academicJournals

Vol. 8(10), pp. 994-1000, 5 March, 2014
DOI: 10.5897/AJMR11.1401
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Impact of media composition and growth condition of antifungal production by *Streptomyces ambofaciens* S2

Jeffrey, L. S. H.* and Halizah, H.

Strategic Resources Research Centre, Malaysian Agriculture Research and Development Institute, Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia.

Received 23 November, 2011; Accepted 17 February, 2014

Anthracnose caused by fungus *Colletotrichum gloeosporioides* is an important disease of chilli in Asia countries such as Malaysia. This fungus makes the chilli fruits to be unmarketable. In this study, *Streptomyces ambofaciens* S2 which gave a high antifungal activity was selected and underwent cultural condition optimization. The optimization involved selecting the best carbon and nitrogen sources, seed age, incubation time, pH, percent NaCl (w/v), and fermentation temperature. It was observed that *S. ambofaciens* S2 gave the highest activity when grown in the present of chitin and peptone as carbon and nitrogen sources, respectively. *S. ambofaciens* S2 also gave optimal cultural growth and antifungal production at pH 8, incubation temperature of 28°C, 2% of NaCl, 6 days of seed age and 3 days of cultural incubation. By studying the impact of each of the cultural conditions, antifungal activity of *S. ambofaciens* S2 has been increased to about 30% in comparison with the use of original conditions.

Key words: *Streptomyces ambofaciens*, anthracnose, chilli, antifungal production, actinobacteria.

INTRODUCTION

Anthracnose fruit rot of chillies is often caused by fungus from the genus of *Colletotrichum* sp. This disease often posed a major constraint for farmers to obtain good profit (Rajapaske, 1999). It is widely spread and often cause severe disease to chilli planted in lowland of Peninsular Malaysia (Mah, 1989). According to Poonpolgul and Kumphai (2007), Thailand suffer severe loses in their chilli production due to anthracnose infection. The use of chemical fungisides not only will pollute the environment but also may cause the microbes to develop resistance towards it (Lodish et al., 1995).

Actinomycetes had been known to be the producers of various antimicrobial activities. Actinomycetes, mainly from the genus of Streptomycetes are well known as biological control agents that inhibit or lyse plant pathogenic fungi (Nakai and Kanehisa, 1991; Nakashima and Nishikawa, 1994). Marten et al. (2000) reported that Rhizovit^{R} from *Streptomyces rimosus* is used for the control of several plant fungal pathogens.

In a study done by Yu et al. (2008), the authors stated that antifungal activity of *S. rimosus* MY02 increased when the culture conditions were adjusted to the optimal

*Corresponding author. E-mail: shlim@mardi.gov.my. Tel: +603-8743 7430. Fax: +603-8743 6766.

metabolite for the control of anthracnose in chilli.

MATERIALS AND METHODS

Isolation of soil streptomyces and screening for antifungal activity

Soil samples were collected from random areas at the agriculture land in Pontian, Johor. Soil samples were taken at the depth of about 15 cm from the top soil and kept in a double zip lock bag. The soil samples were transported back to the laboratory in a cooler box containing ice bags to maintain the natural condition of the soil. Soil samples were air dried for about 1-2 weeks.

Ten grams of the pre-treated soil samples were then added to 100 ml of sterile distilled water and was vigorously shaken with a orbital shaker at 200 rpm for about an hour. Hundred and fifty microliter of the supernatant was later pipetted and spread onto Strach Casien Agar (soluble starch; 10.0 g, casein hydrolysate; 0.3 g, KNO_3; 2.0 g NaCl; 2.0 g; K_2HPO_4; 2.0 g; $MgSO_4.7H_2O$; 0.05 g, $CaCO_3$; 0.02 g, $FeSO_4.7H_2O$; 0.01 g, Agar; 18.0 g, 1.0 ml of Cyclo-heximide (50mg/ml); at pH 7). The plates were later incubated at room temperature (28 ± 2°C) for 14 days. Emerging colonies of *Streptomyces* were later subcultured onto fresh SCA plates and further incubate for 14 days prior to used.

Streptomyces isolated were then tested for their antifungal activity against *Colletotrichum gleosporidease* in a dual culture test. In this test, the Colletotrichum was placed in the middle of the petri dish surrounded by the two plugs of same Streptomyces culture, a positive control disc (50 mg/ml) and a negative control disc. The Petri dish was then incubated at 28 ± 2°C for 5 days. Tests were conducted in triplicate manner.

Effect of incubation period on antifungal activity

To test the incubation period, one plug of the *S. ambofaciens* S2 was inoculated into a 250 ml Erlenmeyer flask contaning 100 ml Starch Casein Broth (SCB). *S. ambofaciens* S2 were grown in triplicate manner for 7 days at 28 ± 2°C to observe the growth pattern. The culture filtrates of each day were tested for their antifungal activity. All results were presented as mean inhibition zone ± standard deviation (SD).

Biomass of *S. ambofaciens* S2 for each day was collected and air dried before weighted. The weight observed for each day was then plot on graph. All results were presented as mean weight ± standard deviation (SD).

Effect of pH and temperature on antifungal activity

S. ambofaciens S2 was grown in a 250 ml Erlenmeyer flask with 100 ml SCB at pH 5.0, 6.0, 7.0, 8.0 and 9.0. *S. ambofaciens* S2 was also test for its ability to produce antifungal activity under controlled incubation temperature of 15, 30, 35 and 45°C. Tests were conducted in triplicate for 3 days at 28 ± 2°C and tested using disc diffusion method. All results were presented in mean inhibition zone ± standard deviation (SD) manner.

Effect of carbon and nitrogen sources on antifungal activity

To determine the effect of carbon sources on antimicrobial metabolites production, different carbon sources; D-glucose, molasses, mannitol, chitin and maltose were added to the SCB medium by replacing soluble starch in the SCB with these carbon sources (soluble starch; 10.0 g, casein hydrolysate; 0.3 g, KNO_3; 2.0 g NaCl; 2.0 g; K_2HPO_4; 2.0 g; $MgSO_4.7H_2O$; 0.05 g, $CaCO_3$; 0.02 g, $FeSO_4.7H_2O$; 0.01 g).

The effect of nitrogen sources such as soy bean, yeast extract, peptone, L-asparagine and beef extract was used to replace casein hydrolysate in the SCB medium. No change of nitrogen source concentration in the media was done. Tests were conducted under pH 7, for 3 days at 28 ± 2°C for both carbon and nitrogen sources. Antifungal activity was checked by disc diffusion method against *C. gleosporioides* after 5 days of incubation. All results were presented in mean inhibition zone ± standard deviation (SD) manner.

Effect of salt (NaCl) on antifungal activity

Salt concentration had been known to cause certain effect on the production of antibiotic from microorganism (Pelczar et al., 1993). The testing of salt concentration effect was done by altering the concentration of NaCl in the SCB. The tested concentrations were 0.1, 0.2, 0.3, 0.4, 0.5 and 1.0% (w/v). Tests were conducted under pH 7, for 3 days at 28 ± 2°C. Disc diffusion method against *C. gleosporioides* was done. Tests were conducted in triplicate and all results were presented as mean inhibition zone ± standard deviation (SD).

Effect of the culture seed age on antifungal activity

Seed age of *S. ambofaciens* S2 was evaluated for their effect on the antifungal production by using different seed age of *S. ambofaciens* S2. *S. ambofaciens* S2 was grown on SCA and one plug (diameter of 0.5 cm) of the culture was taken every 2, 4, 6, 8 and 10 days using a borer (size 5 mm in diameter). The plug of colony was then inoculated into a 250 ml of Erlenmeyer flask with 100 ml of SCB. Tests were conducted under pH 7, for 3 days at 28 ± 2°C. Each test was conducted in triplicate manner. All results were presented as mean inhibition zone ± standard deviation (SD).

Antifungal activity test

Fifteen microliter of *S. ambofaciens S*2 extract from original SCB media and the modified optimal media impregnated separately onto sterile paper disc and placed next to *Coletotrichum gloesporidesea* at a distance of 5 cm apart from each other. Cyloheximide (50 mg/ml) impregnated disc was used as positive control while disc impregnated with sterile distilled water act as negative control. Tests were conducted in triplicate manner and the plates were incubated at 28 ± 2°C for 5 days under sterile condition.

RESULTS

Isolation of actinomycetes

From the total of 110 isolates of *Streptomyces* spp. 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 was in the range of $4.2 \times 10^3 – 6.0 \times 10^4$.

Antifungal activity screening

From the total of 110 *Streptomyces* spp. isolated,

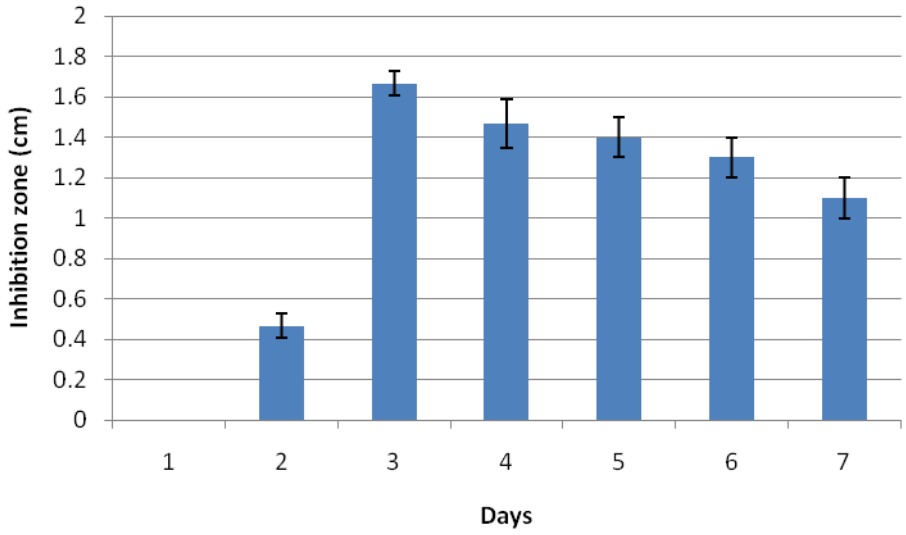

Figure 1. *Streptomyces ambofaciens* S2 incubation period against inhibition zones.

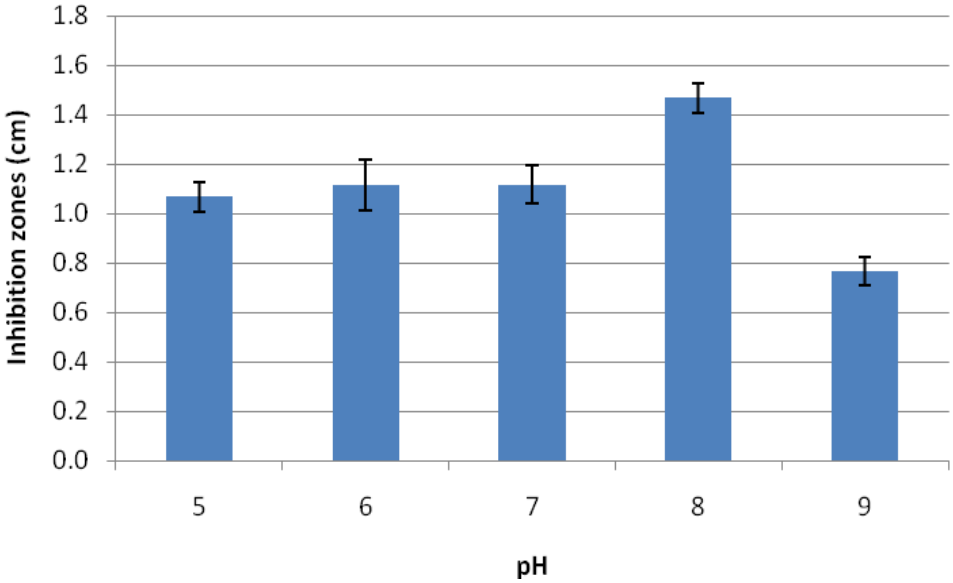

Figure 2. *Streptomyces ambofaciens* S2 pH against inhibition zones.

preliminary screening showed that 44 isolates of *Streptomyces* spp. gave antifungal activity towards *C. gleosporioides*. From these, only one isolate of *Streptomyces* spp. gave the best antifungal activity with the inhibition zone of 15 mm. This *Streptomyces* sp. was later known as *S. ambofaciens* S2.

Impact of cultural condition on the antifungal activity

During the process, we observed that *S. ambofaciens* S2 produced better inhibition zone in these conditions: 3 days of incubation, pH 8, under 28°C, chitin as carbon source, peptone as nitrogen source, when 2% of NaCl was used and 6 days of seed age. Details of the tests are shown in Figures 1 to 7. *S. ambofaciens* S2 showed its lag phase on the 3rd day and followed by the death phase on the 7th day.

Effect of cultural media optimization on antifungal activity

When *S. ambofaciens* S2 was cultured in the optimal cultural media, we observed that *S. ambofaciens* S2 gave an inhibition zone of 20 mm as compared to initial

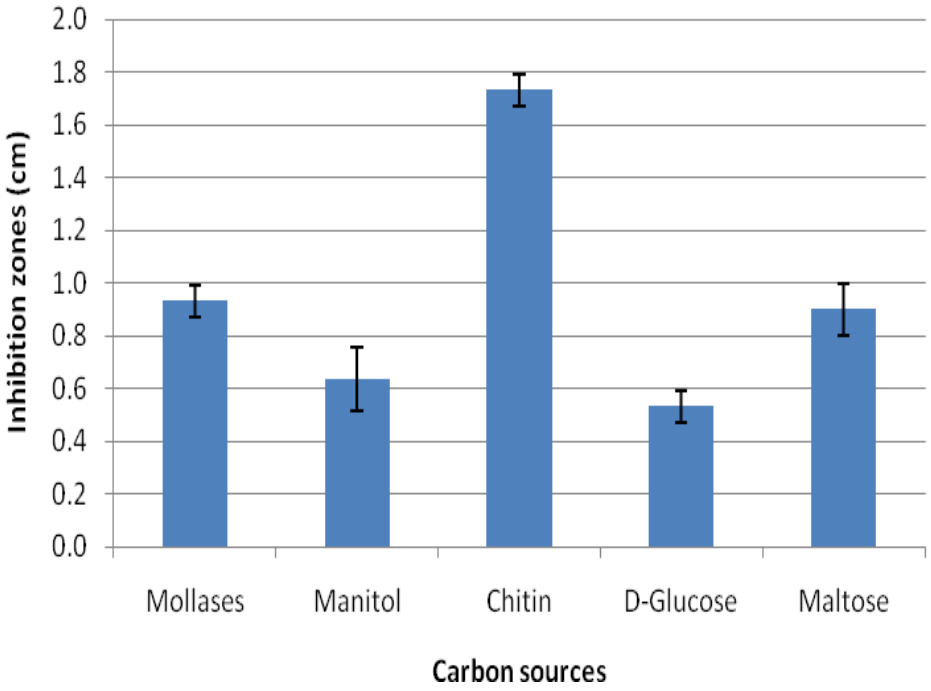

Figure 3. *Streptomyces ambofaciens* S2 carbon sources utilization against inhibition zones.

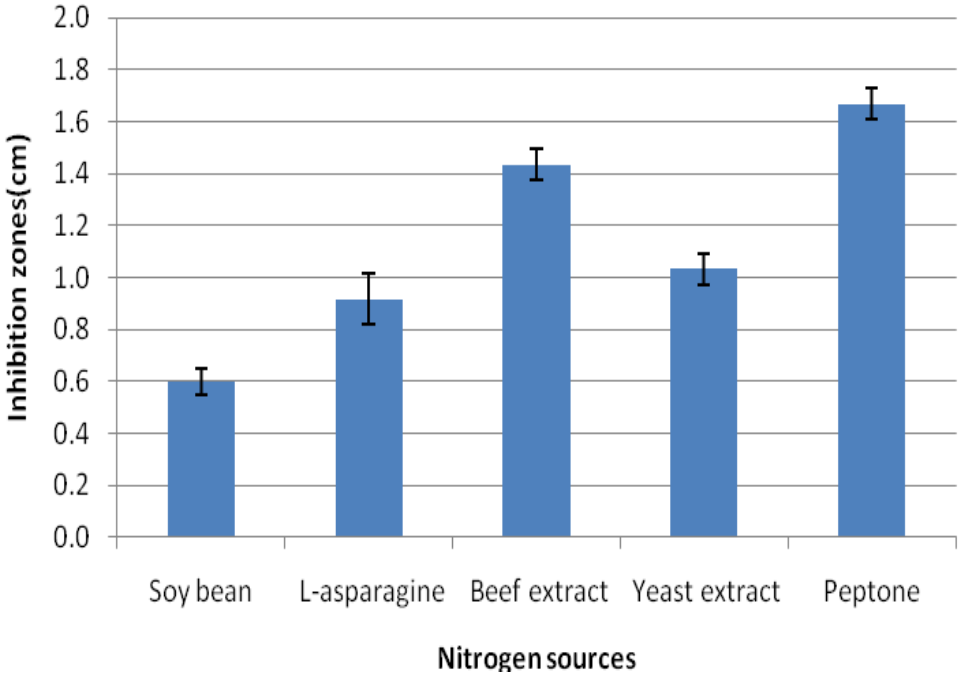

Figure 4. *Streptomyces ambofaciens* S2 nitrogen sources against inhibition zones.

inhibition zone of 15 mm.

DISCUSSION

It has been widely known that soil rhizosphere present a unique biological niche with a diverse of microorganisms living in it (Merckx et al., 1987). The use of 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 diseases issues. Gohel et al. (2006) stated that, fungal plant diseases accounted for approximately one

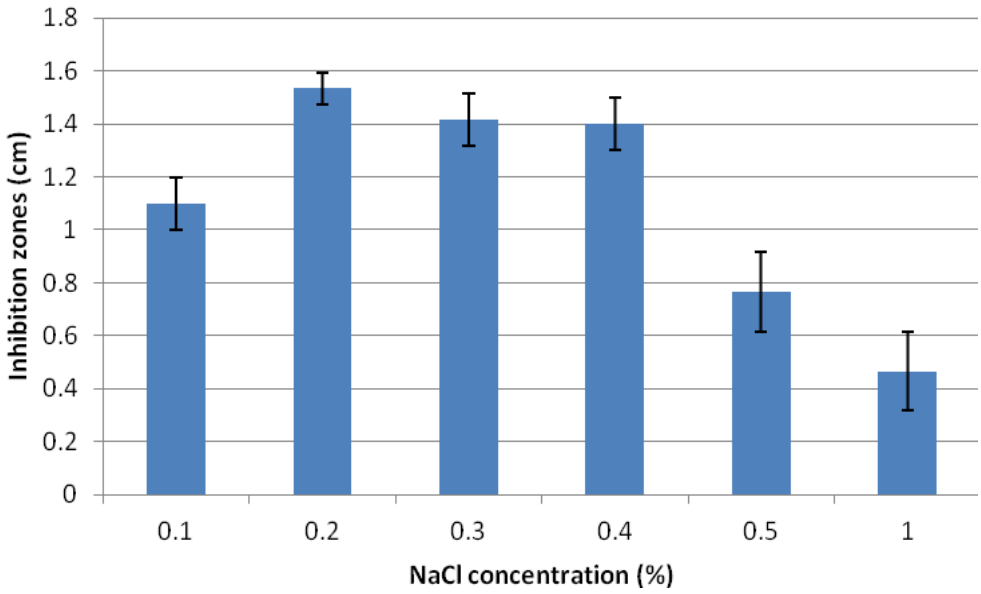

Figure 5. *Streptomyces ambofaciens* S2 NaCl concentration against inhibition zones.

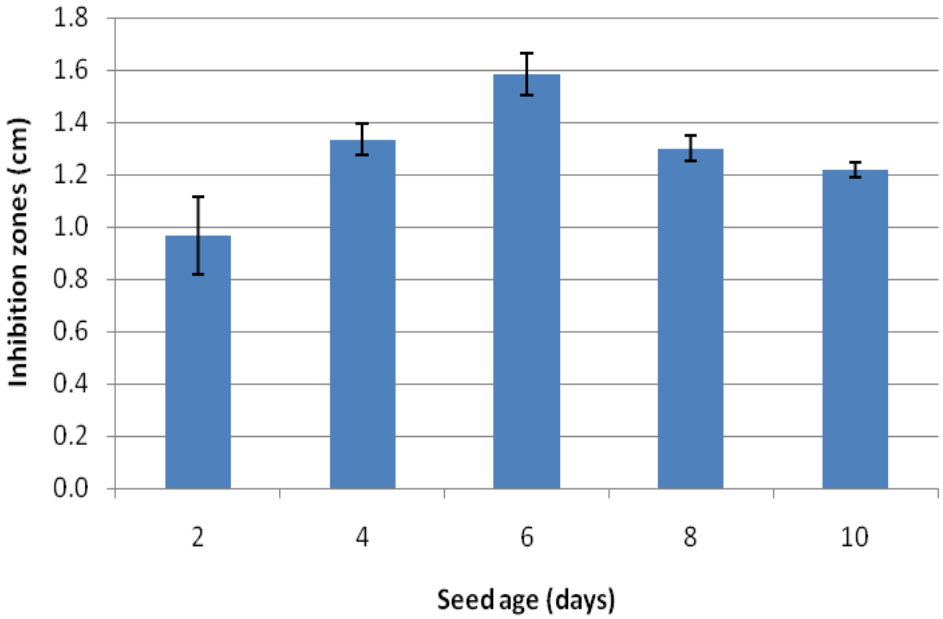

Figure 6. *Streptomyces ambofaciens* S2 seed age against inhibition zones.

third of the world fungal-plant infection. By optimizing the cultural condition of the microbes, we can increase the antifungal activity for microbes of our choice.

From the results we observed that *S. ambofaciens* S2 reached it stationary phase on the 3rd day of incubation. This could be the reason why *S. ambofaciens* S2 gave the highest inhibition zones on the 3rd day of incubation. Study done by Kavitha et al. (2010), showed that *Streptomyces* spp. (A2 and A4) gave the highest antifungal activity on the 5th day and *Streptomyces* sp. (A1) gave the highest antifungal activity on the 4th days although these *Streptomyces* spp. (A1, A2 and A4) reached their stationary phase on the 3rd day of incubation.

Study done by El-Mehalawy et al. (2005) showed that actinomycetes isolated, gave the biggest inhibition zone at pH 7. This however was different from our study whereby,

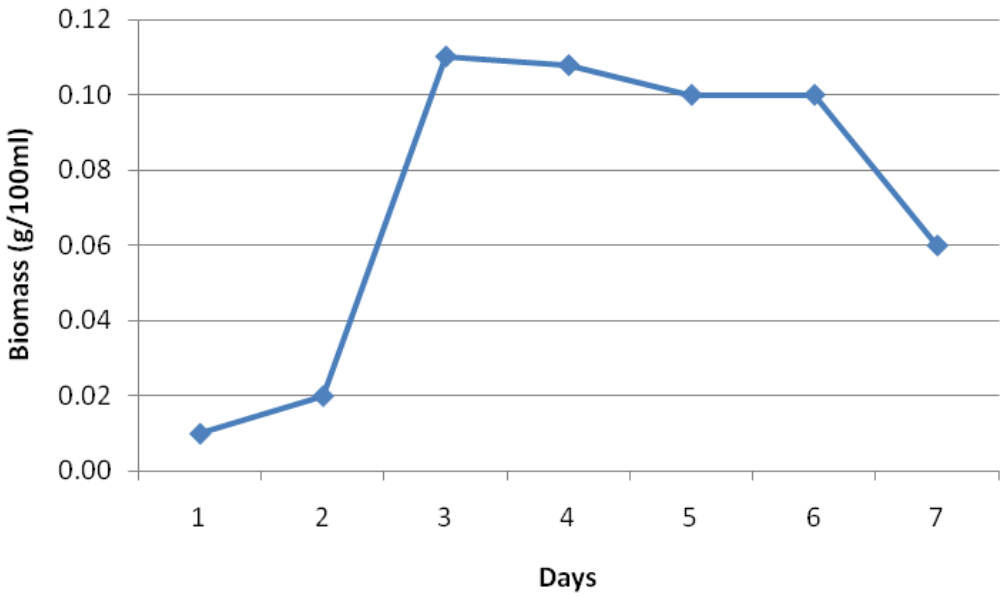

Figure 7. Culture growth pattern of *Streptomyces ambofaciens* S2 on starch casein broth (SCB).

largest inhibition zone were observed at pH 8. El-Abyad et al. (1996) showed that highly acidic or basic media, were not suitable for the antifungal production by many *Streptomyces* species and that neutral media (pH 7.0) were the most favorable for antifungal production. However, in this study this hypothesis was null as *S. ambofaciens* S2 produces the highest antifungal activity when grown on pH 8.

In this study, we observed that chitin and peptone were the two best carbon and nitrogen sources respectively. However study done by Yu et al. (2008) showed that *Streptomyces rimosus* MY02 gave the best antifungal activity when starch and defatted peanut powder were used. El-Mehalawaty et al. (2005), showed best antifungal activity were observed when glycerol was used as the carbon source for all 4 *Streptomyces* spp. used in the test. While ammonium sulphate was the best for *Streptomyces lydicus* and *Streptomyces ederenses*, glutamic acid for *Streptomyces erumpens* and soybean for *Streptomyces antimycoticus.* This shows that each of the *Streptomyces* spp. have their own choices of carbon and nitrogen sources that help them increased their antifungal activity.

S. ambofaciens S2 gave the highest inhibition zone when 0.2% of NaCl was used but the zone size become smaller as the NaCl concentration increased. This showed that *S. ambofaciens* strain S2 could grow on high percentage of NaCl but the secretion of antifungal compounds would be inhibited.

In the cultural age testing, *S. ambofaciens* S2 showed that the highest antifungal activity was obtained when 6 days old seed culture was used. This result however was parallel to the results obtained for *S. rimosus* MY02 which gave the highest antifungal activity when 4-6 days old seed were used (Yu et al., 2008) and S. *hygroscopicus* ATCC 29253 produced the highest amount of rapamycin when 5 and 7 days of seed culture were used (Hamdy et al., 2011).

Conclusion

S. ambofaciens S2 isolated from Malaysia soil showed its antifungal ability towards anthracnose on chilli. The optimized cultural condition showed an improvement in the activity of the antifungal compound to approximately 30%.

Conflict of Interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGEMENT

The authors would like to thank Malaysia government for the fund provided under Sciencefund (RS1250SF10).

REFERENCES

El-Abyad MS, El-Sayed MA, El-Shanoshowry AR, El-Sabbagh SM (1996). Antimicrobial activities of *Streptomyces pulchen*, *Streptomyces canescens* and *Streptomyces citriofluorescens* against fungal and bacterial pathogens of tomato in *vitro.* Folia Microbiol. 41: 321-328.

El-Mehalawy AA, Abd-Allah NA, Mohamer RM and Abu-Shady MR (2005) Actinomycetesantagonizing plant and human pathogenic fungi. II. Factor affecting antifungal production and chemical characterization of the active components. Int. J. Agri. Biol. 7(2):188-196.

Gohel, V., Singh, A., Vimal, M., Ashwini, P, Chhatpar H.S. (2006). Bioprospecting and antifungal potential of chitinolytic microorganisms. Afr. J. Biotechnol. 5: 54-57.

Hamdy AA, El-Refai AF, Sallam LAR, Osman ME, Om Kalthoum HK, Mohamed MA (2011). Seed Stage Manipulation as a Tool for Improving Rapamycin Production by *Streptomyces hygroscopicus* ATCC 29253. Aust. J. Basic Appl. Sci, 5(2): 1-7,

Kavitha A, Vijayalakshmi M, Sudhakar P and Narasimha G (2010). Screening of Actinomycetes strains for the production of antifungal metabolite Afr. J. Microbiol Res. 4: 27-32.

Lodish H, Baltimore D, Berk A, Zipursky SI, Matsudaira P and Darnell J (1995). Molecular Cell Biology, 3rd edition, Scientific American Books, Chapter 3, New York.

Mah, S. T. 1989. Effects of anthracnose fruit rot fungus on chilli variety MC4. *Teknologi Sayura-sayuran.*5:13-16.

Marten P, Bruckner S, Minkwitz A, Luth P, Berg G. (2000). RhizovitR: Impact and formulation of a new baterial product. In: Koch E, Leinonen P (eds), Formulation of Microbial Inoculants, COST Action 830. Microbial inoculants for agriculture and environment, Berlin, German, pp. 78–82.

Merckx R, Dijkra A, Hartog AD, Veen JAV (1987). Production of root-derived material and associated microbial growth in soil at different nutrient levels. Biol. Fertil. Soils 5: 126-132.

Nakai K and Kanehisa M (1991). Expert system for predicting protein localization sites in Gram negative bacteria. Protein:Structure, Func. Gen. 11(1): 95-110.

Nakashima H and Nishikawa K (1994). Discrimination of intracellular and extracellular proteins using amino acid composition and residue-pair frequencies. J. Mol. Biol. 238: 54-61.

Pelczar MJ, Chan ECS, krieg NR (1993). Microbiology: Concept and Application International edition McGraw-Hill, USA, pp 281-324.

Poonpolgul, S. & S. Kumphai (2007). *Chilli pepper anthracnose in Thailand*. In: *First Int. Symp. Chilli Anthracnose.* Convention Center, Seoul National University, Korea: 23. http://www.avrdc.org/publications/proceedings/abstracts_chili_anthracnose.pdf. Accessed on 5th September 2009.

Rajapakse, R. G. A. S. (1999). Mode of anthracnose development in chilli (Capsicum annuum L.) pods. Proceeding of the annual symposium of the Department of Agriculture, Peradeniya, Sri Lanka, pp. 247-266.

Yu J, Liu Q, Liu Q, Liu X, Sun Q, Yan J, Qi X and Fan S (2008). Effect of liquid culture requirements on antifungal antibiotics production by *Streptomyces rimosus* MY02 Bioresource Technol. 99: 2087-2091.