

Research Article

Open Access

Streptomyces ambofaciens S2 - A Potential Biological Control Agent for *Colletotrichum gleosporioides* the Causal Agent for Anthracnose in Red Chilli Fruits

Jeffrey Lim Seng Heng^{1*}, Umi Kalsom Md Shah², Nor 'Aini Abdul Rahman², Khozirah Shaari³ and Halizah Hamzah¹

¹Strategic Resources Research Centre, Malaysia Agricultural Research and Development Institute, Mardi Headquarters, Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia

²Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

³Faculty of Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

Abstract

Streptomyces ambofaciens S2 was chosen to study its ability to control *Colletotrichum gleosporioides* in chilli fruits. Soil samples were collected from Malaysia Agriculture Research Development Institute (MARDI) Pontian Research Station in Johor Darul Takzim, Malaysia. *Streptomyces* were later isolated from the soil samples and subjected to antifungal screening, metabolites characterization and *in vivo* testing of the potential microbes. In this study, 110 isolates of streptomycetes were successfully isolated from peat soil samples collected from Malaysia Agriculture Research Development Institute (MARDI) Pontian Research Station in Johor Darul Takzim, Malaysia. Screening for antifungal activity showed that 10 isolates of streptomycetes gave antifungal inhibition zone of 8-16 mm separately. *Streptomyces ambofaciens* S2 was later chosen for further testing based on the widest antifungal inhibition zone exhibited (16 mm). Characterization of *S. ambofaciens* S2 using both light microscope and scanning electron microscope showed that, *S. ambofaciens* S2 spores appeared to be rough while the spore chain arrangement was long and spiral. *In vivo* testing on *S. ambofaciens* S2, showed that *C. gleosporioides* infected chilli fruits sprayed with *S. ambofaciens* S2 extract did not showed any sign of infection when compared with chilli fruits sprayed with ethyl acetate. Minimal inhibition concentration (MIC) performed on *S. ambofaciens* S2 against *C. gleosporioides* was observed to be 0.8125 mg/ml. The test conducted showed that *S. ambofaciens* S2 maybe an alternative biopesticide for control of *C. gleosporioides*. However, further tests should be in place to ascertain the viability and toxicity of the extract towards human health and environment.

Keywords: Anthracnose; Biocontrol agent; Antifungal activity; *Streptomyces ambofaciens* S2; Chilli

Introduction

Anthracnose disease on chilli fruits has been known to be a serious problem faced by most of the chilli planters other than Chilli Mosaic Virus (CMV). Once the fruit had been infected by anthracnose the fruit is deemed useless and need to be sorted out to reduce further infection on other fruits. Typical symptoms of anthracnose attack on chilli fruits are sunken necrotic tissues, with concentric rings of acervuli. The most widely spread *Colletotrichum* spp. found in the red chilli fruits are the *Colletotrichum capsici* and *Colletotrichum gleosporioides*.

Soil borne *Streptomyces* had been long known as the main producer of antibiotics in the world [1,2]. Scientists stated that at least 5000 documents relating to the production of bioactive compounds by actinomycetes were produced by the genus streptomycetes [3]. Jeffrey has stated that actinomycetes isolated from Sarawak farming soil were capable of inhibiting the growth of plant pathogens such as *Fusarium palmivora*, *Pantoea dispersa* and *Ralstonia solanacearum* [4]. Ndonde and Semu [5] in their study also noted that most of the *Streptomyces* spp. isolated from Tanzanian soils gave potential antimicrobial activities towards *Clavibacter michiganensis* sp. *michiganensis* (CMM IPO 542) and *Xanthomonas vasicatoria*. Taechowisan et al. [6] showed that *Streptomyces* spp. SRM1 isolated from Nakorn Pathom, Thailand produced antagonistic activity on *Colletotrichum musae* on banana fruits. Shimizu et al. [7] and Yang et al. [8], stated that biocontrol agents have the potential to replace the currently widely used chemical pesticides. In agriculture practices, actinomycetes particularly the genus of streptomycetes has been used to control several plant diseases [9,10].

In this present study, isolation of *Streptomyces* spp. from peat soil

were carried out and tested for their capability to produce antifungal substances towards anthracnose disease on chilli fruits.

Materials and Methods

Isolation and enumeration of streptomycetes

Soil samples were collected 15 cm below the surface of the soil under the canopy of selected plants at MARDI Research Station Pontian, Johor which was located at latitude 1°30'U and longitude 103°27'T. This research station was meant for the plantation of peat soil plants. The soil suspension was agitated with an orbital shaker at 250 rpm for approximately 1 h. Hundred and fifty microliter of the suspension was pipetted and spread onto Starch Casein Agar (SCA) [11] after 1 h of agitation. The plates were then incubated at 28 ± 2°C for 14 days before selection of emerging streptomycetes [5]. Selected streptomycetes were then subcultured onto fresh SCA plates and further incubated for 7 days before the pure colonies were used for the test and kept as stock in 20% (v/v) glycerol.

***Corresponding authors:** Jeffrey Lim Seng Heng, Strategic Resources Research Centre, Malaysia Agricultural Research and Development Institute, Mardi Headquarters, Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia, Tel: +603-8943 7430; Fax: +603-8943; E-mail: shlim11@yahoo.com

Received October 14, 2014; **Accepted** May 26, 2015; **Published** June 15, 2015

Citation: Heng JLS, Shah UK, Rahman NAA, Shaari K, Hamzah H (2015) *Streptomyces ambofaciens* S2 - A Potential Biological Control Agent for *Colletotrichum gleosporioides* the Causal Agent for Anthracnose in Red Chilli Fruits. J Plant Pathol Microbiol S1: 006. doi:[10.4172/2157-7471.S1-006](https://doi.org/10.4172/2157-7471.S1-006)

Copyright: © 2015 Heng JLS, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Preparation of pathogens test strain

Colletotrichum gloeosporioides were isolated from infected red chili fruits using surface sterilization method. In this method, infected chili fruits were cut at the infected site and dipped into 2% sodium hypochloride for 2 min. After which, the infected sites were washed with sterile distilled water before being submerged into 75% ethanol for 30 sec. The pieces of cut chilli fruits were then left to dry in laminar flow for about 30 min before being placed onto fresh Potato Dextrose Agar plates (PDA). The plates were then incubated for about 21 days for the emerging of *Colletotrichum* spp. The emerging fungal colonies were picked and grown on fresh PDA for another 8 days to ensure the purity of the isolates. The pure isolates of *Colletotrichum* spp. were identified based on their conidia morphology using research microscope (Meiji MX 5000) and also molecular method using the ITS 1 and ITS 4 primers [12], before proceeding to be screened for the antagonistic activities.

Primary Screening for antimicrobial activity

Antimicrobial test was performed by using agar diffusion technique. In this technique, the test pathogens were inoculated on the middle of the petri dish surrounded by two plugs of the streptomycetes, one plug of PDA which act as negative control and one plug containing cycloheximide as positive control were placed in the petri dish (Figure 1). All the plates were then incubated at $28 \pm 2^\circ\text{C}$ for 5 days. Positive results were determined with the formation of clear zones. All tests were conducted in a triplicate manner. Antifungal activity was evaluated using rating suggested by Baniyadi et al. [13] from the modification of rating by Lee and Hwang [14], whereby 0-4 mm= no inhibition, 5-9 mm=weak inhibition, 10-19 mm=moderate inhibition and ≥ 20 mm=strong inhibition.

Secondary screening

Selected streptomycetes isolates were grown in a 250 ml Erlenmeyer flask containing 100 ml of SCB at $28 \pm 2^\circ\text{C}$ for 3 days. Broth containing streptomycetes were later extracted using ethyl acetate. Equal volume of ethyl acetate was added to the 250 ml Erlenmeyer flask containing 100 ml of three days cultures. The flasks were later incubated at $28 \pm 2^\circ\text{C}$ with continuous agitation using an orbital shaker at 250 rpm for one hour. After one hour, the organic phase was pipetted into the 50 ml centrifuge tubes and was centrifuged at 4°C for 10 min at 8000 rpm. The supernatant obtained were then filtered using $0.45 \mu\text{m}$ nitrocellulose filter. Fifteen microliter of the filtrate which contained the crude antifungal extract was inoculated onto each sterile filter paper discs.

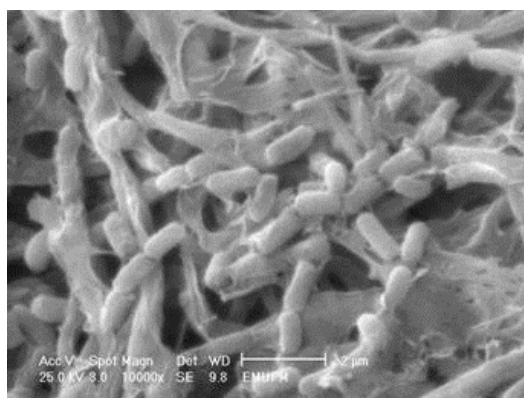


Figure 1: Spores morphology of *Streptomyces* sp. S2 viewed under scanning electron microscope using 10000x magnification.

The discs were air dried in a laminar flow for about 30 min. Dried filter paper discs were then placed onto the prepared assay plates and further incubated at $28 \pm 2^\circ\text{C}$ for 5 days. The sizes of the clear zones formed were measured on the 5th day of the incubation. Tests were conducted in triplicate manner.

Identification of selected actinomycetes

DNA extraction method as described by Vivantis was followed (<http://www.vivantis.com>) with little modification. Supernatant containing R1 buffer and lysozyme (50 mg/ml) was incubated at 37°C for 40 min. Another modification was the incubation time after the addition of R2 buffer and Proteinase K, where the time was prolonged to 40 min at 65°C . All other protocols were as stated by the manufacturer. DNA extracted was later used for Polymerase Chain Reaction (PCR).

PCR parameter and conditions used were as stated by Jeffrey [4]. The PCR products were later subjected to purification using Vivantis GF-1 Gel DNA recovery kit. Protocol used was as stated by the manufacturer (<http://www.vivantis.com>). The purified PCR products were sent for sequencing at First Base Laboratories Sdn. Bhd., Selangor using ABI PRISM 377 DNA Sequencer (Applied Biosystems). Results obtained from the sequencing were blast thru NCBI database [15].

Microscopic examination

Microscopic identification of selected *Streptomyces* spp. were conducted using cover slip methods [16]. In this method, streptomycetes were streaked onto SCA. Sterilized cover slips were carefully stabbed onto the agar at the angle of 45° and the culture plates were incubated at 30°C for 6 days. Cover slip was then taken out from the agar and put onto prepared slide. Crystal Violet dye was used for the staining of the slide. Spore chain arrangement was then viewed using a light microscope (Meiji MX 5000 series). To view the spore morphology of selected streptomycetes, Scanning Electron Microscope (EL-30) was used.

Biochemical characterization of *Streptomyces ambofaciens* S2

Biochemical characterizations of *Streptomyces ambafaciens* S2 was performed using BIOLOG Gen III microplate. Protocol used was as stated by the manufacturer (<http://www.biolog.com>).

Characterization of *Streptomyces ambofaciens* S2 cultural characteristic

Pure colonies of *Streptomyces ambafaciens* S2 was grown in different International Streptomyces Project (ISP) Media for 7 days at $28 \pm 2^\circ\text{C}$. After 7 days, the morphology of *Streptomyces ambafaciens* S2 in different media were evaluated and recorded.

Characterization of *Streptomyces ambofaciens* S2 physiological characteristic

Physiological characterization includes the growth of streptomycetes isolate at selected temperature, pH [17] and also the ability of the isolate to produce various enzymes (lipase, cellulase, protease, mannanase and xylanase).

Spore germination test

Spore germination test was conducted by preparing the spore concentration in a serial dilution manner (10^{-6} - 10^{-1}). The dilution of spores were later plated onto freshly prepared potato dextrose agar (PDA). Spores were incubated at 30°C for 24 hours before being observed using a microscope to determine the germination of spores.

In vivo testing for the effectiveness of *Streptomyces ambofaciens* S2 to control anthracnose

Chilli fruits were bought from the local market for the testing purposes. Chilli fruits were surface sterilized by dipping the fruits in a 2% sodium hypochloride solution for 2 min and 75% ethanol for 30 sec, the fruits were left to dry before being dipped into solution containing 1×10^5 *C. gleosporioides* spores. The inoculated chilli fruits were then incubated for 1 h in a moist container. After 1 h the chilli fruits were sprayed with 0.5 mg/ml of *S. ambofaciens* S2 crude extract. The chilli fruits were then incubated at $28 \pm 2^\circ\text{C}$ for 5 days. After 5 days the chilli fruits were evaluated for any infection by *C. gleosporioides*. Tests were conducted in triplicates manner. Negative control for the test is pure ethyl acetate while 50 mg/ml of cycloheximide was used as the positive control.

Minimal Inhibitory Concentration test

Minimal inhibitory concentration (MIC) of the selected *Streptomyces* sp. crude extract towards *C. gleosporioides* was investigated. The MIC test was conducted in a serially diluted two-fold of concentration ranging from 60 mg/ml, 30 mg/ml, 15 mg/ml, 7.5 mg/ml, 3.25 mg/ml, 1.625 mg/ml, 0.8125 mg/ml, 0.4062 mg/ml, 0.2031 mg/ml, 0.1015 mg/ml and 0.0508 mg/ml. All tests were conducted in triplicate. A standard curve was plotted using cycloheximide to estimate the relative concentration of the crude extract towards a known antifungal compound.

Results

Isolation and enumeration of streptomycetes

From the total of 110 isolates of streptomycetes isolated, observation on the aerial mycelium colour showed that 45.5% (50 isolates), 21.8% (24 isolates), 20% (22 isolates) and 12.7% (14 isolates) of the isolates were found to be white, grey, dark grey and red, respectively. A total of 38 isolates were observed to produce diffusible pigmentation on the agar plate. It was also observed that the colony forming unit per gram (cfu/g) of dry soil for actinomycetes range from 4.2×10^3 to 6.0×10^4 (Table 1).

Identification of pathogen strains

Fungal *Colletotrichum gleosporioides* was identified according to the blast results obtained from the National Center for Biotechnology Information (NCBI) database.

Antifungal activity

Test results of the primary screening, revealed that 44 isolates (40%) of the streptomycetes gave antifungal activity towards *C. gleosporioides*. Upon secondary screening was done only 10 isolates of actinomycetes were selected based on the inhibition zone of 8 -15 mm (Table 2). It was observed that highest antifungal activities of streptomycetes were obtained from rhizosphere of *Stevia rebaudiana* plant while the lowest antifungal activities were obtained from rhizosphere of *Ananas comosus* plant. Streptomycetes isolate number S2 and S4 gave the highest antifungal activities towards *C. gleosporioides* with 15 mm in diameter of clear zone observed. Streptomyces sp. S2 was selected for further tests.

Molecular identification of *Streptomyces* sp. S2

From the blast result performed, it was confirmed that Streptomyces sp. S2 was from the genus of streptomycetes and species of ambofaciens. This isolate was given the name as *Streptomyces ambofaciens* S2.

Metabolite fingerprinting of *Streptomyces ambofaciens* S2

Streptomyces ambofaciens S2 was observed to utilize simple sugar such as Gentiobiose, D-Turanose, Stachyose, D-Raffinose, D-Melibiose, A-Acetyl-β-D-Mannosamine, α-D-Glucose, D-Manose, D-fructose and D-Galactose. Detailed of the results were tabulated in Table 3.

Cultural characterization and microscopic identification of *Streptomyces* sp. S2

Microscopic identification of *Streptomyces ambofaciens* S2 using SEM showed that the spore surface was rough (Figure 1) while the spore chain arrangement was observed to be long and straight (Figure 2) when viewed using research microscope. Physiological test showed that *S. ambofaciens* S2 grew on 4% NaCl, pH 5 and 11 and temperature of 42°C (Table 3). From the observation on different ISP media plates, it was observed that *S. ambofaciens* S2 produces only grey, white or yellow aerial mycelium when grown on different agar media (Table 4).

In vivo testing

Chilli fruits that were sprayed with 0.5 mg/ml of crude extract from *S. ambofaciens* S2 were observed to be free from the inhibition of *C. gleosporioides* after 5 days. Meanwhile, chilli fruits which were sprayed with the ethyl acetate (negative control) were observed to be fully colonized by the fungi after 5 days of incubation (Figure 3).

MIC test for *Streptomyces ambofaciens* S2

The MIC test conducted showed that *S. ambofaciens* S2 has the MIC of 0.8125 mg/ml (0.3 cm of clear zone) towards *C. gleosporioides*. The MIC produced by *S. ambofaciens* S2 was approximately 3.0 mg/ml of cycloheximide

Discussion

The ability to use effective microorganisms or its secondary metabolite to control plant pathogens had open up a doorway to a more sustainable and environmentally friendly solution to pests and

Soil sample collected from the rhizosphere of:	Number of actinomycetes isolated	cfu/g of dry soil
<i>Stevia rebaudiana</i>	25	6.0×10^4
<i>Coffea Arabica</i>	20	3.2×10^4
<i>Musa</i> sp.	16	8.0×10^3
<i>Ananas comosus</i>	22	4.2×10^3
<i>Ipomoea batatas</i>	27	1.4×10^4

Table 1: Colony forming unit of actinomycetes for the soil samples collected.

Isolate no.	Antifungal activity (mean \pm SD*)		Aerial mycelium colour	Pigmentation
	<i>Colletotrichum capsici</i>	<i>Colletotrichum gleosporioides</i>		
B12	8 ± 0.5	10 ± 0.5	White	Nil
B17	9 ± 1.0	11 ± 1.32	White	Nil
K4	10 ± 0.5	8 ± 0.87	White	Nil
K19	9 ± 1.32	12 ± 0.5	White	Nil
N2	9 ± 0.5	8 ± 0.5	Grey	Nil
P1	10 ± 1.32	11 ± 0.87	Grey	Nil
S2	16 ± 0.87	15 ± 0.5	Dark grey	Brown
S4	12 ± 0.5	15 ± 0.5	Dark grey	Nil
V5	8 ± 1.32	10 ± 1.32	White	Nil
V10	10 ± 0.87	9 ± 1.32	White	Brown

*SD: Standard Deviation.

Table 2: Antifungal activity of 10 potential isolates of actinomycetes.

Parameter	Selected actinomycetes isolates
Strain number	S2
Strain identity	<i>Streptomyces</i> spp.
Morphology characterization	
Colour of substrate mycelium	White
Colour of aerial mycelium	Dark Grey
Spore chain arrangement	Long and Spiral
Spore surface	Smooth
Pigment production	+
Enzymatic activity	
Lipase activity	-
Cellulase activity	+
Protease activity	-
Mannanase activity	-
Xylanase activity	+
Hydrolysis of starch	+
Hydrolysis of casein	+
Physiological properties	
Grow on:-	
Starch Casien Agar	+++
Nutrient Agar	+
1% NaCl	++
4% NaCl	+
25°C	+++
37°C	+++
42°C	+
pH 5	+
pH 7	+++
pH 9	++
pH 11	+
Carbon source utilization	
Dextrin	-
D-Maltose	-
D-Trehalose	-
D-Cellobiose	-
Gentiobiose	+
Sucrose	+
D-Turanose	+
α-D-Glucose	+
D-Raffinose	+
α-D-Lactose	-
N-Acetyl-D-Galactosamine	+
N-Acetyl Neuraminic Acid	+
D-Melibiose	+
D-Mannose	+
D-Fructose	+
D-Galactose	+
3-Methyl Glucose	-
L-Rhamnose	-
D-Glucose-6-PO4	+
D-Sorbitol	-
D-Mannitol	-
D-Arabitol	-
Pectin	-
Glycerol	-
Gelatin	-
L-Histidine	-
L-Serine	+

D-Galactonic acid	+
D-Glutamic acid	+
D-Glucuronic acid	+
Mucic acid	+
α-Keto-Butyric acid	+
Propionic acid	-
Acetic acid	-
Acetoacetic acid	-
L-Lactic acid	-

Note: + indicate positive reaction, - indicate negative reaction for all test except for physiological properties test whereby; + indicate less growth, ++ indicate moderate growth and +++ indicate good growth.

Table 3: Morphological, enzymatic and physiological characteristics of *Streptomyces* sp. S2.

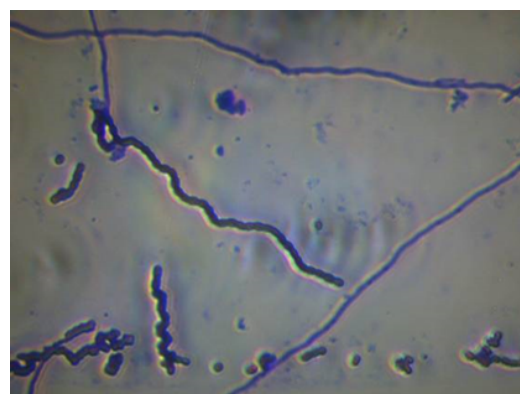


Figure 2: Spore chain arrangement for *Streptomyces* sp. S2 viewed under research microscope using 1000X magnification as shown by red arrows.

Cultural characteristic	Aerial mycelium/substrate mycelium (Colour)
ISP 1	Grey/brown
ISP 2	White/yellow
ISP 2 (with 5% NaCl)	Yellow/yellow
ISP 3	Yellow/yellow
ISP 4	Grey/white
ISP 4(addition of yeast extract)	Grey/white
ISP 4(addition of glucose)	Grey/white
ISP 5	Yellow/yellow
ISP 6	Grey/white

Table 4: Cultural characteristics of *Streptomyces* sp. S2.

diseases issues. According to Gohel et al. [18] fungal plant diseases accounted approximately one third of the world fungal-plant infection.

It has been widely known that soil rhizosphere representing a unique biological niche with a diverse of microorganisms living in it [19]. A total of 110 isolates of streptomycetes were isolated from 5 soil samples collected from this study. It was observed that the majority of the streptomycetes showed the aerial mycelium of white followed by grey. The results obtained, however did not correlate with study done by Jeffrey [4] and Ndonde and Semu [5], where grey aerial mycelium streptomycetes were noted as the most frequently and predominantly isolated streptomycetes from both Sarawak and Tanzania soil, respectively. The differences of population may be due to the pH and soil moisture content of the soil.

The population density of streptomycetes (cfu/g) in this study

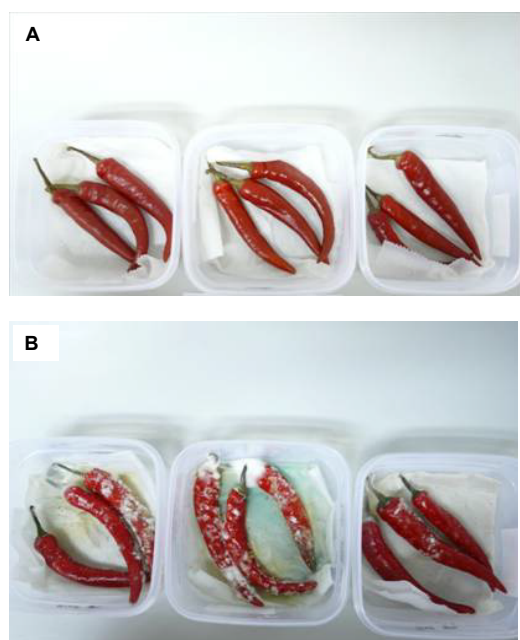


Figure 3: *In vivo* testing of *Streptomyces* spp. S2 extract on chilli fruits which have been infected with anthracnose. [A; Spray with *Streptomyces* Sp. S2 extract from ethyl acetate extraction, B; Spray with ethyl acetate (negative control)].

was much lower compared to study by Jeffrey [4]. He showed that the range for streptomycetes isolated from an agriculture site at Semonggok, Sarawak was 9.8×10^6 – 8.0×10^7 cfu/g [4]. The low number of actinomycetes population in this study may be due to the condition of the rhizosphere itself whereby the actinomycetes were isolated from acidic soil. According to Suzuki et al. [20], soil with low acid level will suppress the population of streptomycetes. This is because streptomycetes can be widely found in soil with pH value of 5.0 to 8.0 while other genera of actinomycetes could be normally isolated from the neutral or alkaline soil [20].

Highest antifungal activity was observed from the rhizosphere of *Stevia rebaudiana* plant while the lowest antifungal activity was observed from the rhizosphere of *Ananas comosus* plant. This could be due to the reason that diversity of a microbial community in specific environment was dependent upon the type of plant species. Only 9.1% (10 potential isolates) of the total isolates gave showed potential for as a biocontrol agent in the secondary screening. Khamna et al. [21] stated that the negative results obtained from other actinomycetes may suggest that the bioactive compounds produced are not meant for the tested fungi but for other untested microorganisms [4]. Another reason may be due to the antifungal compounds produced was not strong enough to inhibit the *Colletotrichum* sp. used in the study. Third reason for the low antifungal activity may be related to the cultivation condition of *S. ambofaciens* S2. According to Porter [22], different cultivation condition might trigger the production of different antimicrobial metabolite.

The ability of *Streptomyces ambofaciens* S2 to inhibit the infection of anthracnose disease on chilli fruits during the *in vivo* trial, may suggest to the alternative application of the antifungal compounds for post-harvest usage other than pre-harvest usage.

Conclusion

Streptomyces ambofaciens S2 showed the ability of producing antifungal activity towards *C. gleosporioides* was able to grow on 4% NaCl, pH 5 and 11 and temperature of 42°C. Apart from this *S. ambofaciens* also showed the ability to utilize a high number of carbon sources such as Gentiobiose, D-Turanose, Stachyose, D-Raffinose, D-Melibiose, A-Acetyl-β-D-Mannosamine, α-D-Glucose, D-Manose, D-fructose and D-Galactose. The ability to inhibit the growth of chilli anthracnose showed that *S. ambofaciens* S2 could be used for the control of anthracnose on field or postharvest.

Acknowledgement

The authors would like to take this opportunity to thank the Malaysian government and MARDI for the funding we received under the e-science with the project number 08-03-05-SF0155.

References

- Bérdy J (2005) Bioactive microbial metabolites. J Antibiot (Tokyo) 58: 1-26.
- El-Naggar MY, El-Aassar SA, Abdul-Gawad SM (2006) Meroparamycin production by newly isolated local *Streptomyces* sp. Strain MAR01: taxonomy, fermentation, purification and structural elucidation. J Microbiol 44: 432-438.
- Anderson AS, Wellington EM (2001) The taxonomy of *Streptomyces* and related genera. Int J Syst Evol Microbiol 51: 797-814.
- Jeffrey LSH (2008) Isolation, characterization and identification of actinomycetes from agriculture soils at Semongok, Sarawak. Afr J Biotechnol 7: 3700-3705.
- Ndondwe MJM, Semu E (2000) Preliminary characterization of some *Streptomyces* species from four Tanzanian soils and their antimicrobial potential against selected plant and animal pathogenic bacteria. World J Microbiol Biotechnol 16: 595-599.
- Taechowisan T, Chuaychot N, Chanaphat S, Wanbanjob A, Tantiwachwutikul P (2009) Antagonistic effects of *Streptomyces* sp. SRM1 on *Colletotrichum musae*. Biotechnol 8: 86-92.
- Shimizu M, Nakagawa Y, Sato Y, Furumai T, Igaroshi Y, et al. (2000) Studies on endophytic actinomycetes *Streptomyces* sp. isolated from *Rhododendron* and its antifungal activity. J Gen Plant Pathol 66: 360-366.
- Yang L, Xie J, Jiang D, Fu Y, Li G, et al. (2007) Antifungal substances produced by *Penicillium oxalicum* strain PY-1 potential antibiotics against plant pathogenic fungi. World J Microbiol Biotechnol 24: 909-915.
- Baniasadi F, Shahidi Bonjar GH, Baghizadeh A, Karimi Nik A, Jorjandi M, et al. (2009) Biological control of *Sclerotinia sclerotiorum*, causal agent of sunflower head and stem rot disease, by use of soil borne actinomycetes isolates. Amer J Agri Biol Sc 4: 146-151.
- Tahtamouni MEW, Hameed KM, Saadoun I (2006) Biological control of *Sclerotinia sclerotiorum* using indigenous chitinolytic actinomycetes in Jordan. Plant Pathol J 22: 107-114.
- Kuester E, Williams ST (1964) Selection of media for isolation of streptomycetes. Nature 202: 928-929.
- White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR protocols. Eds. Innis MA, Gelfand DH, Sninsky JJ and White TJ. Academic Press, San Diego. PP 315-322.
- Baniasadi F, Shahidi Bonjar GH, Baghizadeh A, Karimi Nik A, Jorjandi M, et al. (2009) Biological control of *Sclerotinia sclerotiorum*, causal agent of sunflower head and stem rot disease, by use of soil borne actinomycetes isolates. Amer J Agri Biol Sc 4: 146-151.
- Lee JY, Hwang BK (2002) Diversity of antifungal actinomycetes in various vegetative soils of Korea. Can J Microbiol 48: 407-417.
- Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, et al. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25: 3389-3402.
- Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST (1994) Bergey's manual of determinative bacteriology. 9th edition. Williams and Wilkins, Baltimore.
- Singh LS, Baruah I, Bora TC (2006) Actinomycetes of Loktak habitat: Isolation and screening for antimicrobial activities. Biotechnol 5: 217-221.

18. Gohel V, Singh A, Vimal M, Ashwini P, Chatpar HS (2006) Bioprospecting and antifungal potential of chitinolytic microorganisms. Afr J Biotechnol 5: 54-57.
19. Merckx R, Dijkstra A, Denhartog A, van Veen JA (1987) Production of root-derived material and associated microbial-growth in soil at different nutrient level. Biol Fertil Soils 5: 126-132.
20. Suzuki K, Nagai K, Suzuki Y (1994) Search for actinomycetes in screening for new bioactive compounds. Actinomycetologica 8: 122-127.
21. Khamna S, Yokota A, Peberdy JF, Lumyong S (2009) Antifungal activity of *Streptomyces* spp. isolated from rhizosphere of Thai medicinal plants. Int J Integr Biol 6: 143-147.
22. Porter JN (1971) Prevalence and distribution of antibiotic-producing actinomycetes. Adv Appl Microbiol 14: 73-92.