yeast extract 4 g, malt 10 g, glucose 4 g, agar 20g/L (Shirling and Gottlieb, 1966) for isolation into pure culture. Pure

cultures were transferred to Emerson's agar slant (Gottlieb *et al*., 1948) which consisted of 4.0 g beef extract, 4.0 g peptone, 2.6 g NaCl, 1.0 g yeast extract, 10.0 g glucose and 20 g agar/L of water as stock cultures.

Isolation of pathogen and pathogenicity test

C. gloeosporioides causing chilli anthracnose was isolated from fruits of long cayenne chilli pepper (*C. annuum* var *acuminatum*). The fruiting bodies of pathogen were taken from anthracnose lesion and placed onto potato dextrose agar (PDA), incubated at room temperature to get pure culture. The pathogen isolates were identified and tested for pathogenicity on chilli fruits.

Screening of antifungal activities of actinomycetes

Dual culture method was used to screen effective antagonistic actinomycetes against *C. gloeosporioides*. Each actinomycete isolate was cultured on glucose yeast malt agar (GYM) which consisted of glucose 4 g, yeast extract 4 g, malt extract 10 g, $CaCO_3$ 2 g, agar 20 g/L (Shirling and Gottlieb, 1966). Culture of actinomycete was transferred by a single streak on one side of GYM plate at a distance of 1.5 cm and incubated at 27^{o}C for 7 days (modified from Rothrock and Gottlieb, 1981). Thereafter, the pathogenic isolate of *C. gloeosporioides* growing on PDA for 7-days was cut using sterilized cork borer at peripheral colony to get mycelial agar plug, then transferred to the middle of dual culture plate. Data were collected as percent inhibition of colony growth (PICG) (modified from Soytong, 1989; Lokesha and Benagi, 2007). The most effective isolates of actinomycetes were used for the next experiment.

Identification of the effective soil actinomycetes

Morphological and chemotaxonomy identification: The antagonistic effective actinomycete isolates were identified into genera based on morphological characteristics and chemotaxonomy of cell wall (Williams *et al*., 1989; Miyadoh, 1997). The morphological characters were observed as colony shape and color, sporulation, pigment diffusion etc. on GYM and IMA2 (Inhibitory mold agar consisted of glucose 5 g, soluble starch 5 g, beef extract 1 g, yeast extract 1 g, NaCl 2 g, $CaCO_3$ 1 g and agar 20 g/L) (Shimizu *et al*., 2000). Chemotaxonomy of cell wall component was determined by thin layer chromatography (TLC) to detect the diaminopimelic acid (DAP or A2 pm) isomeric forms either *LL* or *meso* forms or both by using 0.01 M *LL- meso* A2 pm as a standard (Lechevalier and Lechevalier, 1970). All actinomycetes

isolates were grown on YEME agar plates for 14-days. The culture of each isolate was harvested and used as whole cells, 100 mg of biomass cells were placed in 1.5 ml microcentrifuge tube contained with 0.1 ml of 6 N HC1 and homogenized for 6 min vigorously with vortex and sterilized by autoclaving 20 min at 121°C. After cooling, the lyses cells were centrifuged for 5 min at 10000 rpm. Three µl of supernatants were spotted directly on a thin cellulose sheet. Three µl of 0.01 M *LL- meso* A2 pm was spotted on the same sheet as a standard. Ascending chromatography was performed on the solvent system, methanol:distilled water:6 N HCl:pyridine (80: 26: 4:10, v/v) for 3 hr. After the sheet was dried, it was sprayed with 0.2% ninhydrin reagent and heated at 100° for 5 min to reveal the spots (modified from Hasegawa *et al*., 1983).

16s rDNA sequencing and phylogenetics analysis

Total genomic DNA of effective actinomycetes were extracted and amplified for the16S rDNA fragments from the crude lysates. The products were amplified by PCR by using universal primers 27f (5′-AGA GTT TGA TCC TGG CTCAG -3′) and 1492r (5′-GGC TAC CTT GTT ACG ACTT-3′) (Lane, 1991) under the following condition: 96^{o}C for 5 min, 35 cycles of 94^{o}C for 60 sec, 55^{o}C for 60 sec, 72^{o}C for 120 sec and final extension at 72^{o}C for 3min. The amplicons were separated by 1% gel electrophoresis analysis and purified by using a Nucleospin® Extraction Kit (Biogen Ltd.) and 16S rDNA were sequenced. The resultant of 16S rDNA sequences were aligned against corresponding sequences of the type strains of *Streptomyces* species, retrieved from the GenBank and RDP databases, using the PHYDIT and TREECON programs.

Test of culture filtrates from effective actinomycetes against C. gloeosporioides

The effective actinomycete isolates were separately cultured in enzyme producing medium (EPM) modified from Rattanakit *et al*. (2002); consist of 3.0 g/L glucose, 1.0 g/L bacto peptone, 0.3 g/L urea, 1.4 g/L $(NH_4)_2SO_4$, 0.3 g/L $MgSO_4 \cdot 7H_2O$, 0.3 g/L $CaCl_2 \cdot 6H_2O$, 200 mL/L colloidal chitin (described previously on Hsu and Lockwood, 1975), trace salt solution 1.0 ml/L (5.0 g/L $FeSO_4 \cdot 7H_2O$, 1.6 g/L $MnCl_2 \cdot 7H_2O$, 1.4 g/L $ZnSO_4 \cdot 7H_2O$, 2.0 g/L $CoCl_2$) and incubated at 35^{o}C for 15 days with continuous shaking at150 rpm. The cultures were centrifuged for 20 min at 6,000 rpm (4^{o}C) and the supernatants were collected as non culture filtrate (NF). The supernatant was then filtrated through membrane filter pore size 0.2-µm (Minisart®) to get culture filtrate (F) (Chareunrat, 1999). Agar well method was done by following the technique of

Bauer *et al*. (1996) which performed on double-layer PDA plates to test the culture filtrates (F) and non culture filtrates (NF) of each actinomycete isolate. Wells were made on upper layer of PDA plates after solidification, the upper layer of agar was punched by cork borer (5 mm diameter) about 3 cm away from center for 3 wells in each plate. One agar disc (5 mm diameter) of *C. gloeosporioides* culture was cut from peripheral colony of 5 day-old fungal culture grown in PDA at 28°C and transferred to the center of the plate. Thirty microlitre of F, NF and EPM were put to each well, respectively and the plates were incubated at 28°C for 7 days. Data were collected as percent inhibition of colony growth (PICG) modified from Soytong (1989), Lokesha and Benagi (2007).

Effect of the actinomycetes culture filtrates on chilli seeds germination

Seeds of long cayenne chilli pepper, local variety and Chomthong 2 (F1), were soaked with sterilized water overnight at room temperature. The experiment was performed using two factors in Completely Randomized Design (CRD) with three replications. Factor A was actinomycetes isolates and factor B was NF, F, EMP and distilled water. The soaked chilli seeds were placed on water-soaked blotting paper and incubated for 14 days at room temperature. Data were collected as seed germination.

Effect of actinomycetes culture filtrates as biocontrol of chilli anthracnose

The effective antagonistic actinomycetes isolates against *C. gloeosporioides* causing chilli anthracnose were screened and used in this experiment. The experiment was done using CRD with three replications. First experiment was done as the application before inoculation of the pathogen and treatments were NF, F from each isolate of actinomycetes and pathogen alone served as control. The application before inoculation was done by spraying five milliliter of NF and F from each isolate of actinomycetes and inoculated control on wounded leaves, 12 hours before inoculation with 10^6 conidia/mL of *C. gloeosporioides*. The second experiment was done as the application after inoculation of the pathogen and treatments were NF, F from each isolate of actinomycetes and pathogen alone served as control. The application after inoculation of the pathogen was done by inoculated 10^6 conidia/mL of *C. gloeosporioides* on wounded leaves for 12 hours, then applied NF and F from each isolate of actinomycetes and inoculated control. Both experiments, leaves of 45-days chilli seedling were used to inoculated, 4 leaves/plant were wounded by using needle pricked gently (20 spots/leaf). Data were collected as the number of anthracnose lesions per plant and computed to disease reduction as

following formula:- number of lesions in control –number of lesions in treatment/ number of lesion in control X 100.

Statistical analysis

Data were computed analysis of variance (ANOVA). Treatments mean were compared using Duncan's Multiple Range Test (DMRT) at P=0.05 and 0.01.

Results

Isolation soil actinomycetes and screening of antifungal activities of actinomycetes

One-hundred and ninety one isolates of actinomycetes were encountered from Suthep-Pui National Park and soil planted to chilli. Anthracnose on fruits of long cayenne chilli pepper was isolated and identified as *C. gloeosporioides* which was virulent isolate to the same host plant in pathogenicity test. Dual culture test showed that six isolates of soil actinomycetes revealed the highest antifungal activity against *C. gloeosporioides* as 100 % inhibition of colony growth as follows:- NSP1, NSP2, NSP3, NSP4, NSP5 and NSP6.

Identification of the effective soil actinomycetes

Morphological and chemotaxonomy identification: The actinomycetes isolates were incubated for 7-day on GYM and IMA-2 plates for morphological studies. The isolates NSP4, NSP1, NSP2, NSP6 and NSP3 are whitish mass color, rough and convex colonies whereas NSP5 showed whitish mass color and flat colony. Under microscopic observation of the six actinomycetes form coiled and flexible spore chain with more than 10 spores in chain; no diffusible soluble pigment produced on agar and showed it is consisted of *LL*-diaminopimelic acid isomer type of cell wall component (Fig. 1). Both taxonomic criteria showed that these screened isolates are similar to *Streptomyces* spp.

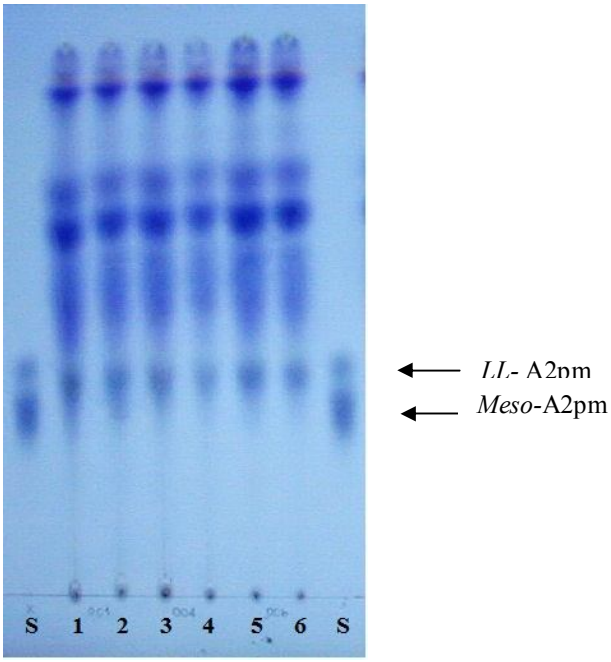

Fig. 1. Separation of A2pm isomers from NSP4 (1), NSP1 (2), NSP2 (3), NSP6 (4), NSP3 (5), NSP5 (6) and 0.01 M *LL*, *meso* A_2 pm (S) by thin-layer chromatography.

16s rDNA sequencing and phylogenetics analysis

Six actinomycete isolates were subjected to a 16S rDNA sequencing to reveal its phylogenetic relationship with representative *Streptomyces*. The fragment of 16S rDNA sequences were determined (0.5 kb). The six actinomycetes isolates were clearly separated from a clade containing 27 *Streptomyces* species and closely related to *Kitasatospora* species. Their similarity to nucleotide sequences of thirty representatives of the genus, the similarity among *Kitasatospora griseola* (Syn. *Streptomyces griseolosporeus*) (Hamano *et al*., 2002) and the isolates showed that NSP3 and NSP4 exhibited comparable levels of similarity 93.08 and 91.33%, respectively whereas NSP1, NSP2, NSP5 and NSP6 exhibited comparable levels of similarity 89.60, 89.31, 85.84 and 89.02%, respectively (Fig. 2). The evident from the 16S rDNA similarity values of the 6 representative actinomycetes indicated that the six actinomycetes are candidate to be a new *Streptomyces* spp.

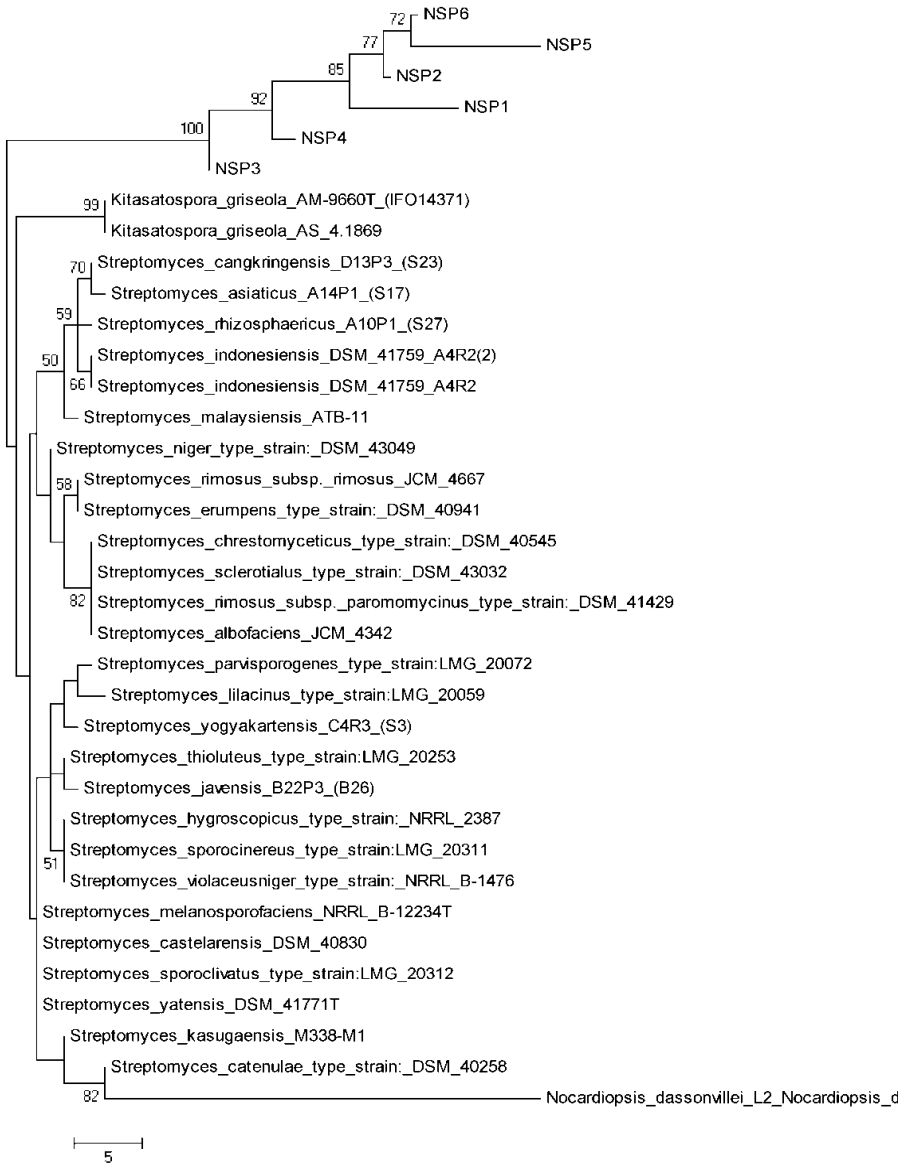

Fig. 2. Phylogenetic tree of the 16S rDNA sequences from NSP4, NSP1, NSP2, NSP6, NSP3 and NSP5 isolates. The sequence data for several closely related *Kitasatospora griseola* (Syn. *Streptomyces griseolosporeus*) were recovered from GenBank and included in the tree. The percentage of replicate tree in which the associated taxa clustered together in the bootstrap test is shown above the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances. *Nocardiopsis dassonvillei* are designated outgroup.

Test of culture filtrates from effective actinomycetes against C. gloeosporioides

The non filtrate (NF) of NSP1 and NSP2 was significantly highest to inhibit the colony growth of *C. gloeosporioides* of 66.67 and 70.00 %, respectively, and followed by NSP3 (58.33%). NSP1 and NSP5 were not significantly differed which inhibited the colony growth of 55.00 %. The filtrates (F) of NSP1 gave significantly highest colony inhibition (55.00 %) and followed by NSP2 (50.00%). The filtrates from other isolates showed colony inhibition lower than 50 %. (Table1).

Table 1. Culture and non-culture filtrates of effective *Streptomyces* sp. against *C. gloeosporioides*

Isolates of *Streptomyces* sp.	Inhibition of colony growth (%)	
NSP4	NF	53.33cd[1]
	F	15.00f
NSP1	NF	66.67a
	F	55.00bc
NSP2	NF	70.00a
	F	50.00d
NSP6	NF	53.33cd
	F	0.00g
NSP3	NF	58.33b
	F	1.67g
NSP5	NF	55.00bc
	F	25.00c

[1]Means of three replications. Means followed by a common letter in each column are not significantly different by DMRT at $P < 0.05$.

Effect of the actinomycetes culture filtrates on chilli seeds germination

The actinomycetes isolates NSP1, NSP2 and NSP3 which gave very good results of colony inhibition were then selected to test the effect of filtrates (F) and non filtrates (NF) from those isolates for seed germination of two varieties of chilli. Result showed that F and NF of isolates NSP1, NSP2 and NSP3 were significantly differed in seed germination in local variety, which lower seed germination than non-treated control. While, in Chomthong 2 variety showed that F of NSP1, NSP2 and NF of NSP2 and NSP3 were not significantly differed in seed germination when compared to the non-treated one (Table 2).

Table 2. Culture and non-culture filtrates of effective *Streptomyces* sp. affected to chilli seed germination for 12 hours

Treatments	Isolates	Chilli seed germination (%)	
		local variety	Chomthong 2
Distillated water		99.00a[1]	95.00abc
EPM		93.00b	98.00a
NF	NSP1	96.00ab	97.00ab
	NSP2	95.00ab	94.00abc
	NSP3	97.00ab	94.00abc
F	NSP1	97.00ab	92.00bc
	NSP2	99.00a	91.00c
	NSP3	98.00ab	96.00ab

[1]Means of three replications. Means followed by a common letter in each column are not significantly different by DMRT at $P < 0.05$.

Effect of actinomycetes culture filtrates as biocontrol of chilli anthracnose

The application of NF-NSP1 before inoculation of pathogen was significantly lowest number of anthracnose lesions and highest in disease reduction was 81.66 %, and followed by NF-NSP2, F-NSP1, F-NSP3, NF-NSP3 and F-NSP2, respectively. On the other hand, the application of NF-NSP1 after inoculation of pathogen was also significantly lowest number of anthracnose lesions and highest in disease reduction was 66.66 %, and followed by NF-NSP2, NF-NSP3, F-NSP1, F-NSP2 and F-NSP3, respectively when compared to the inoculated control (Table 3).

Table 3. Number of anthracnose lesions and disease reduction after application of actinomycete culture filtrate and non-filtrate for 10 days inoculation

Treatments	**applied before inoculated pathogen**		**applied after inoculated pathogen**	
	Lesion no.	Disease reduction	Lesion no.	Disease reduction
Pathogen	20.00 a[1]	0.00 d	20.00 a	0.00 e
NF-NSP1	3.67 d	81.66 a	6.67 e	66.66 a
NF-NSP2	7.67 cd	61.66 ab	9.33 d	53.33 b
NF-NSP3	9.33 bc	53.33 bc	12.00 c	40.00 c
F-NSP1	8.00 c	60.00 b	13.33 bc	33.33 cd
F-NSP2	13.00 b	35.00 c	14.00 bc	30.00 d
F-NSP3	7.00 cd	65.00 ab	20.00 a	0.00 e
C.V.(%)	24.97	24.04	6.21	13.24

[1]Means of three replications. Means followed by a common letter in each column are not significantly different by DMRT at $P < 0.05$.

Discussion

Six isolates of soil actinomycetes of NSP1, NSP2, NSP3, NSP4, NSP5 and NSP6 exhibited high antagonistic efficiency against *C. gloeosporioides* causing chilli anthracnose which were identified as *Streptomyces* based on morphological and chemotaxonomic of cell wall component (Becker *et al*., 1964; Lechevalier and Lechevalier, 1970; Cummins and Harris, 1985; Williams *et al*., 1989; Miyadoh, 1997) and 16S rDNA sequence (Kumar *et al*., 2007). With this, these isolates of *Streptomyces* were confirmed based on *LL*-A2pm isomer of DAP in cell wall component, on the contrary *meso*-A2pm isomer of DAP were found in rare actinomycetes which stated by Lechevalier and Lechevalier (1970).

However, those actinomycetes isolates were clearly separated from a clade that comparing 27 *Streptomyces* species and closely related to *Kitasatospora* species. Their similarity to nucleotide sequences of representatives showed 16S rDNA similarity to *Kitasatospora griseola* (Syn. *Streptomyces griseolosporeus*). These effective antagonistic actinomycetes are then identified as candidates to be a new *Streptomyces* spp.

The potential of antifungal activity of the soil actinomycetes culture as non-filtrate (NF) and filtrate (F) to inhibit growth of *C. gloeosporioides* showed that NF and F of isolates NSP1, NSP2 and NSP3 gave the highest per cent inhibition of colony growth and no adverse effect on seed germination of chlli. The inhibition of fungal colony of *C. gloeosporioides* in dual culture test in this experiment may be due to the presence of extracellular metabolites both hydrolytic enzyme and secondary antifungal compound might be produced by six isolates of *Streptomyces* (Prapagdee, 2008).

The application of NF-NSP1 before inoculation of *C. gloeosporioides* was significantly lowest number of anthracnose lesions of long cayenne chilli pepper (*C. annuum* var *acuminatum*) and highest in disease reduction was 81.66 %. In this study the non filtrates showed better inhibition than filtrates. The non filtrates are usually contained living cells of actinomycetes. Bibb (2005) and Prapagdee (2008) stated that the non filtrate of actinomycetes shown higher potent than filtrate treatment due to active actinomycetes cells remained in culture and may be produced antibiosis and secondary metabolites against the pathogen cells. In this study, the tested actinomycetes were cultured for 15 days to obtain filtrates and it showed efficacy to control chili anthracnose. Abyad *et al*. (1993) and Chang (2007) reported that degree of growth inhibition were decreased in association with incubation period of the fungal culture were increased (El-Abyad *et al*., 1993, Chang, 2007). It is suggested that further study would be concerned on incubation period of culture to get the highest potential filtrates to control the disease. It is recommended

that the six actinomycetes isolates from soil in this study are successfully candidates as potentially biological control agent against chilli anthracnose caused by *C. gloeosporioides*

Acknowledgements

This Research is partially supported by the Center of Excellence on Agricultural Biotechnology, Science and Technology Postgraduate Education and Research Development Office, Commission on Higher Education, Ministry of Education. (AG-BIO/PERDO-CHE) Bangkok, Thailand, 10900.

References

Bibb, M.J. (2005). Regulation of secondary metabolism in streptomycetes. Curr Opin Microb. 8:208–215.

Chang, W.T., Chen, Y.C. and Jao, C.L. (2007). Antifungal activity and enhancement of plant growth by *Bacillus cereus* grown on shellfish chitin wastes. Bioresour Technol. 98: 1224-1230.

Cummins, C.S. and Harris, H. (1985). Studies on the cell-wall composition and taxonomy of actinomycetales and related groups. J Gen Microbio. 18: 173-189.

El-abyad, M.S., El-sayad, M.A., El-shanshoury, A.R. and El-sabbagh, S.M. (1993). Towards the biological control of fungal and bacterial diseases of tomato using antagonistic *Streptomyces* spp. Plant Soil. 149: 185-195.

El-tarabily, K.A. and Sivasithamparam, K. (2006). Non-streptomycete actinomycetes as biocontrol agents of soil-borne fungal plant pathogens and as plant growth promoters. Soil Biol Biochem. 38: 1505–1520.

El-tarabily, K.A., Soliman, M.H., Nassar, A.H., Al-hassani, H.A. Sivasithamparam, K. McKenna, F. and Hardyb, Hardy, G.E.ST.J. (2000). Biological control of *Sclerotinia minor* using a chitinolytic bacterium and actinomycetes. Plant Pathol. 49. 573-583.

Gottlieb, D., Bhattacharyya, P.K., Anderson, H.W. and Carter, H.E. (1948). Some properties of an antibiotic obtained from a species of *Streptomyces*. J Bacteriol. 55: 409–417.

Hamano, Y., Dairi, T., Yamamoto, M., Kuzuyama, T., Itoh, N., Seto, H. (2002). Growth-phase dependent expression of the mevalonate pathway in a terpenoid antibiotic-producing *Streptomyces* strain. Biosci Biotechnol Biochem. 66:808-819.

Hasegawa, T., Takizawa, M. And Tanida, S. (1983). A rapid analysis for chemical grouping of aerobic actinomycetes. J Gen Appl Microbiol. 29: 319-322.

Hsu, S.C. and Lockwood, J.L. (1975). Powdered chitin agar as a selective medium for enumeration of Actinomycetes in water and soil3. Appl Microbiol Biot. 29: 422-426.

Kumar, Y., Aiemsum-ang, P., Ward, A.C. and Goodfellow, M. (2007). Diversity and geographical distribution of members of the *Streptomyces violaceusniger* 16S rRNA gene clade detected by clade-specific PCR primers. FEMS Microbiol Ecol. 62: 54–63.

Lane, D.J. (1991). 16S/23S rRNA sequencing. *In*: Nucleic acid techniques in bacterial systematics. Stackebrandt, E. and Goodfellow, M., eds. John Wiley and Sons, New York, NY. 115-175.

Lechevalier, M.P. and Lechevalier, H.A. (1970). Chemical composition as a criterion in the classification of aerobic actinomycetes. Int J Sys Bacteriol. 20: 435-443.

Lokesha, N.M. and Benagi, V.L. (2007). Biocontrol management of pigeonpea dry root caused by *Macrophomina phaseolina*. Karnataka J Agric Sci. 20: 54-56.

Mananahar, J.B. Hartman, G.L. and Wang, T.C. (1995). Anthracnose development on pepper fruits inoculated with *Colletotrichum gloeosporioides*. Plant Dis. 79, 1995, 380–383.

Miyadoh, S., Hamada, M., Hotta, K., Kudo, T., Seino, A., Vobis, G. and Ypkpya, A. (1997). Atlas of Actinomycetes. The Society for Actinomycetes Japan. 233 p.

Prapangdee, B., Kuekulvong, C. and Mongkolsuk, S. (2008). Antifungal Potential of Extracellular Metabolites Produced by *Streptomyces hygroscopicus* against Phytopathogenic Fungi. Int J Biol Sci; Short Research Communication. 4: 330-337.

Rattanakit, N., Plikomol, A., Yano, S., Wakayama, M. and Tachiki, T. (2002). Utilization of shrimp shellfish waste as a substrate for solid-state cultivation of *Aspergillus* sp. S1-13: Evaluation of a culture based on chitinase formation which is necessary for chitin-assimilation. J Biosci Bioeng. 93: 550–556.

Rothrock, C.S. and Gottlieb, D. (1984). Role of antibiosis in antagonism of *Streptomyces hygroscopicus var. geldanus* to *Rhizoctonia solani* in soil. Can J Microbiol 30: 1440–1447.

Rothrock, C.S. and Gottlieb, D. (1981). Importance of antibiotic production in antagonism of selected *Streptomyces* species to two soil-borne plant pathogens. J Antibiot. 34: 830.

Shimizu, M., Nakagawa, Y., Sato, Y., Furumai, T., Igarashi, Y., Onaka, H., Yoshida, R. and Kunoh, H. (2000). Studies on endophytic actinomycetes (I) *Streptomyces* sp. isolated from rhododendron and its antifungal activity. J Gen Plant Pathol. 66: 360-366.

Shimizu, M., Yazawa, S. and Ushijima, Y. (2009). A promising strain of endophytic *Streptomyces sp.* for biological control of cucumber anthracnose. J Gen Plant Pathol. 75: 27–36.

Soytong, K. (1989). Biological Control of Plant Pathology. 362p.

Takefumi, H., Motosama, S., Ryosuke, F., Yasuko, J., Takayuki, K., Akira, T., Kikuo, S. and Hiroshir, S. (2005). Isolation of novel bacteria and Actinomycetes using soil extract agar medium. J Biosci Bioeng. 99: 485-492.

Than, P.P., Jeewom, R., Hyde, K.D., Pongsupasamit, S., Monkholporn, O. and Taylor, P.W.J. (2008). Characterization and pathogenicity of *Colletotrichum* species associated with anthracnose disease on chilli (*Capsicum* spp.) in Thailand. Plant Pathol. 57: 562–572.

Waller, J.M., Lanne, J.M. and Waller, S.J. (2002). Plant Pathologist's Pocketbook 3rd edition, Oxon, UK, New York: CABI Pub. 516 p.

Xiao, K., Kinkel, L.L. and Samac, D.A. (2002). Biological control of *Phytophthora* root rots on alfalfa and soybean with *Streptomyces*. Biol Control. 23: 285–295.

Xu, L., Li, Q. and Jiang, C. (1996). Diversity of soil actinomycetes in Yunnan, China. Appl Environ Microb. 62: 244-248.

Zhang, R.L. and Huang, J.S. (2007). Cloning of a carbendazim-resistant gene from *Colletotrichum gloeosporioides* of mango in South China. Afr J Biotech. 6: 143-147.

(Published in March 2012)