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Effect of Different Carbon Sources and Peptones on the Production of Antimicrobial Substances from Bacteria Against *Schizophyllum commune* FR.

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ABSTRACT

The improvement of quality of antimicrobial substances from bacteria is required in order to obtain strong formulated antimicrobial substances to suppress *Schizophyllum commune*. Antimicrobial substances from *Burkholderia multivorans* and *Microbacterum testaceum* through the fermentation in the liquid medium with basal ingredient 10 g neo peptone, 10 g lactose dissolved in 1 L of distilled water and 10 g peptone, 10 g maltose and dissolved in 1 L distilled water, respectively had good value of minimum inhibitory concentration (MIC). The mixed concentrate antimicrobial substances from *B. multivorans* and *M. testaceum* were compatible substances to suppress *S. commune.*

Key Words: Carbon sources; Peptones; Antimicrobial substances; Bacteria

INTRODUCTION

Bacteria produce antimicrobial substances as important compounds with the function as self defense from other organisms or bio-control activity. The production of antimicrobial substances was depending upon the substrate medium for the optimal bacterial growth, temperature, pH and the concentration of nutrition in the medium (Leifert *et al*., 1995). Quality of antimicrobial substances was produced by bacteria for the suppressing seed-borne pathogen of oil palm, *S. commune* as the major factor to reach the successful treatment. Furthermore, the antimicrobial substances are also expected to have broad spectrum to control pathogenic fungi. Carbon source as the part of ingredient medium was required for bacteria on the production of antimicrobial substances. *P. fluorescent* in the liquid medium by adding glucose and sucrose produced more antimicrobial substances, 2, 4-diacetylphloroglucinol (Duffy & Defago, 1999). Similarly, *Pseudomonas aeruginosa* produced phenazine throughout their secondary metabolism, when grew aerobically in phosphate-poor medium (Tambong & Hofte, 2001). In contrast, *B. cepacia* in limited nutrition produced more phenylpyrrol than full nutrition medium (Roitman *et al*., 1990). Balanced ingredient of medium as the nutrition for bacterial growth was important factor for producing antimicrobial substances.

The objective of study was to use different carbon sources and peptones on the production of antimicrobial substances against pathogenic fungi from oil palm seeds.

MATERIALS AND METHODS

Bacterial and fungal cultures. Bacterial and fungal cultures were used in the study namely *Burkholderia cepacia, B. multivorans, Microcabterium testaceum* and *Schizophyllum commune.*

Optimization of antimicrobial substances. Bacterial isolates, *B. multivorans, B. cepacia* and *Microbacterium testaceum* were inoculated to the modified liquid medium (Medium-5: Neo Peptone 10 g, Lactose 10 g, Distilled water 1 L). The ingredient of liquid medium was prepared with different carbon sources (Maltose, Lactose, glucose, Myo-Inositol, Sorbitol, Arabinose, Galactose, Dextrose, Mannitol) as the first factor and different sources of peptone (Neo Peptone, Proteose Peptone & Peptone) as the second factor. Liquid media consisted of 2 g of carbon source, 2 g kinds of peptone and dissolved in 200 mL of distilled water. Each liquid medium was inoculated with *B. cepacia, B. multivorans, M. testaceum* and then placed on electric rotator (New Brunswick Scientific G-25 KC) for 5 days at 100 rpm. Liquid medium was added methanol for inactivating bacterial cells. Supernatant of liquid medium was separated by using centrifugation (Beckman J2 - 21) at 10.000 rpm for 10 min and then concentrated up to 20 mL with rotary evaporator (Buchii rotavopor R-200). The concentrate substances were determined their potential suppressing against *S. commune.* 100 µL substances were dropped onto five mm diameter of fungal plug onto WA plate. Plates were incubated 7 days and measured diametric radial growth of fungi. Each treatment was replicated 3

times.

Determination of the inhibitory concentration of antimicrobial substances. The prepare concentrate antimicrobial substances produced from *M. testaceum, B. cepacia* and *B. multivorans* in the liquid medium content peptone maltose, peptone lactose and neo-peptone lactose, respectively were determined their inhibitory concentration. Concentrate antimicrobial substances was sequently diluted with distilled water with concentration ratio of antimicrobial substances and distilled water (1: 9; 2: 8; 3: 7; 4: 6; 5: 5; 6: 4; 7: 3; 8; 2; 9: 1). 100 mL of spores of *S. commune* (10^7spores/mL) were seeded into 900 mL melted PDA medium at 50°C and stirred. 25 mL of seeded spores PDA medium was poured into 9 cm diameter of plate. One 5 mm diameter of paper dish was placed onto seeded spores PDA medium and dropped 100 µL diluted substances and air dried in laminair chamber. The diametric clear zone on PDA medium was measured at 2 days after incubation. Each treatment was replicated 3 times.

Compatibility of mixed concentrate antimicrobial substances against *S. commune.* Prepared three concentrate antimicrobial substances from *M. testaceum, B. cepacia* and *B. multivorans* in liquid medium with ingredient peptone maltose, peptone lactose and neo peptone lactose, respectively were used for determination of compatibility mixing both substances. The same volume of each prepared concentrate substance was mixed well. The combination of 2 concentrate substances is presented in Table I. One 5 mm diameter of paper disk was placed on the prepared seeded spores PDA medium. 100 µL of mixed antimicrobial substances were dropped on PDA medium and incubated for 48 h. Diametric clear zone against *S. commune* was measured. Each treatment was replicated 3 times.

Statistical analysis. The designed experiments were conducted with Completely Randomized Design (CRD). Recorded data were analyzed with SAS® System release version 8 (TS M1), SAS Institute Trial Site, Cary, North California, USA. The significant data from ANOVA was evaluated the comparison by using Least Significant Different Test at level of 5%.

RESULTS

Optimization of antimicrobial substances. *B. cepacia, B. multivorans* and *M. testaceum* produced substances in the modified liquid medium-5 with mixing the peptones and carbon sources. The substances were produced from *B. cepacia,* which was significant to suppress ($P \leq 0.05$) the mycelial growth of *S. commune* (Table II). Substances from *B. cepacia* suppressed mycelial growth of *S. commune* with smallest diametric mycelial growth from 3 liquid media content peptone maltose, neo peptone dextrose and peptone lactose.

The concentrate substances from *B. multivorans* significantly suppressed ($P \leq 0.05$) the mycelial growth of *S. commune* (Table III). The concentrate substances from *B. multivorans* inhibited mycelial growth of *S. commune* with smallest diametric mycelial growth, which were produced from liquid medium in all combination peptones and sugars and except the liquid medium content proteose peptone L-arabiose, proteose peptone dextrose, proteose peptone mannitol, proteose peptone glucose, peptone L-arabinose and peptone itself.

The concentrate substances from *M. testaceum* significantly suppressed ($P \leq 0.05$) the mycelial growth of *S. commune* (Table IV). The substances from *M. testaeum* inhibited the mycelial growth of *S. commune* with smallest diametric mycelial growth from liquid media content proteose peptone D + sorbitol, proteose peptone L-arabinose, proteose peptone dextrose, proteose peptone mannitol, proteose peptone lactose, peptone D + sorbitol, peptone maltose, peptone lactose, peptone itself and neo-peptone galactose.

Kinds of peptone namely peptone, proteose peptone and neo peptone affected the production of substances from *B. cepacia, B. multivorans* and *M. testaceum,* which represented on the suppressing diametric mycelial growth of *S. commune* after treatment.

Determination of the inhibitory concentration of antimicrobial substances. Three selected highest spectrum antimicrobial substances against *S. commune* were determined their inhibitory concentration. The inhibitory concentration of antimicrobial substances from *B. multivorans* with basal ingredient liquid medium neo-peptone lactose in the 2 parts of antimicrobial substances diluted with 8 parts of sterilized water reached the mean diametric clear zone 18 mm. In the edge of clear zone in PDA spores medium found non-germinating spores and retarded germinating spores (Plate 1).

The inhibitory concentration of antimicrobial substances from *M. testaceum* with basal ingredient liquid medium peptone maltose in the 2 parts of antimicrobial substances diluted with 8 parts of sterilized water reached the mean diametric clear zone 16.3 mm. The inhibitory concentration of antimicrobial substances from *B. cepacia* with basal ingredient liquid medium neo-peptone lactose in the one part of antimicrobial substances diluted with 1 part of sterilized water reached the mean diameter clear zone 11.1 mm (Table V).

Based on the Table V, the minimum inhibitory concentration of antimicrobial substances from *B. cepacia* shows the lowest ratio at one part of antimicrobial substances diluted with one part of sterilized water with mean diametric clear zone 11.1 mm. For *B. multivorans* and *M. testaceum* have ratio 1 part of antimicrobial substances with 4 parts of sterilized water with mean diametric clear zone 18 mm and 16.3 mm, respectively.

Compatibility of mixed both antimicrobial substances against *S. commune.* Determination of the compatible mixing two antimicrobial substances among *B. cepacia, B. multivorans* and *M. testaceum* showed the varied responses

Table I. Combination of mixing 2 concentrate antimicrobial substances against *S. commune*

	Peptone, Maltose, *M. testaceum* (PepMal Mt)	**Neopeptone, Lactose, *B. multivorans* (NeoLac Bm)**	**Peptone, Lactose, *B. cepacia* (PepLac Bc)**
Peptone, Maltose, *M. testaceum* (PepMal *Mt*)	PepMal *Mt* + PepMal *Mt*		
Neopeptone, Lactose, *B. multivorans* (NeoLac *Bm*)	NeoLac *Bm* + Pep Mal *Mt*	Neo Lac *Bm* + Neo Lac *Bm*	
Peptone, Lactose, *B. cepacia* (PepLac *Bc*)	Pep Lac *Bc* + Pep Mal *Mt*	Pep Lac *Bc* + NeoLac *Bm*	Pep Lac *Bc* + Pep Lac *Bc*

Table II. Diametric mycelial growth of *S. commune* on WA medium after treated with concentrate substances from *B. cepacia* with different combinations of peptones and carbon sources at 7 days after incubation (mm)

Carbon source	**Kind of peptone**		
	Proteose peptone	**Peptone**	**Neo Peptone**
D+ Sorbitol	32bH	32bH	21aH
Maltose	6bA	5aA	18cF
L-Arabinose	30cF	27bG	16aD
Myo-Inositol	23bE	25cF	19aG
Dextrose	35cI	20cF	5aA
Mannitol	15bC	15bC	13aC
Galactose	9bB	7aB	32cJ
Glucose	18aD	24bE	28cI
Lactose	40cJ	5aA	17bE
Blank	31cG	25bF	8aB

Means followed by different small letters within row and different capital letters in column are significantly different.

Table III. Diametric mycelial growth of *S. commune* on WA after treated with substances from *B. multivorans* with different combination peptones and carbon sources at 7 days after incubation (mm)

Carbon source	**Kind of peptone**		
	Proteose peptone	**Peptone**	**Neo Peptone**
D+ Sorbitol	5aA	5aA	5aA
Maltose	5aA	5aA	5aA
L-Arabinose	23.3cE	6bB	5aA
Myo-Inositol	5aA	5aA	5aA
Dextrose	6bB	5aA	5aA
Mannitol	6.6bC	5aA	5aA
Galactose	5aA	5aA	5aA
Glucose	8bD	5aA	5aA
Lactose	5aA	5aA	5aA
Blank	5aA	7bC	5aA

Means followed by different small letters within row and different capital letters within column are significantly different.

on the formation of diametric clear zone (Table VI).

In the Table VI, the mixed antimicrobial substances from *B. multivorans* and *M. testaneum* are significantly different ($P \leq 0.05$) with mean diametric clear zone 29.6 mm, higher than individual antimicrobial substances with mean diametric clear zone 24 mm and 20 mm, respectively. Antimicrobial substances from *B. cepacia* mixed with antimicrobial substances from *B. multivorans* with mean diametric clear zone 19.6 mm. The mixed antimicrobial substances from *M. testaceum* and *B. cepacia* showed the mean diametric clear zone 20.6 mm.

Plate 1. Diametric clear zone of antimicrobial substances against *S. commune*: (a). mixed concentrate neopeptone lactose plus *B. multivorans* and peptone maltose plus *M. testaceum.* (b). peptone maltose plus *M. testaceum.* (c). neopeptone lactose plus *B. multivorans*

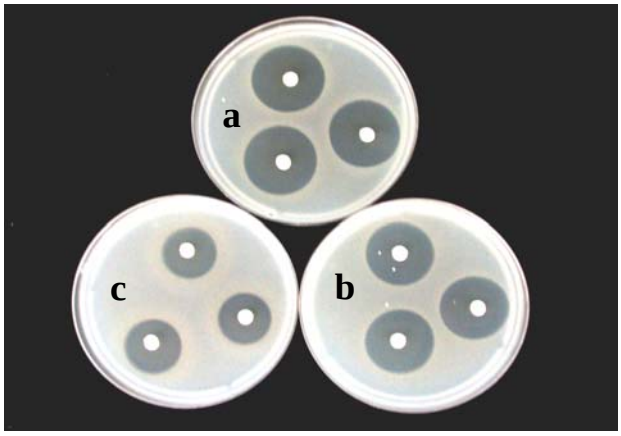
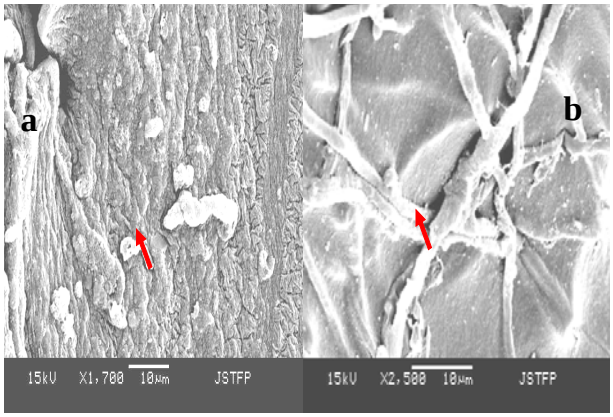


Plate 2. (a). Retarded germinating spore of *S. commune* at the edge area of clear zone (arrow sign); (b). Retarded hyphae of *S. commune* at the edge area of clear zone (arrow sign)



DISCUSSION

B. cepacia, B. multivorans and *M. testaceum* had the varied capability to produce antimicrobial substances in the same basal ingredient of liquid medium. The varied capability of each species of bacteria to hydrolyze each carbon sources was commonly used to distinguish one species to other species based upon the biochemical character. *B. cepacia* hydrolyzes such as maltose, galactose, mannitol, and glucose. Biochemical characteristics of *B. cepacia* based on the Biolog® identification system are not to utilize maltose and to utilize mannitol, glucose and galactose. The biochemical characteristics of *B. cepacia*

Table IV. Diametric mycelial growth of *S. commune* on WA after treated with substances from *M. testaneum* with different combination peptones and carbon source at 7 days after incubation (mm)

Carbon source	Kind of peptone		
	Proteose peptone	Peptone	Neo Peptone
D+ Sorbitol	5aA	5aA	7.6bCD
Maltose	8cD	5aA	6bB
L-Arabinose	5aA	7bC	11.3cE
Myo-Inositol	22cE	11.6aD	12bF
Dextrose	5aA	11cD	7.3bC
Mannitol	5aA	6bB	8.6cD
Galactose	6bB	18.6cE	5aA
Glucose	13.3cD	6aB	8.6bD
Lactose	5aA	5aA	8bD
Blank	8.6bC	5aA	26cG

Means followed by different small letters within row ($LSD_{0.05}$ =0.3 mm) and different capital letters within column ($LSD_{0.05}$= 0.7 mm) are significantly different.

Table V. The inhibitory concentration of antimicrobial substances from *B. multivorans, M. testaceum* and *B. cepacia* against *S. commune* in the PDA medium at 3 days after incubation

Ratio (antifungal substances: sterilized water)	Mean diametric clear zone (mm)		
	Antimicrobial substances of *B. multivorans*	Antimicrobial substances of *M. testaceum*	Antimicrobial substances of *B. cepacia*
10:0	24a	20a	15.5a
9: 1	24a	20a	15.6a
8: 2	24a	20.3a	14.5b
7: 3	23.6a	20a	13.3c
6: 4	23b	19b	13.1c
5:5	22c	17.3c	11.1d
4:6	22c	17.6c	5e
3: 7	21d	16.3d	5e
2: 8	18e	16.3d	5e
0:10 (Sterilized Water)	5g	5f	5e
Mancozeb (0.2%)	15f	15e	15ab
Daconil (0.2%)	15f	15e	15ab

Means followed by different letters within column are significantly different (LSD P≤0.05).

Table VI. The compatibility of mixing two antimicrobial substances against *S. commune*

Source of antifungal substances	Mean diametric clear zone (mm)*
Peptone Maltose, *M. testaceum*	20cd
Peptone Maltose, M. tesatceum + Neopeptone Lactose *B. multivorans*	29.6a
Neopeptone Lactose, *B. multivorans*	24b
Neopeptone Lactose *B. multivorans* + Peptone Lactose, *B. cepacia*	19.6c
Peptone Lactose, *B. cepacia*	15.5e
Peptone Lactose, *B. cepacia* + Peptone Maltose, *M. testaceum*	20.6c

Means followed by different small letters within column are significantly different (LSD P≤0.05).

were to utilize D-ribose, D-arabinose, D-fucose, trehalose, cellobiose, alicin and not to utilize maltose, D-tartrate, erythritol (Ballard *et al*., 1970). Difference of *B. cepacia* hydrolyzed maltose for production antimicrobial substances and *B. cepacia* did not utilize maltose for identification was caused by different conditions of medium for growth. In the biochemical characteristics of bacteria on fermentation and oxidative against carbon source use solid medium (Fahy & Hayward, 1983), while in the production of antimicrobial substances was done in the liquid medium.

L-Arabinose in proteose peptone did not enhance *B. multivorans* to produce antimicrobial substances. This combination of basal medium was not compatible for bacterial growth. *M. testaceum* hydrolyzed carbon sources of lactose, galactose, mannitol, dextrose, L-arabinose and D + sorbitol in proteose peptone and produced antimicrobial substances.

B. cepacia, B. multivorans and *M. testaceum* produced the antimicrobial substances in the peptones without carbon source. These species grew without depend on carbon sources and extracted antimicrobial substances in the minimal ingredient as the nutrition.

Kinds of peptone (proteose peptone, peptone & neo peptone) enhanced *B. cepacia, B. multivorans* and *M. testaceum* on the production of antimicrobial substances in the same of carbon source of the liquid medium as showed on the variability of antimicrobial substance to suppress the fungal growth. The variation response of bacterial growth and production of antimicrobial substances in the peptones is due to different compounds of each peptone. The peptone itself provides isotonic environment for microorganisms (Yousef & Carlstrom, 2003). Peptone as important supplement to enhance the production of antifungal metabolites was showed by *Bacillus subtilis* and *Erwinia herbicola* in concentration 0.25 and 2%, respectively to inhibit the fungus *Eutypa lata*, causal agent of dieback of gravepine (Schmidt *et al*., 2001). *P. fluorescens* Pf- 5 was enhanced the production of pyrrolnitrin in the liquid medium of diluted corn meal broth and containing 2 µg/mL of supernatant (Rodriguez & Pfender, 1997).

Antimicrobial substances were produced from *B. multivorans, B. cepacia* and *M. testaceum*, had broad spectrum against some species of pathogenic fungi. The spectrum of antimicrobial substances was related to the active compounds in the supernatant. The one of active compound, pyrrolnitrin is broad spectrum antimicrobial substance from *P. cepacia* (Homma *et al*., 1989).

The potential broad spectrum of antimicrobial substances against some species of pathogenic fungi provided more benefit for treatment with the occurred infection more than one pathogen.

The quality of antimicrobial substances was recognized in the minimum inhibitory concentration (MIC). The value of MIC of antimicrobial substances represents the ability of active compounds in the minimum quantity is still able to suppress the growth of fungus. Among 3 sources of antimicrobial substances from 3 species of bacteria had varied MIC. Antimicrobial substances from *B. multivorans* and *M. testaceum* had the higher value of MIC than *B. cepacia*. Both concentrate antimicrobial substances from *B.*

multiviorans and *M. testaceum* had the value of MIC higher than synthetic fungicide with application dose 0.2% such as daconil and mancozeb. These antimicrobial substances from *B. multivorans* and *M. testaceum* in the certain basal liquid medium can be considered for potential bio-pesticide. The compatibility of mixing two antimicrobial substances from 2 sources of bacteria showed stronger formulation than individual antimicrobial substances from one source of bacteria.

CONCLUSION

Antimicrobial substances from *B. cepacia, B. multivorans* and *M. testaceum* through the fermentation in the liquid medium with basal ingredient certain combination carbon sources and peptones can be extracted. Optimal production of antimicrobial substances from *B. multivorans* in the liquid medium with ingredient 10 g neo peptone, 10 g lactose dissolved in 1 L of distilled water. *M testaceum* grows in the liquid medium with ingredient 10 g peptone, 10 g maltose and dissolved in 1 L distilled water.

The mixed concentrate antimicrobial substances from *B. multivorans* and *M. testaceum* were strong substances to suppress *S. commune* 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 bio-pesticide.

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REFERENCES

Ballard, R.W., N.J. Palleroni, M. Doudoroff, R.Y. Stanier and M. Mandel, 1970. Taxonomy of the aerobic Pseudomonas *Pseudomonas cepacia, P. marginata, P. alliicola* and *P. caryophylli*. *J. General Microbiol.,* 60: 199–214

Duffy, B.K. and G. Defago, 1999. Environmental factors modulating antibiotic and siderophore biosynthesis by *Pseudomonas fluorescens* biocontrol strains. *Appl. Environ. Microbiol.,* 65: 2429–38

Fahy, P.C. and A.C. Hayward, 1983. Media and methods for isolation and diagnostic tests. *In*: Fahy, P.C. and G.J. Persley (eds.), *Plant Bacterial Diseases a Diagnostic Guide*, 16: 337–78. Sydney: Academic Press

Homma, Y., Z. Sato, F. Hirayama, K. Konno, H. Shirahama and T. Suzui, 1989. Production of antibiotics by *Pseudomonas cepacia* as an agent for biological control of soilborne plant pathogens. *Soil Biol. Biochem.,* 21: 723–8

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* CL 27 and *Bacillus pumilus* CL 45. *J. Appl. Bacteriol.,* 78: 97–108

Rodriguez, F. and W.F. Pfender, 1997. Antibiosis and antagonism of *Sclerotinia homoeocarpa* and *Drechlera poae* by *Pseudomonas fluorescens* Pf-5 *in vitro* and in planta. Biological Control. *Phytopathology,* 87: 614–21

Roitman, J.N. N.E. Mahoney and W.J. Janisiewicz, 1990. Production and composition of phenylpyrrole metabolites produced by *Pseudomonas cepacia*. *Appl. Microbiol. Biotechnol.*, 34: 381–6

Schmidt, C.S., D. Lorenz, G.A. Wolf and J. Jager, 2001. Biological control of the gravepine dieback fungus *Eutypa lata* II: Influence of formulation additives and transposon mutagenesis on the antagonistic activity of *Bacillus subtilis* and *Erwinia herbicola*. *J. Phytopathol.,* 149: 437–45

Tambong, J.T. and M. Hofte, 2001. Phenazines are involved in biocontrol of *Pythium myriotylum* on cocoyam by *Pseudomonas aeruginosa* PNA1. *European J. Pl. Pathol.,* 107: 511–21

Yousef, A.E. and C. Carlstrom, 2003. *Practical Basic Technique. Food Microbiology. A Laboratory Manual,* P: 277. Wiley-Interscience. A John Wiley and Sons, Inc. Publication

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