INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
10–394/STS/2011/13–1–1–8
http://www.fspublishers.org

Full Length Article

Effect of Carbon Sources on Bacterial Production of Metabolites against *Fusarium oxysporum* and *Colletotrichum gloeosporioides*

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ABSTRACT

This study was conducted to determine the effect of carbon sources on the production of bacteria metabolites against *Fusarium oxysporum* and *Colletotrichum gloeosporioides*. Antagonistic bacteria were identified by using Biolog® Identification System known as *Malikia spinosa* and *Stenotrophomonas maltophilia*, while the unidentified was named as UPMKB4. For optimizing antimicrobial substances, *M. spinosa*, UPMKB4 and *S. maltophilia* were cultured individually or combination in liquid media with addition of 2 g carbon sources derived from glucose, fructose and glycerol. Results revealed that different types of carbon source amended in liquid media determined the efficacy on the antimicrobial substances. Specific or special types of carbon sources are required to suppress different types of plant pathogenic pathogens effectively. Malformed, shrunken and vacuolar hyphae were observed on the treated plant pathogenic fungi in the presence of antimicrobial substances. In compatibility mixture of antimicrobial substances, combination *M. spinosa* + UPMKB4 proved to be the best treatment to inhibit the growth of *F. oxysporum* and *C. gloeosporioides* with suppression values of 11.00 mm and 12.67 mm, respectively, which were the maximum values recorded in this study. Some modification and improvement are required to find out the most suitable types and quantity of carbon sources in the basal liquid medium under appropriate conditions in order to produce more effective antimicrobial substances against *F. oxysporum* and *C. gloeosporioides.*

Key Words: Glucose; Fructose; Glycerol; Compatibility mixture; *Malikia spinosa*; *Stenotrophomonas maltophilia*; *Fusarium oxysporum*; *Colletotrichum gloeosporioides*

INTRODUCTION

Antagonistic bacteria are considered as ideal biological control agents due to their rapid growth, easy handling and aggressive colonization at rhizosphere of plant roots. These bacteria may mediate biocontrol activities by one or more types of mechanisms of diseases suppression (Weller, 1988). However, a primary mechanism of pathogen inhibition is by producing secondary metabolites. Such bacteria are mostly involved in the biological control of plant pathogenic bacteria and fungi. Antagonistic bacteria produce antimicrobial substances as important compound for self defense function towards other organisms e.g., *Bacillus* sp. producing antimicrobial compounds have been used as biocontrol agents against plant pathogenic fungi (Yilmaz *et al*., 2005). Similarly, *Burkholderia cepacia* complex formerly known as *Pseudomonas cepacia* is a group of nine closely related bacterial species that have useful properties in the natural environment (Chiarini *et al*., 2006) have emerged as powerful biocontrols agents for plant pathogens (Bevivino *et al*., 1998). However, the production of antimicrobial substances depends upon the substrate medium for their optimal growth, temperature, pH and the concentration of nutrients in the medium (Leifert *et al*., 1995). Many studies exploring of beneficial organisms have been carried out, such as *P. fluorescens*, which was one of the examples used for the control of *Fusarium* wilt of tomato. Similarly, *P. fluorescens* were found to be effective biocontrol agents against the *Phytophthora* disease in black pepper (Diby *et al*., 2005).

Carbon as a part of an ingredient in the medium is required for bacterial growth and to enhance the production of antimicrobial substances. Antimicrobial substances produced by bacterial species were greatly influenced by variation of carbon sources (El-Banna *et al*., 2006). Galactose and glucose strongly enhanced the antimicrobial activity of *Corynebacterium kutscheri* and *C. xerosis* respectively, while ribose and lactose repressed their activity (Gebreel *et al*., 2008). *P. fluorescens* in the liquid medium with the addition of glucose and sucrose produced more antimicrobial substances like 2, 4-diacetylphloroglucinol (Duffy & Defago, 1999). Balanced ingredient in medium as nutrition for bacterial growth and production of antimicrobial substances is important. Hence, the objectives of this study were to identify the potential antagonistic bacteria and to study the effect of different carbon sources

To cite this paper: Farhana, M.S.N., M.R. Bivi and A. Khairulmazmi, 2011. Effect of carbon sources on bacterial production of metabolites against *Fusarium oxysporum* and *Colletotrichum gloeosporioides. Int. J. Agric. Biol*., 13: 1–8

on the production of antagonistic bacteria metabolites against selected plant pathogenic fungi.

MATERIALS AND METHODS

Identification of antagonistic bacteria using Biolog® Identification System: Selected of potential antagonistic bacteria isolates collected from previous study (Nur Aimi, 2009) was identified using Biolog® Identification System. The procedures for identification included the used of 95 different dried carbon sources plus one control well. Bacterial isolates were initially determined for gram reaction and oxidase test for categorizing into enteric bacteria or non-enteric bacteria. Bacteria were then cultured onto Biolog Universal Growth (BUG) medium for 24 h at 26±2°C to promote growth and retention of its metabolic activity. Bacterial suspension was prepared in the inoculants' solution (0.1 g Gellan Gum, 4 g NaCl, 0.3 g Pluronic F-68 & 1 L distilled water) with concentration for non-enteric bacteria at 52%, enteric bacteria at 63% and positive gram bacteria at 25-28% transmission using Biolog's spectrophotometer. Bacterial suspension was inoculated into GN or GP micro plates (145 µL per well using the 8-channel repeating pipette) depending on gram reaction cluster. Microplate was covered with its lid and incubated at 28°C to 30°C for 24 h to allow the utilization of carbon sources. Reading result was directly done after inserting the incubated microplate into the Biolog's reader apparatus for identifying the bacteria up to species level (Anonymous, 2001).

Liquid medium preparation: Three liquid media containing (w/v): 0.1 % (w/v); KH_2PO_4, 0.5% $(NH_4)_2SO_4$, 0.05 % (w/v) $MgSO_4$, 0.1% (w/v) KNO_3, 0.05% (w/v) NaCl, 0.1% (w/v) $CaCO_3$ and added with distilled water until the final volume to 80 mL were prepared. Then, 2 g of fructose, glucose and glycerol were incorporated into the liquid medium individually. The liquid medium was autoclaved at 120°C for 15 minutes. After autoclaving, the pH of the liquid medium was adjusted to 7.

Optimizing quality of supernatant containing antimicrobial substances: Three selected bacterial isolates from previous (Nur Aimi, 2009) studies namely *Malikia spinosa* (UPMKB1), UPMKB4 (unknown species) and *Stenotrophomonas maltophilia* (UPMKB9) were used to optimize the quality of antimicrobial substances by using the modified liquid medium. Liquid medium was inoculated with 20 mL of pure bacteria suspension solution from fresh cultures of antagonistic bacteria and placed on orbital shaker rotator at 180 rpm at 37°C for 5 days. Fifty mL methanol was added to the fermented liquid medium to kill bacteria. The supernatant of the liquid medium was separated from bacterial cells using centrifugation (Beckman J2-21) at 9,500 rpm for 15 min at 2°C temperature. The supernatant containing antimicrobial substances were concentrated to reach up to 20 mL with rotary evaporator machine at 45°C. Supernatant containing antimicrobial substances was tested for their potential to suppress pathogenic fungi by dropping 10 µL supernatant onto one 5 mm diameter of fungal plug in Water Agar (WA) plate. The inhibition of diametric mycelial growth of *F. oxysporum* and *C. gloeosporioides* on WA medium was measured after 2 days of incubation at 37°C (Dikin *et al*., 2005). Optimizing quality of supernatant containing antimicrobial substances was based on Dikin *et al*. (2005) methods with some modification at the final volume and the centrifuge speed.

Spore germination test: About 100 µL of suspension (10^{-6} spore/mL spore) of *F. oxysporum* and *C. gloeosporioides* were spread over the Potato Dextrose Agar (PDA) plate using a sterile bent glass rod. Three sterilized filter paper discs (0.6 mm diameter) were placed 3 cm apart on the agar. About 50 µL of antimicrobial substances was pipetted onto each of the paper disc. Discs received 50 µL of sterilized Nutrient Broth (NB) for the control plate. Spore occurring around each disc were examined after 2 days of incubation at 28±2°C (Dikin *et al*., 2005).

Compatibility mixture of supernatant containing antimicrobial substances against plant pathogenic fungi: Three supernatants containing antimicrobial substance from the three antagonistic bacteria in the fermentation of liquid medium were selected. The supernatant containing antimicrobial substances was used to determine the compatibility of combinations supernatant containing antimicrobial substances from 3 bacteria isolates. Equal volume of each prepared supernatant containing antimicrobial solution was mixed well. A 6 mm diametric paper disk was placed on the prepared PDA medium with spores of *F. oxysporum* and *C. gloeosporioides*. Then 100 µL of mixed supernatant containing antimicrobial substances then was spotted on PDA medium with 6 mm diameter of paper disk, and then incubated for 48 h. Formations of diametric clearing zone against these pathogenic fungi were recorded.

Statistical analysis: The experiment in the optimization of antimicrobial substances was conducted in a completely randomized design (CRD). The experiments in the inhibitory concentration and the compatibility of mixed antimicrobial substances against pathogenic fungi were also conducted in a CRD. Recorded data was analyzed with SAS® Software. Treatment effect was tested by ANOVA and the means compared using Duncan's Multiple Range Test (DMRT) at a 5% probability level (Gomez & Gomez, 1984).

RESULTS

Identification of antagonistic bacteria: Three antagonistic bacterial isolates from the previous study were selected based on their suppression potential. These antagonistic bacteria were clustered into Gram-negative and Gram-positive bacteria based on gram test. These bacteria were then further identified using Biolog® Identification System. Results of showed that, two potential antagonistic bacterial

isolates were identified namely, *M. spinosa* and *S. maltophilia*. On the other hand, one isolate of potential antagonistic bacteria could not be identified and this isolate was referred to as UPMKB4 (Table I).

Optimizing quality of supernatant containing antimicrobial substances: *M. spinosa*, UPMKB4 and *S. maltophilia* were made to produce antimicrobial substances in modified liquid medium amended with difference types of carbon sources. Supernatant containing antimicrobial substances produced by *M. spinosa*, UPMKB4 and *S. maltophilia* showed significant suppression ($P<0.05$) of the mycelia growth of *F. oxysporum* and *C. gloeosporioides* (Table II & Table III).

Supernatant containing antimicrobial substances produced by *M. spinosa* was suppressed mycelia growth of *F. oxysporum* significantly as compared to control plate. It also gave the minimum diametric growth value of 4.67 mm in the present of glucose as carbon source in liquid medium. Similarly, the isolate also managed to suppress the growth of *C. gloeosporioides* significantly (2.33 mm). However, for this fungus a different type of carbon source namely fructose was added in liquid medium.

In case of UPMKB4, the antimicrobial substances that produced in the present of fructose as carbon source managed to inhibit diametric mycelia growth of *F. oxysporum* and *C. gloeosporioides* with the minimum diametric values of 3.67 mm and 3.33 mm, respectively. In our study, fructose proved to be the best carbon source as compared to glucose and glycerol in terms of inhibiting diametric mycelia growth of *F. oxysporum*. Similar findings were also observed on *C. gloeosporioides*.

Supernatant containing antimicrobial substances obtained from *S. maltophilia* was also able manage to inhibit mycelia growths of *F. oxysporum* and *C. gloeosporioides*. Use of this antimicrobial substance in the presence of glycerol as carbon source against those plants pathogenic fungi, yielded the minimum diametric mycelia growth (3.00 & 3.33 mm, respectively). *S. maltophilia* cultured in glycerol amended media showed significantly different effect as compared to control plate as well as with those amended media with fructose and glucose. Results of our study proved that different types of carbon sources used in liquid media determine the quality of antimicrobial substances and specific or special types of carbon source is required to suppress different types of plant pathogens. The quality of the antimicrobial substances was measured according to their ability to suppress the mycelia growth of that particular fungus.

The effect of the antimicribial substances activity was futher analyzed using light microscopy (Fig. 1 & 2). The changes in the hyphal tips of the *F. oxysporum* treated with *M. spinosa*, UPMKB4 and *S. maltophila* in the different carboun sources such as glucose, fructose and glycerol were observed at 400 magnification. The hyphal tips of the plant pathogenic fungi became thickened after treatment with *M. spinosa* and UPMKB4 as compared with hyphae in the control plate. Hyphae of *F. oxysporum* treated with *S. maltophila* were swollen (Fig. 1D). The hyphae of *C. gloeosporioides*, were shriveled (Fig. 2D). Moreover, both treated fungi were also showed reduced spore production.

Spore germination test: In this study, antimicrobial substances from *M. spinosa*, UPMKB4 and *S. maltophilia*

Table I: List of antagonistic bacteria identified against *Fusarium oxysporum* and *Colletotrichum gloeosporioides* based on Biolog® Identification System

Bacterial code	Identified antagonistic bacteria
UPMKB1	*Malikia spinosa*
UPMKB4	Unknown species
UPMKB9	*Stenotrophomonas maltophilia*

Table II: Mycelia growth of *F. oxysporum* on WA medium after treatment with supernatant containing antimicrobial substances from different antagonistic bacteria (mm)*

Carbon source	*Malikia spinosa*	UPMKB4	*Stenotrophomonas maltophilia*
Fructose	4.67^{c}	3.67^{c}	6.00^{c}
Glucose	2.33^{d}	5.50^{b}	7.33^{b}
Glycerol	6.33^{b}	6.33^{b}	3.00^{d}
Control	15.67^{a}	15.00^{a}	16.00^{a}
CV	8.28%	4.81%	5.19%

Note: *Average three replications inhibition diametric mycelia growth of *Fusarium oxysporum* at 7 days after incubation. Means in a column with the same letter(s) do not show significantly different at $P<0.05$ according to DUNCAN

Table III: Mycelia growth of *C. gloeosporioides* on WA medium after treatment with supernatant containing antimicrobial substances from different antagonistic bacteria (mm)*

Carbon source	*Malikia spinosa*	UPMKB4	*Stenotrophomonas maltophilia*
Fructose	2.33^{d}	3.33^{c}	5.67^{c}
Glucose	5.67^{c}	6.33^{b}	7.33^{b}
Glycerol	7.33^{b}	6.50^{b}	3.33^{d}
Control	16.00^{a}	16.33^{a}	15.33^{a}
CV	9.51%	8.33%	7.29%

Note: *Average three replications of inhibition diametric mycelia growth of *C. gloeosporioides* at 7 days after incubation. Means in a column with the same letter(s) do not show significantly different at $P<0.05$ according to DUNCAN

Table IV: Clearing zone on the spore germination of *F. oxysporum* after treatment with supernatant containing antimicrobial substance (mm)*

Antimicrobial substances	Mean of inhibition zone (mm)
Control	0.00^{c}
Malikia spinosa	$10.33^{a} \pm 0.33$
UPMKB4	9.00^{b}
Stenotrophomonas maltophilia	$8.33^{b} \pm 0.33$
CV (%)	9.22%

Note: *Average three replications of inhibition diametric clearing zone on the spore germination of *F. oxysporum* at 2 days after incubation. Means in a column with the same letter(s) do not significantly different at $P<0.05$ according to DUNCAN

Table V: Clearing zone on the spore germination of *C. gloeosporioides* after treatment with supernatant containing antimicrobial substance (mm)*

Antimicrobial substances	Mean of inhibition zone (mm)
Control	0.00^{d}
Malikia spinosa	$12.00^{a} \pm 0.58$
UPMKB4	$10.67^{b} \pm 0.33$
Stenotrophomonas maltophilia	10.00^{c}
CV (%)	3.53%

Note: *Average three replications of inhibition diametric clearing zone on the germination of *C. gloeosporioides* at 2 days after incubation. Means in a column with the same letter(s) do not significantly different at P<0.05 according to Duncan

Table VI: Compatibility of mixture supernatant containing antimicrobial substances against spore germination of *F. oxysporum*

Compatibility Mixture Supernatant Containing Antimicrobial Substances	Diametric clearing zone (mm)#
Malikia spinosa	$10.00^{ab} \pm 0.58$
UPMKB4	$9.67^{b} \pm 0.33$
Stenotrophomonas maltophilia	$9.00^{bc} \pm 0.58$
Malikia spinosa+UPMKB4	11.00^{a}
Malikia spinosa+*Stenotrophomonas maltophilia*	10.00^{ab}
UPMKB4+*Stenotrophomonas maltophilia*	$8.33^{c} \pm 0.33$
Control	0.00^{d}

Note: #Average of three replications. Means in a column with the same letter(s) are not significantly different at P<0.05 according to Duncan. CV= 7.13%

Table VII: Compatibility of mixture supernatant containing antimicrobial substances against spore germination of *C. gloeosporioides*

Compatibility Mixture Supernatant Containing Antimicrobial Substances	Diametric clearing zone (mm)*
Malikia spinosa	11.00^{b}
UPMKB4	$10.33^{b} \pm 0.33$
Stenotrophomonas maltophilia	$9.00^{c} \pm 0.58$
Malikia spinosa+UPMKB4	$12.67^{a} \pm 0.33$
Malikia spinosa+*Stenotrophomonas maltophilia*	11.00^{b}
UPMKB4+*Stenotrophomonas maltophilia*	$10.00^{bc} \pm 0.58$
Control	0.00^{d}

Note: *Average of three replications. Means in a column with the same letter(s) do not significantly different at P<0.05 according to Duncan. CV= 5.61%

were tested to determine their ability to inhibit spore germination. Treated spore of *F. oxysporum* and *C. gloeosporioides* showed a clear inhibition zone at the edge of filter paper discs placed in the centre of Petri dishes (Fig. 3 & 4). Results of our study showed that these supernatant containing antimicrobial substances managed to inhibit spore germination significantly after two days of incubation period compared with control plate. The mean values of spore inhibition for *F. oxysporum* and *C. gloeosporioides* are given in Table IV and V. In this study, the maximum diameter of inhibition zone (10.33 mm) was recorded on *F. oxysporum*, by *M. spinosa*. Interestingly, this bacterium also gave the maximum inhibition rate (12.00 mm) on *C. gloeosporioides*. On the other hand, *S. maltophilia* gave the minimum inhibition rate (8.33 mm) on *F. oxysporum* and 10.00 mm on *C. gloeosporioides*. No significant difference of inhibition was observed between UPMKB4 and *S. maltophilia* on *F. oxysporum*. While on *C. gloeosporioides*, it showed significant difference.

Compatibility of mixture of supernatant containing antimicrobial substances: Results of this study showed that antimicrobial substances obtained from *M. spinosa*, UPMKB4 and *S. maltophilia* and their combination exhibited were significantly different effect (P<0.05) in suppressing *F. oxysporum* as compared to control treatment. Combination of *M. spinosa* + UPMKB4 proved to be the best treatment to inhibit the growth of *F. oxysporum*, 11.00 mm, which was the maximum value recorded in this study (Table VI). However, the combination of antimicrobial substances of *M. spinosa*+*S. maltophilia* was not significantly different with the individual bacteria tested. On the other hand, it proved significantly different when compared with combination of *S. maltophilia*+UPMKB4.

In a separate study, by using *C. gloeosporioides* as a tested sample, was found that all treatments were significantly different (P<0.05) in suppressing the growth of *C. gloeosporioides* as compared to control treatment. The best result was obtained when mixed supernatant of *M. spinosa*+UPMKB4 was used. This combination yielded the maximum suppression value, 12.67 mm followed by treatments 5 and 1, 2, 6 and 3 (Table VII).

DISCUSSION

Amongst the three antagonistic bacteria isolates, two (*M. spinosa* & *S. maltophilia*) were positively identified using Biolog® Identification System. Based on the previous reports, *S. maltophilia* has been reported as a biocontrol agent (Burkhead *et al*., 1994; Kobayashi *et al*., 1995; Suparman *et al*., 2002; Szczech & Shoda, 2004). *M. spinosa* was also reported as potential antagonistic bacteria which produce volatile substance which can affect the growth of *Phytopthora palmivora* (Bulter) in coco fruits (Ahmad, 2004). In another study, *M. spinosa* has been reported to have a potential to enhanced biological phosphorus removal from wastewater containing acetic and propionic acids (Spring *et al*., 2005; Liu *et al*., 2007).

Many species of bacterial antagonist have been reported able to suppress the growth of many fungal and, bacterial pathogen and nematodes. Among them are *Burkholderia cepacia, B. gladioli, Serratia marcescens, Serratia sp., Pseudomonas fluorescent, P. aeruginosa, P. putida, P. syringae, Sternotrophomonas maltophilia, Agrobacterium radiobacter, Bacillus subtillis, B. megaterium, B. pumilus, B. cereus* and *Pasteuria penetrans* (Bertagnolli *et al.*, 1996; Fulton *et al.*, 1996; Heydari & Misaghi, 1998; Miyagawa, 2000; Vesudevan *et al.*, 2002). Based on our findings we strongly believe that there are still many unknown antagonistic bacteria still not been

Fig 1: Hyphal morphology of *F. oxysporum* as affected by *M. spinosa*, UPMKB4 and *S. maltophilia* after staining with Lactophenol blue. Observations are at 400 magnifications by compound optics. Normal hyphae are from control treatment (A). Shrivelling of hypae by *M. spinosa* (B) and UPMKB4 (C). Occurrence of vacuoles and swelling in hyphae by *S. maltophilia* (D)

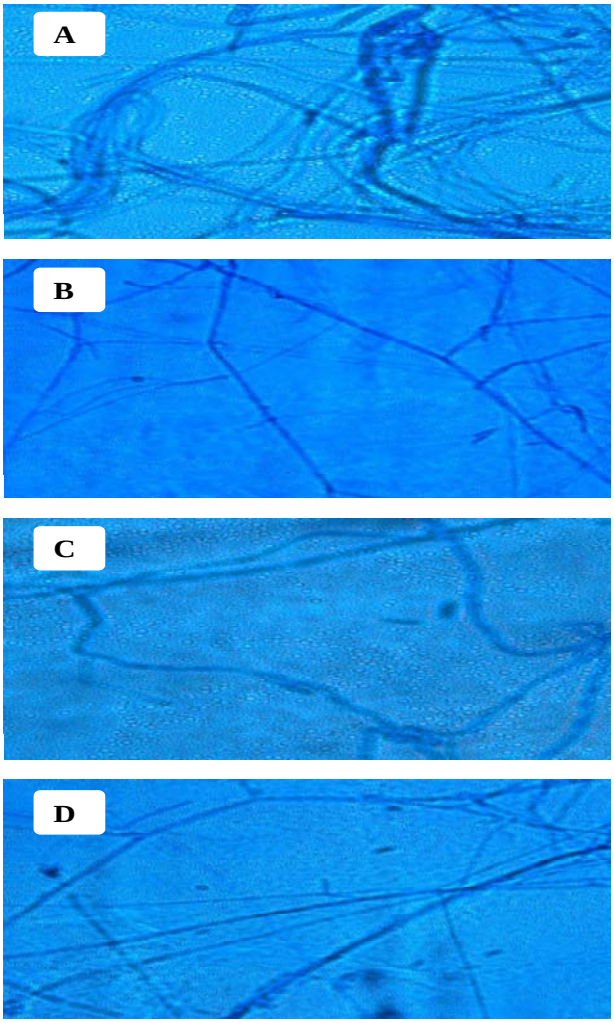
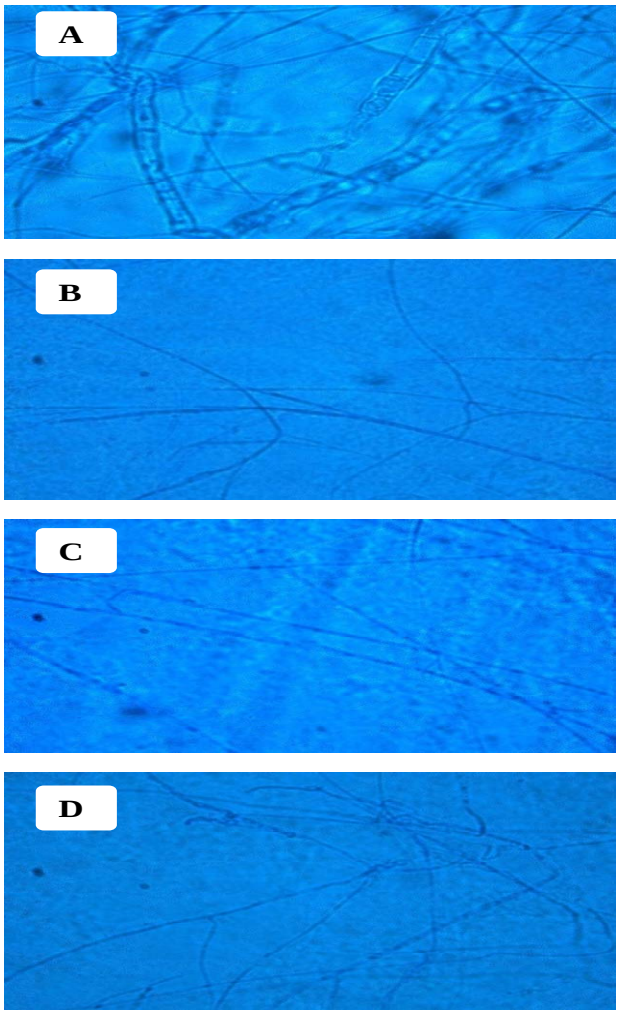


Fig 2: Hyphal morphology of *C. gloeosporioides*. as affected by *M. spinosa*, UPMKB4 and *S. maltophilia* after staining with Lactophenol blue. Observations are at 400 magnifications by compound optics. Normal hyphae are from control treatment (A). Shrivelling of hypae by *M. spinosa* (B) and UPMKB4 (C). Occurrence of vacuole and shrivel in hyphae by *S. maltophilia* (D)



identified. In our study, we found one isolate of potential antagonistic bacterium, UPMKB4 that could not be identified based on Biolog® Identification System. Further study is required to identify this bacterium. There are many methods which could be used to achieve this purpose and one of the options is the use of polymerase chains reaction (PCR) test or fatty acids methyl esters (FAME) analysis.

M. spinosa, UPMKB4, and *S. maltophilia* have showed differential capability in producing supernatant containing antimicrobial substances even from the same basal ingredient of liquid medium. The variability in this capability of each species of bacteria is mostly due to variation in ability of hydrolyzing each carbon source provided.

Regarding the effect of different carbon source on the antifungal production by *M. spinosa*, glucose was found to be the best carbon source for suppressing the growth of *F. oxysporum* colony and fructose for *C. gloeosporioides.* Interestingly, our findings showed similar results as obtained by many other researchers in the world (Slininger & Shea-Wilbur, 1995; Duffy & Defago, 1999; El-Banna, 2006). In our study, fructose was found to be the best carbon sources for UPMKB4 and glycerol for *S. maltophilia* for suppressing the growth of *F. oxysporum* and *C. gloeosporioides*. According to Qureshi *et al*. (2001) and Fukuda *et al*. (2005) glycerol was very important medium component for the production of antifungal antibiotics from microorganisms. Our findings conform that secondary

Fig 4: Formation of clearing zone at the edge of filter peppers discs showing suppression of the growth of *C. gloeosporioides* after treatment with supernatant containing antimicrobial substances (A-antimicrobial substances *M. spinosa*; B-antimicrobial substances UPMKB4; C-antimicrobial substances from *S. maltophilia*)

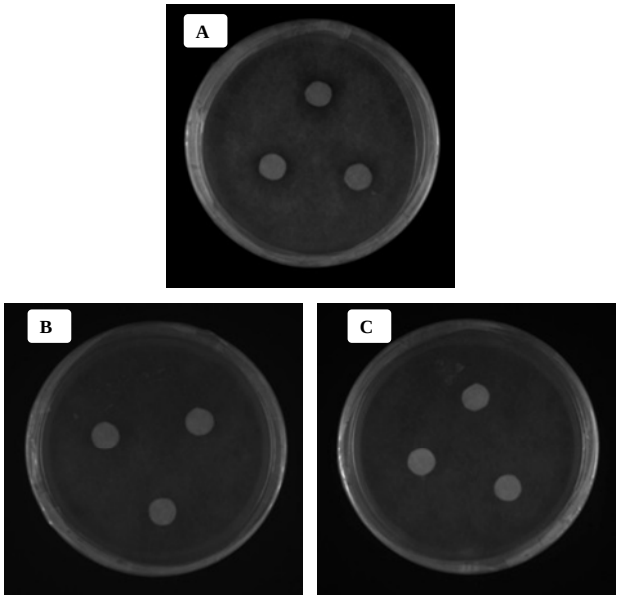
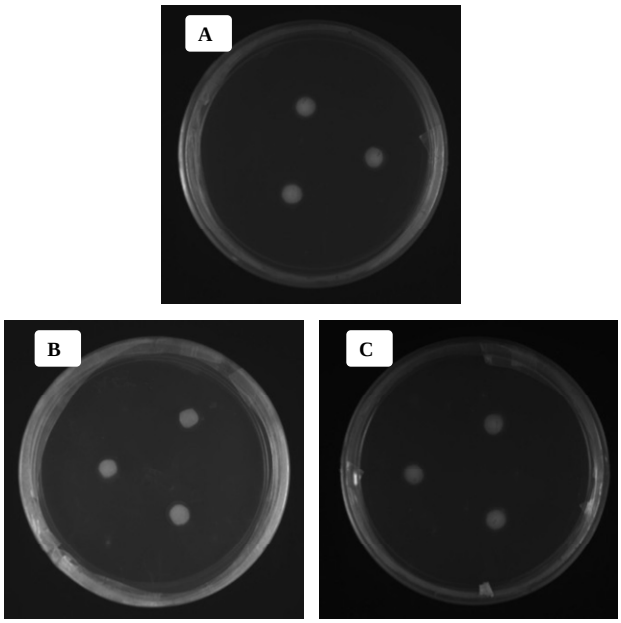


Fig 3: Formation of clearing zone at the edge of filter peppers discs showing suppression of the growth of *F. oxysporum* after treatment with supernatant containing antimicrobial substances (A-antimicrobial substances *M. spinosa*; B-antimicrobial substances UPMKB4; C-antimicrobial substances from *S. maltophilia*)



metabolite production from these bacteria was often stimulated by slowly assimilated complex carbohydrates or oils from the production media and is decreased when more rapidly utilized monosaccharide's such as glucose are present (Bertasso *et al*., 2001). A possible explanation for this phenomenon is that glucose or other carbon sources caused catabolite repression in which the production of an enzyme of secondary metabolite biosynthesis is inhibited (Drew & Demain, 1977; Iwai & Omura, 1982).

Many factors may represent an important role in the process of antifungal production and consequently affect the antagonistic activity of the bacterial species. Carbon compounds constitute the major requirement for growth as they enter in different metabolic process resulting in the production of primary and secondary metabolites including antifungal substances (Gebreel *et al.*, 2008). The quantity of carbon source in the basal liquid medium affects the production of antimicrobial substances against plants pathogenic fungi. Dikin *et al.* (2005) reported that the quantity of lactose in the basal liquid medium affected the production of antimicrobial substances by *Burkholderia cepacia* RB47 and *Microbacterium testaceum* RU7 against *Schizophyllum commune*. Liquid media which are modified with ingredient of 40 g lactose for growing of *B. cepacia* RB47 and *M. testaceum* RU7 were found managed to promote and produce antimicrobial substances thereby causing strongly inhibition in the growth of *S. commune* mycelia as compared to the ingredient amended with 10 g of lactose in the same liquid medium.

Wicklow *et al.* (1998) reported that optimal quality of antimicrobial substances very much affected by the density of bacterial cells and duration of the incubation of liquid medium. Similarly, Dikin *et al*. (2005) reported that *B. multivorans* RU50 grew faster than *M. testaceum* RU7 within 24 h after incubation to reach a population up to 1.42 x 10^{11} cfu/mL and 5.6 x 10^{9} cfu/mL, respectively. Effective suppression of plant diseases by many microorganisms is largely affected by environmental conditions. It was suggested that environmental and nutritional conditions may influence the survival and the activity of biocontrol agents (Hannusch & Boland, 1996; Guetsky *et al*., 2001; Kurze & Bahl, 2001; Abdel-Gawad, 2002). Some modification and improvement need to be done in this study to find out the most suitable quantity of carbon sources in the basal liquid medium under appropriate conditions in order to produce more effective antimicrobial substances against *F. oxysporum* and *C. gloeosporioides.*

M. spinosa, UPMKB4 and *S. maltophilia* were showed clear inhibition zone around a filter paper discs placed in the center of the Petri dishes. Antimicrobial substances produced by these potential antagonistic bacteria could inhibit the spore germination of *F. oxysporum* and *C. gloeosporioides. C. gloeosporioides* has been reported to be controlled by the application of antifungal compounds produced by biocontrol agents (Rahman *et al*., 2007). Interestingly, we found that the use of different types of carbon sources would give different levels of efficacious effect in inhibition in the spore germination of *F.*

oxysporum and *C. gloeosporioides*.

The failure of the spore of *F. oxysporum* and *C. gloeosporioides* to germinate in 2 days exposure indicated that antimicrobial substances produced by antagonistic bacteria is fungicidal to the spore of the tested fungus. Suppression of spore germination of several fungi by *B. cepacia* has been reported (Janisiewicz & Roitman, 1988). Antifungal activity of microorganisms is mostly due to the effect of lytic enzymes. This enzyme was found degrading the fungal cell wall (Sindhu & Dadarwal, 2001). Other antifungal metabolites such as antibiotics, Fe chelating siderophores, ammonia and cyanide were also reported by many researchers (Lovic *et al*., 1993; Weller, 2007). Because of these activities, the spore of the tested fungus cannot be germinated effectively. Cook (1985) reported that the use of antagonistic bacteria as biocontrol agents is extremely promising in reducing the spore germination of the pathogens. By the formulation of carbon sources as a medium growth for the *M. spinosa*, UPMKB4 and *S. maltophilia*, also proved to reduce or suppress germination of plant pathogen spore.

Compatible mixture of antimicrobial substances from *M. spinosa* and UPMKB4 produced stronger antifungal activity than individual antimicrobial substances. This finding is in agreements with Dikin *et al*. (2005) who has also reported that the compatible mixture of supernatant containing antimicrobial substances from *B. multivorans* and *M. testaceum* produced stronger biopesticides than individual supernatant containing antimicrobial substances. The compatibility mixture of supernatant from *M. spinosa* and UPMKB4 can be used in further study *in vivo*.

In a conclusion, applications of antimicrobial substances have shown significant difference in suppression of growth as compared to the control treatments. Antimicrobial substances obtained from *M. spinosa*, UPMKB4 and *S. maltophilia* through fermentation process in the liquid medium with basal ingredient of carbon sources showed effective control in the presence of glucose, fructose and glycerol, respectively on *F. oxysporum*. However, antimicrobial substances produced by *M. spinosa*, UPMKB4 and *S. maltophilia* most proficient in suppressed the growth of *C. gloeosporioides* in the present of fructose, fructose and glycerol, respectively. The combination of antimicrobial substances from *M. spinosa* and UPMKB4 were strong substances to suppressed *F. oxysporum* and *C. gloeosporioides* and compatible formulation, which represented higher diametric clear zone than individual. The mixed antimicrobial substances are required for further study on the characterization to provide the information for formulating biopesticide.

Acknoeledgement: This project was supported by Universiti Putra Malaysia Bintulu Campus Sarawak (UPMKB). Special gratitude is expressed to Siti Fatimah for her technical support during conducting research activities. Last but not the least special appreciation to all staff of UPMKB who directly or undirectly involved in this project.

REFERENCES

Abdel-Gawad, A.M., 2002. Biological Control of Some Tomato Diseases Caused by *Fusarium* spp. and *Alternaria* spp. *Ph.D. Thesis*, p: 106. Faculty of Science, Cairo University, Cairo, Egypt

Ahmad, K.M.J., 2004. Antagonistic Activities Of Epiphytic Bacteria On Black Pod Disease of Cocoa. *Master Thesis*, p: 26. Universiti Putra Malaysia

Anonymous, 2001. *A Powerful automated system for the rapid identification of bacteria and yeasts. Biolog*. http://www.biolog.com. Accessed on March 13, 2010

Bertagnolli, B.L., F.K.D. Soglio and J.B. Sinclair, 1996. Extracellular enzyme profiles of fungal pathogen *Rhizoctonia solani* isolate 2B-12 and of two antagonists, *Bacillus megaterium* strain B153-2-2 and *Trichoderma harzianum* isolate Th008. I. Possible correlation with inhibition of growth and biocontrol. *Physiol. Mol. Plant Pathol.*, 48: 145–160

Bertasso, M., M. Hulzenkampfer, A. Zeexk, F. Doll Antonia and H. Fiedler, 2001. Bahremycin A and B, novel antibiotics from *Streptomycete* sp. TU. 4128. *J. Antibiotic*, 54: 730–736

Bevivino, A., S. Sarrocco, C. Dalmastri, S. Tabacchioni, C. Cantale and L. Chiarini, 1998. Characterization of a free-living maizerhizosphere population of Burkholderia cepacia: effect of seed treatment on disease suppression and growth promotion of maize. *FEMS Microbiol. Ecol.*, 27: 225–237

Burkhead, K.D., D.A. Schisler and P.J. Slininger, 1994. Pyrrolnitrin production by biological control agent *Pseudomonas cepacia* B37W in culture and in colonized wounded of potatoes. *Appl. Environ. Microbiol.*, 60: 2031–2039

Chiarini, L., A. Bevivino, C. Dalmastri, S. Tabacchioni and P. Visca, 2006. *Burkholderia cepacia* complex species: health hazards and biotechnological potential. *Trends Microbiol.*, 14: 277–286

Cook, J., 1985. Biological control of plant pathogens: Theory to application. *Phytopathology*, 75: 25–29

Diby. P., M. Anandaraj, A. Kumar and Y.R. Sarma, 2005. Antagonistic mechanisms of *fluorescent pseudomonads* against *Phytophthora capsici* in black pepper (*Piper nigrum* Linn.). *J. Spices Aromatic Crops*, 14: 94–101

Dikin. A., K. Sijam, J. Kadir and A.S. Idris, 2005. Extraction of Antimicrobial Substances from Antagonistic Bacteria against *Schyzophyllum commune* Fr. In: *Proc. 27th Malaysian Microbiology Symposium*. Innovation through Microbes. Grand Plaza Parkroyal Penang. Malaysia, 24-27 November 2005

Drew, S.W. and A.L. Demain, 1977. Effect of primary metabolites on secondary metabolism. *Annu. Rev. Microbiol.*, 31: 343–356

Duffy, B.K. and G. Defago, 1999. Environmental factors modulating antibiotic and siderophore biosynthesis by *Pseudomonas fluorescens* biocontrol strains. *Appl. Environ. Microbiol.*, 65: 2429–2438

El-Banna, N.M., 2006. Effect of carbon sources on the antimicrobial activity of *Corynebacterium kutscheri* and *Corynebacterium xerosis*. *African J. Biotechnol.*, 5: 833–835

Fukuda, T., A. Matsumoto, Y. Takahashi, H. Tomoda and S. Omura, 2005. Phenatic acids A and B, new Potentiators of antifungal miconazole activity produced by *Streptomyces* sp. K03-0132. *J. Antibiotic*, 58: 252–259

Fulton, E.W., D.W. Dickson and E.B. Whitty, 1996. Suppression of *Meloidogyne incognita* and *M. javanica* by *Pasteuria penetrans* in field soil. *J. Nematol.*, 28: 43–49

Gebreel, H.M., A.A. El-Mehalawy, I.M. El-Kholy, H.M. Rifaat and A.A. Humid, 2008. Antimicrobial Activities of Certain Bacteria Isolated from Egyptian Soil Against Pathogenic Fungi. *J. Agric. Biol. Sci.*, 4: 331–339

Gomez, K.A. and A.A Gomez, 1984. *Presentation of research results. Statistical Procedures for Agricultural Research*, Vol. 17, pp: 591–627. New York: A Wiley-Interscience Publication

Guetsky, R., D. Shteinberg, Y. Elad and A. Dinoor, 2001. Combining biocontrol agents to reduce the variability of biological control. *American Phytopathol. Soc.*, 91: 621–627

Hannusch, D.J. and G.J. Boland, 1996. Interaction of air temperature, relative humidity and biological control agents on grey mould of bean. *European J. Plant Pathol.,* 102: 133–142

Heydari, A. and I.J. Misaghi, 1998. Biocontrol activity *Burkholderia cepacia* against *Rhizoctonia solani* in herbicide-treated soil. *Plant Soil,* 202: 109–116

Iwai, Y. and S. Omura, 1982. Culture conditions for screening of new antibiotics. *J. Antibiotic,* 35: 12–14

Janisiewicz, W.J. and J. Roitmann, 1988. Biological control of blue mold and gray mold on apple and pear with *Pseudomonas cepacia*. *Phytopathology,* 78: 1697

Kobayashi, D.Y., M. Guglielmoni and B.B. Clarke, 1995. Isolation of the chitinolytic bacteria *Xanthomonas maltophilia* and *Serratia marcescens* as biological control agents for summer patch disease of turfgrass. *Soil Biol. Biochem.,* 27: 1479–1487

Kurze, S. and H. Bahl, 2001. Biological control of fungal strawberry diseases caused by *Serratia plymuthica* HRO-C48. *Plant Dis.,* 85: 529–534

Leifert, C., H. Li, S. Chidburee, S. Hampson, S. Workman, D. Sigee, H.A.S. Epton and A. Harbour, 1995. Antibiotic production and biocontrol activity by *Bacillus subtilis* CL27 and *Bacillus pumilus* CL45. *J. Appl. Bacteriol.,* 78: 97–108

Liu. Y., Y. Chen and Q. Zhou, 2007. Effect of initial pH control on enhanced biological phosphorus removal from wastewater containing acetic and propionic acids. *Chemosphere,* 66: 123–129

Lovic, B., C. Heck, J. Gallian and A. Anderson 1993. Inhibition of the sugar beet pathogens *Phomabetae* and *Rhizoctonia solani* by bacteria associated with sugarbeet seeds and roots. *J. Sugar Beet Res.,* 30: 169–184

Miyagawa, H., 2000. Biocontrol of bacterial seedling blight of rice caused by *Burkholderia gladioli* using with its avirulent isolate. *Japan J. Phytopathol.,* 66: 232–238

Nur Aimi, R., 2009. Isolation and screening of potential antagonistic bacteria against Colletotrichum gloeosprioides, the causal agent of anthracnose disease in papaya. *Bachelor Thesis*. Faculty of Agriculture and Food Sciences, University Putra Malaysia Bintulu Campus Sarawak, Malaysia

Qureshi, A., J.B. Mauger, R.J. Cano, J.L. Galazzo and M.D. Lee, 2001. MF-EA-705a and MF-EA-705b, new metabolites from microbial fermentation of a *Streptomyces* sp. *J. Antibiotic,* 54: 1100–1103

Rahman, M.A., J. Kadir, T.M.M. Mahmud, R. Abdul Rahman and M.M. Begun, 2007. Screening of antagonistic bacteria for biocontrol activities on *Colletotrichum gloeosporioides* in papaya. *Asian J. Plant Sci*., 6: 12–20

Sindhu, S.S. and K.R. Dadarwal, 2001. Chitinolytic and cellulolytic *Pseudomonas* spp. Antagonistic to fungal pathogens enhances nodulation by *Mesorhizobium* spp. Cicer in chickpea. *Microbiol. Rev.,* 56: 662–676

Slininger, P. and M.A. Shea-Wilbur, 1995. Liquid culture pH, temperature and carbon (not nitrogen) source regulate phenazine productivity of teke-all biocontrol agent *Pseudomonas fluorescens,* p. 2-79. *Appl. Microbiol. Biotechnol.,* 43: 794–800

Spring, S., M. Wagner, P. Schumann and P. Kamfer, 2005. *Malikia granosa* gen. nov., sp. nov., a novel polyhydroxyalkanoate- and polyphosphate-accumulating bacterium isolated from activated sludge and reclassification of *Pseudomonas spinosa* as *Malikia spinosa* comb. nov. *Int. J. Syst. Evol. Microbiol.,* 55: 621–629

Suparman, M., K. Sijam, S. Meon and I. Sulaiman, 2002. *In vitro Screening of Antagonistic Bacteria against Fusarium Oxysporum f.sp. Lycopersici, 25th Malaysian Microbiology Society Symposium,* p: 24. 5th Unesco National Workshop on the Promotion of Microbiology in Malaysia. Kota Bharu, September 8-11 2002. Programme and Abstract Book

Szczech, M. and M. Shoda, 2004. Biocontrol of Rhizoctonia damping-off tomato by *Bacillus subtilis* combined with *Burkholderia cepacia*. *J. Phytopathol.,* 152: 549–556

Vesudevan, P., S. Kavitha, V.B. Priyadarisini, L. Bubejee and S.S. Gnanamanickam, 2002. Biological control of rice diseases. *In*: Gnanamanickam, S.S. (ed.), *Biological Control of Crop Diseases*, Vol. 2, pp: 11–32. Marcel Dekker

Weller, D.M., 1988. Biological control of soilborne plant pathogen in the rhizospheres with bacteria. *Annu. Rev. Phytopathol.,* 26: 379–407

Weller, D.V., 2007. *Pseudomonas* Biocontrol agents of soilborne pathogens: Looking back over 30years. *Phytopathology,* 97: 250–256

Wicklow, D.T., B.K. Joshi and W.R. Gamble, 1998. Antifungal metabolites (Monorden, Monocillin IV, and Cerebrosides) from Humicola fuscoatra Traaen NRRL 22980, a mycoparasite of *Aspergillus flavus sclerotia*. *Appl. Environ. Microbiol.,* 64: 4482–4484

Yilmaz, M., H. Soran and Y. Beyatli, 2005. Antimicrobial activities of some *Bacillus* spp. strains isolated from the soil. *Microbiol. Resistance,* 161: 127–131

(Received 26 July 2010; Accepted 01 September 2010)