

Antagonistic *Streptomyces* Selection to Broad Spectrum for Biological Control of *Colletotrichum* spp., Causal Agent of Anthracnose in Chilli

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Antifungal activity on mycelium growth inhibition of *Colletotrichum capsici* (21 isolates), *C. acutatum* (37 isolates), *C. gloeosporioides* (1 isolate) and *C. coccodes* (1 isolate) tested by dual culture method showed that all antagonistic *Streptomyces* spp. inhibited the mycelium growth of the fungi, as *Streptomyces*-PR22 had strongest activity followed by *Streptomyces*-PR78, PR15, PR13, PR87, PR84 and PR33, respectively. The spore germination of 4 species of *Colletotrichum* was inhibited by culture filtrate of *Streptomyces*-PR22 while the other *Streptomyces* presented good results for some species only: PR33 for *C. capsici*, *C. acutatum*, and *C. gloeosporioides*, PR87 for *C. gloeosporioides* and *C. coccodes*, PR15, PR78 and PR84 for *C. coccodes*. All isolates of antagonistic *Streptomyces* produced hydrolytic enzymes such as chitinase, α 1,3 glucanase. For protease enzymes was produced by *Streptomyces*-PR78, PR15, PR84 and PR87. In a greenhouse experiment, *Streptomyces*-PR22 and *Streptomyces*-PR87 sprayed weekly from- seedling or from- flowering stage until fruit harvesting reduced number of diseased fruits in both chilli cultivars var. Prik kheenu and Num Kiew as effective as fungicide benomyl application. The study shows that antagonistic *Streptomyces*-PR22 or PR87 is powerful alternatives to fungicide for control anthracnose disease of chilli caused by *Colletotrichum* and increase crop yields.

Key words: Actinomycetes, biocontrol, anthracnose, hydrolytic enzyme, pepper.

Chilli (*Capsicum annum* L.) is one of the most of economic crop in Thailand; but its production had a problem by plant disease, especially anthracnose same as other countries. Anthracnose disease is a major disease of chilli in worldwide, especially, in tropical and sub-tropical areas. Damage was reported in Indonesia for 10-80%¹ and Thailand up to 80%². The disease is caused by at least four species of *Colletotrichum*; *C. gloeosporioides* (Penz.) Sacc., *C. capsici* (H.Syd.) E. Butl. & Bisby, *C. acutatum* Simmonds

ex Simmonds, and *C. coccodes* (Wallr.) S. Hughes^{3,4}.

Selection of resistant chilli cultivar was success for some species of *Colletotrichum*. Chemical fungicides, such as benomyl, maneb, chlorothalonil and mancozeb were popular to manage the disease. However, some chemical, e.g. benzimidazole affected differently to species of *Colletotrichum* (*C. acutatum* was found to be moderately susceptible to this fungicide, while *C. gloeosporioides* was highly susceptible)⁵. Chemical application normally applied biweekly or monthly which can damage the environment, and lead to development of fungicide resistance strains of *Colletotrichum*⁶. Hence, management by biocontrol agent application can be an alternative

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method to save environment and human health.

Streptomyces, gram -positive bacteria in the order Actinomycetales are able to produce extracellular secretions with different properties such as antibacterial, antifungal, antiviral, nematocidal, antitumor, and enzyme inhibitory activities^{7,8}. Cell- free culture filtrates of *S. hygroscopicus* SRA 14 inhibited growth of *C. gloeosporioides* in orchid, apparently as hyphal swelling and abnormal shapes⁹. The crude extract from *Streptomyces* sp. SRM1 culture also showed antagonistic effects against *C. musae* as abnormal growth of hyphae and inhibition of spore germination¹⁰. Extracellular chitinase and α -1,3-glucanase produced by *S. aureofaciens* inhibited growth of *C. gloeosporioides* and treatment with the bioactive components exhibited a high protective activity against development of anthracnose disease on mango trees and increased fruit yield¹¹. *Streptomyces cavourensis* SY224 produced chitinase, α -1,3 glucanase, and 2-Furancarboxaldehyde inhibited growth mycelium and spore germination of *C. gloeosporioides* infecting pepper plants¹². The solid concentrate of *Streptomyces* sp. A1022 reduced anthracnose by *C. gloeosporioides* in infections of pepper and cherry tomato¹³. Previously, we found that the isolates test of *Streptomyces* were antagonistic to *Colletotrichum capsici* casual agent of anthracnose disease in chilli¹⁴. In this study, we were to 1) screen antagonistic *Streptomyces* isolates for broadly control several species of *Colletotrichum* by testing of their potential to inhibit mycelial growth, spore germination of *Colletotrichum* spp. and to produce hydrolytic enzyme, and 2) to evaluate biological control potential for chilli anthracnose under greenhouse conditions.

MATERIALS AND METHODS

Pathogens

Colletotrichum. capsici 21 isolates, *C. acutatum* 37 isolates, *C. gloeosporioides* 1 isolate and *C. coccodes* 1 isolate were isolated from chilli fruits with anthracnose symptom collected from northeast provinces of Thailand, i.e. Khon Kaen, Mugdahan and Sakhonakon, pathogenicity test on green and red fruits and identification species of *Colletotrichum* followed by Sutton¹⁵ and

Sutaphun et al.³ were used for laboratory test and *C. acutatum* was used for green house experiment in chilli plant.

Antagonistic *Streptomyces*

Seven isolates of *Streptomyces* spp. used in this study: PR13, PR15, PR22, PR33, PR78, PR84 and PR87 provided by Petcharat Thummabenjapone were grown on arginine glycerol mineral salt agar (AGMA) medium for 7 days for testing inhibition of mycelium growth of *Colletotrichum*. Their culture filtrates and micro colonies were prepared by culturing on AGMA medium for 7 days, and transferring to AGMB medium on a rotary shaker (150-160 rpm) at room temperature for 7 days. The micro colonies and culture liquid were separated by filtration through Whatman paper #1. The micro colonies were kept in 200 mL sterile bottles at 4 °C whereas the culture liquid were filtrated by using a millipore membrane filter (0.45 μ m) and kept in 125 mL sterile flasks at 4 °C. Cell suspension of *Streptomyces* were prepared by homogenizing of micro colonies at the speed of 24,000 rpm for 5 min, and preserved at 4 °C until future use.

Evaluation of mycelium growth inhibition by dual culture method

Colletotrichum isolates were cultured on PDA, and *Streptomyces* isolates were grown on AGMA, both for 7 days incubated at room temperature (28-30°C). The hyphal tips of *Colletotrichum* and *Streptomyces* colonies were bored with sterile 8-mm cork borer to produce culture discs. The assay was made on two types of media: PDA (suitable media for *Colletotrichum*) and AGMA (proper media for *Streptomyces*). Each culture disc of *Colletotrichum* and *Streptomyces* was transferred on each media at opposite direction with 30 mm apart and incubated at room temperature. In a control dish, an AGMA plug was used instead of *Streptomyces* disc. The experiment was arranged in completely randomized design (CRD) with four replications. The inhibition zone as distance between hyphal tips of *Colletotrichum* and *Streptomyces* colony was measured, compared with hyphal tips and AGMA plug as a control, after co-culture for 5-16 days depending on species of *Colletotrichum*.

Evaluation of spore germination inhibition

One isolate of each *Colletotrichum* species were selected and cultured on PDA at room

temperature for 7-10 days depending on species. Spores were harvested by flooding the surface of culture with 5 mL of sterile distilled water and gently scraping with sterile L- shape glass rod to displace the spores. The spore suspension was passed through two layers of sterile cheesecloth. The suspensions were sampled to count spore number with haemocytometer under light microscope and adjusted concentration to 10^5 spore mL^{-1} with sterile distilled water. A 1.5 mL sterile tube were filled with 100 μL of culture filtrate free from cell of *Streptomyces* and added with 100 μL of spore suspension of *Colletotrichum*. The volume of 80 μL combined suspension was placed on a sterile slide in moist plate and incubated at room temperature about 7-14 h (depending on species) of the fungal test, based on observations and estimations of the number of spores germinated in sterile distilled water (control). The experimental design was completely randomized with four replication.

After incubation, added one drop of lactophenol blue to stop spore germination and a total of 100 spores (germinated and non-germinated) were counted on each slide, under a light microscope. Spores with germinating tube length of at least 50% greater than the normal spore size were considered germinated¹⁶. Percentage of spore germination inhibition was calculated according to formulation as:

$$\text{Inhibition of spore germination (\%)} = \left[\frac{\text{No. of spores germinated in distilled water} - \text{No. of spores germinated in Treatment}}{\text{No. spores germination in distilled water}} \right] \times 100$$

Seven isolates of antagonistic *Streptomyces* were selected for the test using petri-dish assay. The colonies of *Streptomyces* on AGMA for 7 days were bored with sterile 8-mm cork borer to produce culture discs and transferred to different substrates, i.e. chitinase-detection agar (CHDA)¹⁷, AGMA+ 0.02% laminarin¹⁸, and casein agar medium¹⁹ for evaluation of chitinase, α ,1,3 glucanase, and protease production. In a control dish, an AGMA plug was used instead of *Streptomyces* disc. Each substrate media were tested for five replications in completely randomized design. The clear zone or precipitated zone around the colonies appeared after 7 days was indication of hydrolytic enzyme production, and diameter of the zones was measured. Productions of chitinase and α ,1,3 glucanase were showed after incubating with 0.1% congo red and 1 M NaCl, while that of collagenase was reacted when saturated with $(\text{NH}_4)_2\text{SO}_4$.

Effect of antagonistic *Streptomyces* on chili anthracnose under greenhouse condition

Seven treatments arranged in completely randomized design with 10 replications were as

Table 1. The distance of inhibition zones between antagonistic *Streptomyces* colony and hyphal tips of *C. capsici*, *C. acutatum*, *C. gloeosporioides* and *C. coccodes* isolates after co-cultured on PDA and AGMA media

<i>Streptomyces</i> isolate	<i>C. capsici</i>		<i>C. acutatum</i>		<i>C. gloeosporioides</i>		<i>C. coccodes</i>	
	PDA	AGMA	PDA	AGMA	PDA	AGMA	PDA	AGMA
PR13	10.7cd	11.8b	11.6b	10.6c	19.5bc	5.4c	16.0b	7.3a
PR15	10.7cd	11.2b	6.3d	9.5d	18.5bc	7.3bc	13.5cd	9.8a
PR22	15.8a	16.2a	17.7a	16.3a	25.5a	16.0a	20.5a	10.3a
PR33	9.3e	9.4c	7.2c	11.3b	18.5bc	6.0bc	12.5d	3.0c
PR78	11.9b	11.4b	6.7cd	9.0e	21.0b	9.3bc	14.5c	6.0ab
PR84	9.9de	9.9c	6.3d	8.7e	17.8bc	8.3bc	9.8e	7.8a
PR87	11.1c	10.3c	5.4e	8.1f	16.8c	10.3b	10.0e	7.0ab
Control	0.0f	0.0d	0.0f	0.0g	0.0d	0.0d	0.0f	0.0d
C.V. (%)	3.35	3.84	4.52	2.12	9.48	12.65	4.07	16.78

Control = AGMA plugs

Means of inhibition zone from 21 isolates of *C. capsici*, 37 isolates of *C. acutatum* and each 1 isolate of *C. gloeosporioides* and *C. coccodes*.

Means within column followed by a common letter are not significantly different according to DMRT ($P < 0.05$).

follows: (1) *Streptomyces*-PR22 dressed seed and from-flowering spray + *C. acutatum*, (2) *Streptomyces*-PR22 dressed seed and from-seedling spray + *C. acutatum*, (3) *Streptomyces*-PR87 dressed seed and from-flowering spray + *C. acutatum*, (4) *Streptomyces*-PR87 dressed seed and from-seedling spray + *C. acutatum*, (5) chemical dressed seed and from-flowering spray + *C. acutatum*, (6) untreated + *C. acutatum* (disease check), and (7) untreated, uninoculated (healthy check).

Two Thailand commercial chilli varieties used in this study were bird chilli var. Prik Keenu and long cayane var. Num Kiew (Chia Tai Group). Seeds of chilli were surface disinfested with 70% ethanol for 1 min, rinsed five times with sterile distilled water, and then disinfested again with 0.5% sodium hypochlorite (Mohammed *et al.*, 2008). Seed dressing with *Streptomyces* was prepared by adding 1 mL cell suspension of *Streptomyces* (approximately 10^7 cfu mL⁻¹) into 200 chilli seeds. Seed dressing with chemical was prepared by

Table 2. Inhibition of spore germination of 4 species of *Colletotrichum* by culture filtrate of 7 isolates of antagonistic *Streptomyces*

<i>Streptomyces</i> isolates	% inhibition of spore germination			
	<i>C. capsici</i>	<i>C. acutatum</i>	<i>C. gloeosporioides</i>	<i>C. coccodes</i>
PR13	-4.2c	9.2c	-0.2d	1.6e
PR15	-91.3e	7.4cd	2.3cd	27.1c
PR22	100.0a	100.0a	88.5a	88.8a
PR33	58.2b	87.5b	12.3b	4.4e
PR78	-93.5e	-8.3ef	5.3c	78.0b
PR84	-91.9e	-16.7f	-0.2d	11.3d
PR87	-65.2de	-3.9de	11.6b	89.3a
control-AGMB	-52.2d	1.8cde	0.4d	0.9e
control-distilled water	0.0c	0.0cde	0.0d	0.0e
C.V.(%)	26.95	25.26	19.98	14.01

Means within column followed by a common letter are not significantly different according to DMRT ($P < 0.05$).

Table 3. Diameter of hydrolysis zone of hydrolytic enzymes produced by isolates of *Streptomyces* on different substrates

<i>Streptomyces</i> isolates	Diameter of hydrolysis zone (cm)		
	CHDA	laminarin	casein
PR13	4.12a	4.74a	0.00c
PR15	4.45a	3.90c	1.86b
PR22	4.48a	4.38b	0.00c
PR33	4.76a	2.86d	0.00c
PR78	4.82a	3.86c	2.19a
PR84	4.41a	4.44b	1.77b
PR87	4.78a	3.70c	1.82b
Control	0.00b	0.00e	0.00c
C.V.(%)	14.84	4.69	15.09

Means within column followed by a common letter are not significantly different according to DMRT ($P < 0.05$).

mixing 100 seeds with slurry of benomyl at the rate of 3 g benomyl per 1 kg seed. The dressed seeds were incubated at room temperature for 16 h. Treated and non treated seeds were sown in sterile peatmoss in 104-hole plastic trays.

Seedlings of *Streptomyces*-treated seed were divided into 2 halves. The first half was sprayed with the cell suspension (10^5 cfu mL⁻¹) with 1 ml of Tween 80 at seedling stage and every week. At 45 days old the seedlings were transplanted into each 24-cm pot containing soil mixture (2:1:1,v/v/v proportion of soil: manure: black rice hull). The spray was continued until harvest. The second half of seedlings, thirty days after transplanting into the same soil mixture (at flowering stage) were sprayed with *Streptomyces* cell suspension + Tween 80 every week until harvest.

The 45-day-old seedlings of benomyl-treated seed were transplanted into each 24-cm pot containing same soil mixture. Thirty days after transplanting, chilli plants were sprayed with benomyl (rate 6 g per 20 L) + Tween 80 every week until harvest.

Sixty days after transplanting (fruiting stage), spore suspension of *C. acutatum* (10^5 spores mL⁻¹) was sprayed over 60 mL per plant all chilli plants except uninoculated control treatment.

The data collected at five months after transplanting consists of the number and weight of fruits per plant, and length of fruit (sampling 20 fruits per plant). Numbers of diseased fruits per plant and disease severity were recorded at one month after inoculation of *C. acutatum*. Percentage of anthracnose incidence was then calculated as:

Anthracnose incidence (%) = (No. of diseased fruits/total No. of harvested fruits) × 100
Index of anthracnose disease modified from Phialathounheuanee1 et al.²⁰ as:

1 = No infection

2 = Necrotic lesion or water soaked lesion < 5%

3 = Necrotic lesion or water soaked lesion with few acervuli ranging 6-15%

4 = Necrotic lesion or water soaked lesion with acervuli present ranging 16-30%

5 = Necrotic lesion or water soaked lesion with acervuli present ranging 31-50%

6 = Necrotic lesion or water soaked lesion with abundant acervuli > 51%

Statistical analysis

The total data received from this study were processed for analysis of variance (ANOVA).

The treatments means comparisons were done using Duncan's multiple range test (DMRT).

RESULTS

Evaluation of mycelium growth inhibition by dual culture method

All isolates of *Streptomyces* could inhibit the mycelial growth of any *Colletotrichum* when culturing together on PDA and AGMA media (Fig. 1). The distance of inhibition zone differed significantly between isolates of *Streptomyces*. *Streptomyces*-PR22 showed the greatest inhibition of mycelium growth of 4 species of *Colletotrichum* (Table 1).

Evaluation of spore germination inhibition assay

Culture filtrate of *Streptomyces*-PR22 produced the greatest inhibition of *Colletotrichum* spore germination (Table 2). This isolate completely inhibited spore germination of *C. capsici* and *C. acutatum*, and greatly inhibited those of *C. gloeosporioides* and *C. coccodes* (Fig.2). Great inhibition also occurred in treatments of *C. acutatum* with PR33, and *C. coccodes* with PR78 and PR87

Evaluation of hydrolytic enzymes production

All isolates of antagonistic *Streptomyces* produced hydrolytic enzymes such as chitinase and ²1,3 glucanase as clear zone around colonies showed on CHDA and AGMA + 0.02% laminarin, respectively (Fig. 3). The diameter of hydrolysis zone differed significantly between isolates of *Streptomyces*. For casein agar method to check protease enzyme production, it was found that

Table 4. Yields, percentage of anthracnose incidence and disease severity of bird chili var. Prik Kheenu in greenhouse

Treatments	No. fruits/plant	Fruit weight/plant (g)	Fruit length (cm)	Anthracnose incidence (%)	Anthracnose severity index
Strep-PR22 flowering	191.7	188.65b	4.10	17.0b	3.5c
Strep-PR22 Seedling	209.4	227.54ab	4.71	21.9ab	4.0bc
Strep-PR87 flowering	207.8	186.34b	3.99	16.4b	4.0bc
Strep-PR87 Seedling	237.0	212.89ab	4.23	14.7b	4.0bc
Chemical	216.9	221.14ab	4.42	17.5b	5.0ab
Inoculated control	188.7	176.69b	4.24	33.4a	5.5a
Uninoculated control	297.3	344.85a	4.61	-	-
C.V. (%)	16.81	26.20	6.75	29.54	9.42

Means within column followed by a common letter are not significantly different according to DMRT (P < 0.05).

Streptomyces-PR78, PR15, PR84 and PR87 created clear zone of which *Streptomyces*-PR78 had the greatest one (Table 3).

Effect of antagonistic *Streptomyces* on *C. acutatum* infected chili under green house condition

In brid chilli var. Prik Kheenu almost all *Streptomyces* treatment, except *Streptomyces*-PR22 dressed seed + from-seedling spray, could reduce anthracnose incidence as benomyl treatment did (Table 4). In long yard var. Num Kiew same, all applications of *Streptomyces* reduced anthracnose incidence similarly as chemical treatment (Table 5).

Application of *Streptomyces*-PR22 and PR87 or chemical had no effect on fruit number, fruit weight, and fruit length in both bird chilli (Table 4) and long yard (Table 5).

DISCUSSION

The antagonist *Streptomyces*-PR13, PR15, PR22, PR33, PR78, PR84 and PR87 had board spectra of antifungal activity, indicated by complete inhibition of the mycelial growth for all 4 species of tested *Colletotrichum* isolates on PDA and AGMA media, so these *Streptomyces* can produce secondary metabolite on PDA which suitable for fungal culture same as AGMA was synthetic media suitable for actinomyces culture²¹ which ingredient was arginine, glycerol, phosphorus, potassium, magnesium and pH suitable for enhanced secondary metabolites production of *Streptomyces*^{22,23}. All 7 isolates of *Streptomyces* actively produced several hydrolytic enzymes e.g.

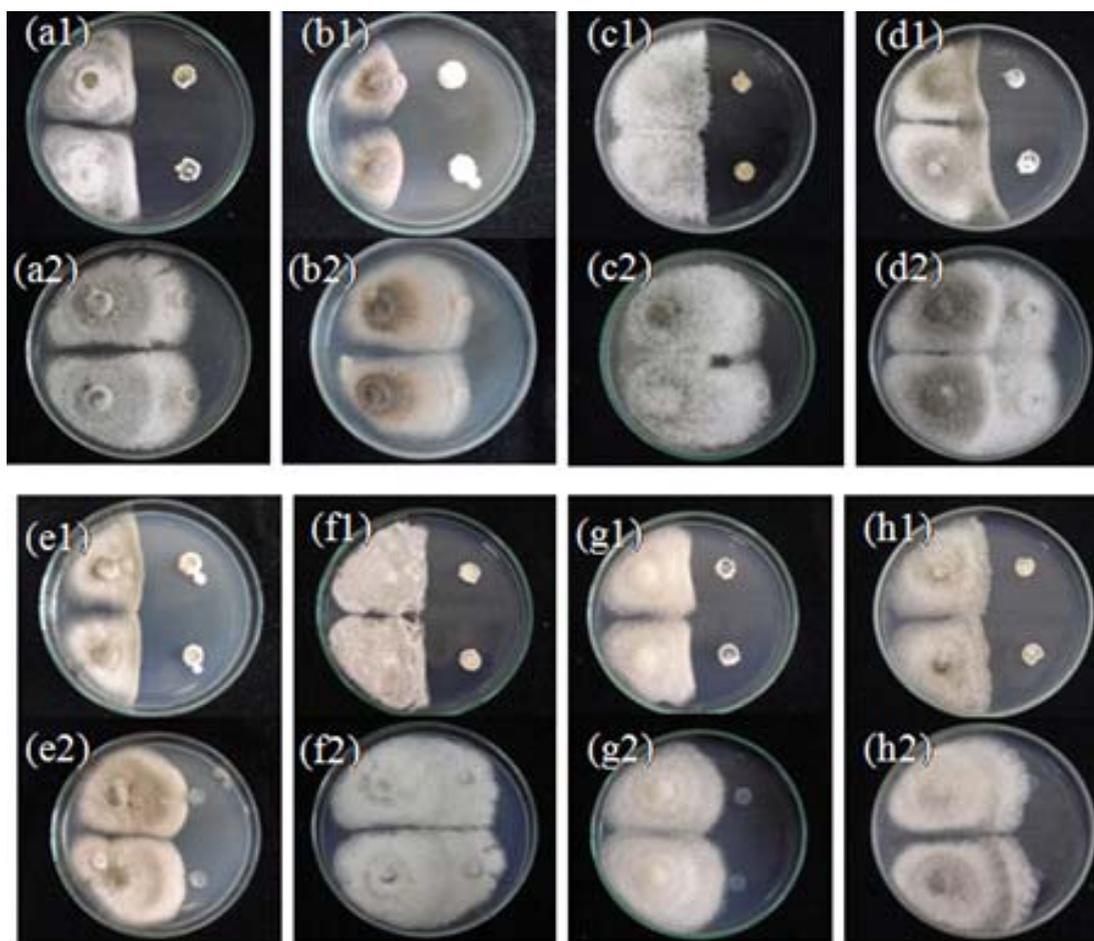


Fig. 1. The antagonistic *Streptomyces*-PR22 inhibited mycelial growth of *C. capsici* (a1, e1), *C. acutatum* (b1, f1), *C. gloeosporioides* (c1, g1) and *C. coccodes* (d1, h1) compared with mycelium growth of 4 species of *Colletotrichum* without *Streptomyces* after dual cultured on PDA (a2-d2) and AGMA (e2-h2) media

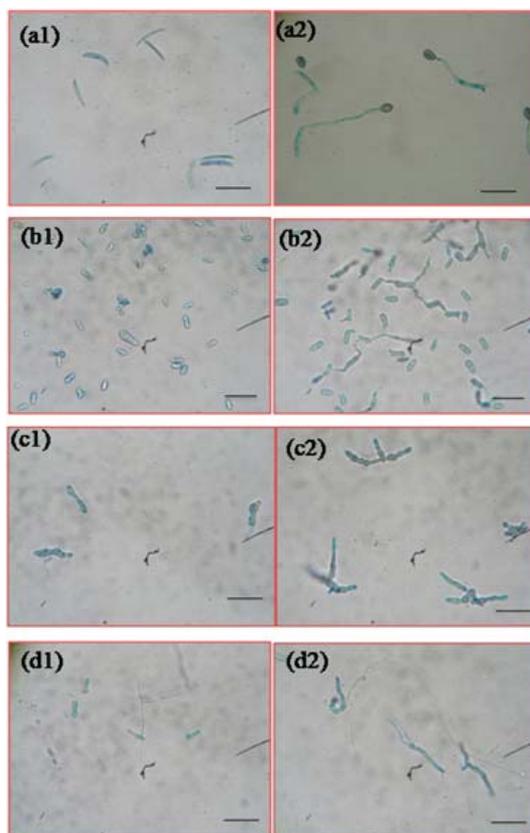


Fig. 2. Effect of culture filtrate produced from *Streptomyces*-PR22 on spore germination of *C. capsici* (a1), *C. acutatum* (b1), *C. gloeosporioides* (c1) and *C. coccodes* (d1) *C. acutatum* compared with sterile distilled water (a2-d2). Scale bar had value 20 μ m

chitinase, 2 1,3glucanase, and protease. These hydrolytic enzymes, especially chitinases and 2 -1, 3-glucanases, may play an important role for inhibition of *Colletotrichum* mycelium growth resulting in degradation of fungal cell wall²⁴. Previous research showed that *Streptomyces aureofaciens* highly produced extracellular chitinase and 2 -1,3-glucanase to inhibit growth of *C. gloeosporioides* of mango anthracnose¹¹ and *S. cavourensis* subsp. *cavourensis* SY224 produced lytic enzymes such as chitinase, 2 -1,3-glucanase, lipase, and protease, resulting in high growth inhibition of *C. gloeosporioides* infecting pepper plants¹². *Streptomyces*-PR22 showed the greatest inhibition of mycelium growth of 4 species of *Colletotrichum* while hydrolytic enzymes production was no significant difference compare with other isolates, so *Streptomyces*-PR22 may be secreted antifungal compound more than other isolates^{7,8}.

Culture filtrate from extracellular of *Streptomyces*-PR22, produced the greatest inhibition of spore germination of all tested *Colletotrichum* species while the other *Streptomyces* presented good results for some species only: PR33 for *C. capsici*, *C. acutatum*, and *C. gloeosporioides*, PR87 for *C. gloeosporioides* and *C. coccodes*, PR15, PR78 and PR84 for *C. coccodes*. This study found that *Streptomyces*-PR15, PR78, PR84 and PR87 could produce both proteolytic and gelatinolytic activity, but *Streptomyces*- PR22 and PR33 had no proteolytic activity, so that component in culture

Table 5. Yields percentage of anthracnose incidence and disease severity of long cayane var. Num Kiew in greenhouse

Treatments	No. fruits/plant	Fruit weight/plant (g)	Fruit length (cm)	Anthracnose incidence (%)	Anthracnose severity index
Strep-PR22 flowering	98.0	901.37	11.73	35.9b	3.0b
Strep-PR22 Seedling	106.0	953.69	12.14	24.3b	3.0b
Strep-PR87 flowering	98.1	880.9	12.14	28.0b	3.0b
Strep-PR87 Seedling	94.3	917.88	12.15	29.4b	3.0b
Chemical	88.7	824.19	11.94	31.3b	3.5ab
Inoculated control	107.9	1037.19	11.54	64.8a	4.0a
Uninoculated control	107.2	1032.75	12.08	-	-
C.V. (%)	20.53	18.24	6.47	26.04	8.88

Means within column followed by a common letter are not significantly different according to DMRT ($P < 0.05$).

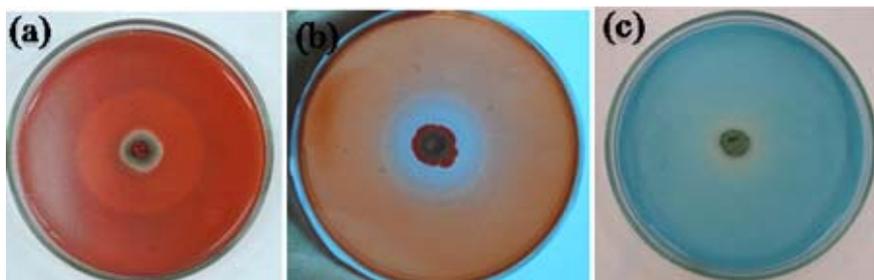


Fig. 3. Production of hydrolytic enzymes by *Streptomyces* isolates: (a) chitinase on CHDA, (b) β 1,3 glucanase on laminarin AGMA and (c) protease on Casein agar medium

filtrate from them may be responsible for the inhibition of spore germination of *Colletotrichum*. Many reports indicated hydrolytic enzymes i.e. chitinase, α -1, 3-glucanase and protease inhibited spore germination^{25,26}. Palaniyandi *et al.*²⁷ showed that *S. phaeopurpureus* ExPro138 produced several extracellular proteases (proteolytic and gelatinolytic activity) inhibiting spore germination, spore adhesion to polystyrene surface, and appressorium formation of *C. coccodes*. *Streptomyces*-PR22 showed a broad spectrum for inhibition of spore germination while creation of hydrolytic enzymes were similar to other isolates. Spores of all 4 species *Colletotrichum* incubated with culture filtrate from *Streptomyces*-PR22 had similar characteristics as spores were normal structure of cell wall but inner component lysis compared with spores in sterile distilled water (Fig. 4). In addition, the researcher reported an antifungal compound inhibited spore germination of fungi such as the iturin-like compound produced by *B. subtilis* YM 10-20 permeabilizes fungal spores and blocks germination of *Penicillium roqueforti* conidiospores²⁸.

The potential of *Streptomyces*-PR22 and -PR87 to control anthracnose disease in chilli plants was evaluated in a greenhouse experiment with two varieties of chilli (*Capsicum annuum* var. Prik kheenu, and *C. annuum* var. Num Khew) and artificial inoculation with *C. acutatum*. The suspension formulation of tested *Streptomyces* spp. was sprayed weekly from seedling or from flowering and continued weekly spray until harvest compared with fungicide benomyl treatment. The results showed that both *Streptomyces*-PR22 and *Streptomyces*-PR87 reduced the number of diseased fruits in both chilli cultivars, similar to fungicide

benomyl application. This result was agreeable with a report of Lee *et al.*²⁹, *Streptomyces* sp. A 1022 formulated into solid concentrate were protective for pepper plants from anthracnose under greenhouse conditions. The application of *Streptomyces*-PR22 and *Streptomyces*-PR87 formulations is a good biocontrol agent for management of anthracnose disease.

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