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Preliminary screening of endophytic fungi isolated from medicinal plants at MARDI Sessang, Sarawak for their bioactivity

(Penyaringan awal fungi endofit daripada tumbuhan herba di MARDI Sessang, Sarawak untuk bioaktiviti)

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Key words: endophytic fungi, medicinal plants, bioactive compounds

Abstract

A total of 100 isolates of endophytic fungi were isolated from 19 species of medicinal plants collected at MARDI Station Sessang, Sarawak. A total of 55% of the endophytic fungi were isolated from the leaves while 45% from the branches. Screening of isolates for enzymatic secretion found that 15, 28 and 12 isolates were able to hydrolyze cellulose, xylan and mannan respectively. All 100 isolates were also tested for their antimicrobial activity towards selected phytopathogenic and human pathogenic microbes. The test indicated that only one isolate showed positive result when tested against *Xanthomonas campestris*. The results indicate that the endophytic fungi isolated from medicinal plant at MARDI Research Station Sessang, Sarawak may have the potential to be further exploited for its bioactivity.

Introduction

Endophytic fungi have attracted great attention in the past few decades due to its ability to produce novel secondary metabolites for medical, agricultural and industrial use. Endophytic fungi are also considered as an outstanding source of bioactive compounds due to its ability to occupy any plants at any environments (Strobel and Daisy 2003).

Endophytic fungi live in their host plants and due to this, they must develop certain chemical strategies that favour their existence. By producing metabolites, the endophytic fungi either protect the host from animal or herbivores attack or from other pathogenic microbes infection that will decrease the fungi's colonization. Tan and Zou (2001) reported that some endophytic fungi produced highly bioactive compounds. Although the potential of endophytic fungi are known but there are only limited experimental data available as references (Zikmundová et al. 2002). Aphelandrine, which is a macrocylic polyamine alkaloid, was found in the root of different species of the genus *Aphelandra* and is metabolized by several species of endophytic fungi that were isolated from the roots of *Aphelandra tetragona* (Werner et al. 1997).

Several studies on the use of bioactive compounds from endophytic fungi have been reported. Endophytic fungi are able to produce antimicrobial, anticancer such as Taxol (Walker and Croteau 2001) and antimalarial activities (Wiyakrutta et al.

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2004). Study done by Woropong et al. (2001) showed that isolated endophytic fungi are able to produce mixture of volatile organic compounds that are lethal to human and plant pathogenic fungi and bacteria.

In Malaysia and Indonesia, extract from many types of local medicinal plants and jungle plants are used traditionally for the treatments of various ailments (Ong and Noralina 1998; 1999). This has lead to a main question, whether the ability of these plants to be used for treatment comes from the plant itself or is it a consequence of a mutualist relationship between the beneficial organisms that reside in the plant tissue. Reports have indicated that in most microbe–plant relationship, endophytes contribute substances that have various bioactivity reactions such as antimicrobial and enzyme production (Son and Cheah 2002). Medicinal plants were chosen in this study due to the ethnobotanical property of these plants.

This study was conducted to preliminary screen parts of medicinal plants collected from MARDI Station at Sessang, Sarawak for endophytic fungi for the antimicrobial and enzymes secretion activities.

Materials and methods

Sample collection

Plant samples were randomly collected from healthy medicinal plants planted at MARDI Station Sessang, Sarawak in June 2003. All the plant samples were then kept in the refrigerator available at the station. Plant samples were transported back to MARDI Serdang in ice bucket containing ice packs (5 °C).

Isolation

Samples from randomly selected plants of healthy leaves and branches were taken. Leaf and branch portions were washed thoroughly in running tap water. Samples were then surface sterilized by submerging them in 75% ethanol for 2 min, 10% sodium hypochlorite solution for 3 min and 95% ethanol for 30 sec and left to dry (Ananda and Sridhar 2002; Son and Cheah 2002). After drying, the leaves were divided into four segments and were placed onto Potato Dextrose Agar (PDA) supplemented with 50 mg/litre of chloramphenicol to suppress bacterial growth (Son and Cheah 2002; Son et. al. 2003). Branch portions were cut to expose their inner tissues and placed on the same medium. All the plates were incubated at 27 °C for up to 21 days and emerging fungi were transferred to fresh PDA plates and incubated for 7 days with periodical check for purity (*Plate 1*).

Screening for the secretion of enzymes

Endophytic fungi grown on PDA were transferred onto the minimal medium agar containing AZO-CM-cellulose, AZO-Carob-galactomannan and AZO-xylan (Oat) as substrate [peptone 1.0 g, yeast extract 1.0 g, $MgSO_4.7H_2O$ 0.5 g, KH_2PO_4 0.5 g, $(NH_4)_2$ 1.0 g, substrate (Megazyme) 1.0 g, agar 15.0 g and distilled water 1,000 ml] at pH 7 and incubated for 5 days to observe the formation of clear zones (*Plate 2*).

Antimicrobial screening

Endophytic fungi were grown on a 5 ml Potato Dextrose Broth and incubated at 27 °C for 5 days. Extraction of crude bioactive compounds from endophytic fungi was done using isopropanol (Cheah

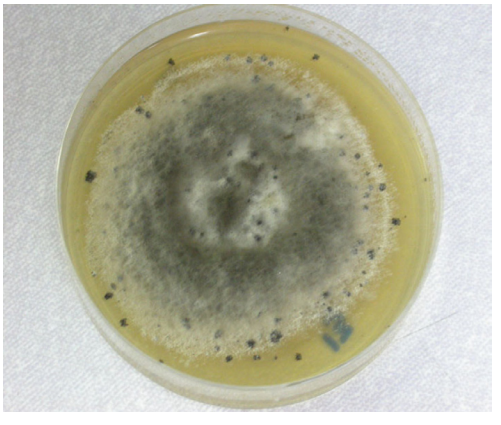

Plate 1. Morphology of endophytic fungi isolate number 13 on Potato Dextrose Agar plate

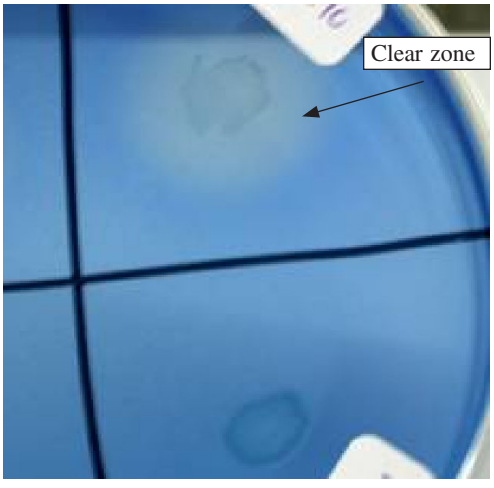

Plate 2. Clear zone formed by endophytic fungi on minimal agar supplemented with cellulose

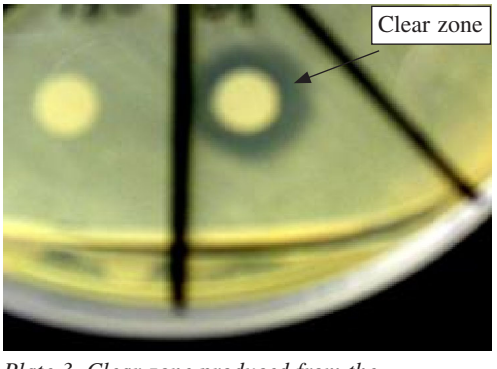

Plate 3. Clear zone produced from the antagonistic reaction between endophytic fungi isolate number 13 with ***Xanthomonas campestris***

2001), ethyl acetate (Pandey et al. 2002) and methanol (Ates and Erdogrul 2003). Each of the endophytic fungi was extracted for its bioactive compounds by adding the selected organic solvent in the ratio of 1:1 (v/v) and shaken vigorously for 1 h using a shaker at 220 rpm. About 20 µl of the organic phase was pipetted and applied onto each paper disc prepared. Paper discs were then left to dry in the laminar airflow for about 60 min before being placed onto selected assay plates.

Assay plates were prepared using different agar media for each test strain. Corn Meal Agar plate was used for *Phytophthora palmivora*, Potato semi-synthetic Agar plate for *Xanthomonas campestris*, Casamino-Peptone-Glucose Agar plate for *Ralstonia solanacearum* and Potato Dextrose Agar plate for *Fusarium* spp. (*Plate 3*).

Results and discussion

Isolation of endophytic fungi

A total of 100 endophytic fungi were isolated from the 19 samples of medicinal plants collected (*Table 1*). Majority (55%) of the endophytic fungi isolated were from the leaves while 45% were isolated from the branches. This indicates that most of the colonization of endophytic fungi in this study maybe localized only on the leaves. This maybe true because local people use mostly extract from the leaves of the medicinal plant (Ong and Noralina 1998). Study done by Glienke-Blance et al. (2002) on citrus plants supported this statement; from the 433 endophytic fungi isolated, 81% were from the leaves. Study done by Son and Cheah (2002) also supported our finding with 110 from 121 endophytic fungi isolated were also from the leaves. More intensive sampling is necessary to further clarify the localization of the fungi in leaves and branches.

Screening for enzymes secretion by endophytic fungi

Isolated endophytic fungi were tested for their ability to secrete cellulase, mannanase and xylanase. Around 15% of the endophytic fungi showed the ability to hydrolyze cellulose, while 12% for mannan and 28% for xylan (*Table 2*) with most of the enzyme producers were isolated from the leaf portions. Endophytic fungi isolated from *Alpinia conchigera* showed the best potential with 50% of the total isolates gave positive reaction to all the three enzymes tested. Endophytic fungi isolated from *Andropraphis paniculata* did not show any reaction towards any of the three enzymes tested. More test on the ability of the endophytes isolated for enzymes secretion

Table 1. Prevalence of endophytic fungi according to host and part of the host

Medicinal plants		Part of plant endophytic fungi isolated from		Total of endophytic fungi isolated
Local name	Scientific name	Leaf	Branch	
Halia hitam	*Zingiber* spp.	3	4	7
Langkunang	*Alpinia conchigera*	3	5	8
Misai kucing	*Orthosiphon stamineus*	5	10	15
Korean ginseng	*Panax* spp.	2	2	4
Jerangau	*Acorus calamus*	2	4	6
Temu pauh	*Curcuma* spp.	2	2	4
Sapooh	*Coleus camosus*	2	2	4
Lemuni	*Vitex negundo linn*	1	0	1
Sambung nyawa	*Gynura procumbens*	1	1	2
Kesum	*Polygonum minus*	3	1	4
Cekur	*Kaempferia galanga*	4	0	4
Pegaga	*Hydrocotyle asiatica linn*	3	0	3
Mengkudu	*Morinda citrifolia*	3	0	3
Kunyit	*Curcuma domestica*	5	4	9
Halia bara	*Zingiber minor*	4	4	8
Ekor anjing	*Plantago major*	5	4	9
Bonglai	*Zingiber cassumunar*	2	0	2
Serai wangi	*Cymbopogon nardus*	5	0	5
Hempedu bumi	*Andropraphis paniculata*	0	2	2
Total		55	45	100

Table 2. Total number of endophytic fungi secreted enzymes

Medicinal plants		Cellulase		Xylanase		Mannanase	
Local name	Scientific name	Leaf	Stem	Leaf	Stem	Leaf	Stem
Langkunang	*Alpinia conchigera*	3	1	3	1	3	1
Misai kucing	*Orthosiphon stamineus*	2	2	2	2	1	1
Jerangau	*Acorus calamus*	1	0	1	0	0	0
Temu pauh	*Curcuma* spp.	0	0	1	1	0	0
Sapooh	*Coleus camosus*	1	0	1	1	1	0
Lemuni	*Vitex negundo linn*	0	0	2	1	1	1
Korean ginseng	*Panax* spp.	1	0	1	1	1	0
Kesum	*Polygonum minus*	0	0	1	0	0	0
Cekur	*Kaempferia galanga*	0	0	2	0	1	0
Mengkudu	*Morida citrifolia*	1	0	0	0	0	0
Sambung nyawa	*Gynura procumbens*	0	0	1	0	0	0
Kunyit	*Curcuma domestica*	0	0	0	1	0	0
Halia bara	*Zingiber minor*	1	0	1	1	0	0
Halia hitam	*Zingiber* spp.	1	0	0	0	0	0
Pegaga	*Hydrocotyle asiatica linn*	1	0	1	0	1	0
Serai wangi	*Cymbopogon nardus*	0	0	1	0	0	0
Ekor anjing	*Plantago major*	0	0	1	0	0	0
Bonglai	*Zingiber cassumunar*	0	0	0	0	0	0
Hempedu bumi	*Andropraphis paniculata*	0	0	0	0	0	0
Total		15		28		12	

should be done in order to diversify the potential of these fungi.

Screening for antimicrobial ability by endophytic fungi

Fermentation broths of 100 endophytic fungi were tested for antimicrobial activities. It was observed that only isolate number 13, which was isolated from leaves, produced antimicrobial activity towards *Xanthomonas campestris.* According to Li et al. (2005), at least one active isolate should be obtained from each plant species but the percentage of active isolates may differ accordingly. Son and Cheah (2002) showed that most of the endophytic fungi with active antimicrobial activities were those isolated from the leaves. In a study conducted by Li et al. (2005), they observed that 62.5% of the total strains of endophytic fungi isolated from *Rhoiptelea chiliantha* exhibited antifungal activity. Other extracts from endophytes that do not show any sign of antimicrobial activity in the disc diffusion bioassay maybe active towards other pathogenic microbes which are not used in this study.

According to Wiyakrutta et al. (2004), one of the reasons that affects the performance or production of bioactive profile is the type of culture medium used. A broad range of culture medium should be employed to test for the bioactivity of the endophytic fungi. Other methods should also be used to explore these isolates as the endophytic fungi may only be an inducer to the immune system in the host itself (Son and Cheah 2002).

The observation of antimicrobial activities in the crude extract, although was detected, but it does not prove that isolate number 13 produced bioactive substances. This is because in the process of screening for bioactive compounds, extract that shows promising results need to be analysed by analytical chemists.

Conclusion

Bioprospecting of microbes such as endophytic fungi for their bioactive properties should not be concentrated only on the production of pharmaceutical products for human. More study should be done by local researchers to further exploit the biodiversity of these endophytic fungi for agricultural purposes. Although not all the endophytic fungi produces bioactive activity, but we believe that endophytic fungi isolated from Malaysian medicinal plants might be a potential source of novel bioactive compounds.

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References

Ananda, K. and Sridhar, K.R. (2002). Diversity of endophytic fungi in the roots of mangrove species on the west coast of India. *Review of Canadian Microbiology 48:* 871–878

Ates, D.A. and Erdogrul, O.T. (2003). Antimicrobial activities of various medicinal and commercial plant extracts. *Turkey Journal of Biology 27:* 157–162

Cheah, Y.K. (2001). Isolation, screening for bioactivities and identification of selected endophyte fungi by sequencing of 18S rRNA/ITS genes. Master Thesis. Universiti Putra Malaysia

Glienke-Blanco, C., Aguilar-Vildoso, C., Ieiro Vieira, M.L., Vianna Barroso, P.A. and Azevedo, J.L. (2002). Genetic variability in the endophytic fungus *Guignardia citricarpa* isolated from citrus plants. *Genetics and Molecular Biology 25:* 251–255

Li, H.Y., Qing, C., Zhang, Y.L. and Zhao, Z.W. (2005). Screening for endophytic fungi with antitumour and antifungal activities from Chinese medicinal plants. *World Journal of Microbiology and Biotechnology 21:* 1515–1519

Ong, H.C. and Noralina, J. (1998). Malay herbal medicine in Gemencheh, Negeri Sembilan, Malaysia. *Fitoterapia 70:* 10–14

—— (1999). Malay enhno-medico botany in Machang, Kelantan, Malaysia. *Fitoterapia 70:* 502–513

Pandey, B., Ghimire, P. and Agrawal, V. P. (2002). Studies on the antimicrobial activity of the actinomycetes isolated from the Khumbu Region of Nepal. Retrieved on 21 June 2004 from http://www.aehms.org/pdf/Panday%20F.pdf

Son, R. and Cheah, Y.K. (2002). Preliminary screening of endophytic fungi from medicinal plants in Malaysia for antimicrobial and antitumor activity. *Malaysian Journal of Medicinal Sciences 9:* 23–28

Son, R., Jeffrey, L., Sahilah, A.M., Cheah, Y.K., Asano, K. and Tomita, F. (2003). Screening for bioactivity from endophyte fungi isolated from botanical garden and forested areas in Sarawak, on the island on Borneo. *Proceedings of Project Seminars in 2002–2003 for JSPS-NRCT/DOST/LIPI/VCC Multilateral Cooperative Research Program in the Field of Biotechnology*, Bangkok, (Murooka, Y., ed.) p. 417–422

Strobel, G. and Daisy, B. (2003). Bioprospecting for microbial endophytes and their natural products. *Microbiology and Molecular Biology Reviews 67:* 491–502

Tan, R.X. and Zou, W.X. (2001). Endophytes: a rich source of functional metabolites. *NaturalProduct Report 18:* 448–459

Walker, K. and Croteau, R. (2001). Taxol biosynthetic genes. *Phytochemistry 58:* 1–7

Werner, C., Petrini, O. and Hesse, M. (1997). Degradation of polyamine alkaloid aphelandrine by endophytic fungi isolated from *Aphelandra tetragona*. *Federation of European Microbiology Societies Microbiology Letter 155:* 147–153

Wiyakrutta, S., Sriubolmas, N., Panphut, W., Thongon, N., Danwisetkanjana, K., Ruangrungsi, N. and Meevootisom, V. (2004). Endophytic fungi with anti-microbial, anti-cancer and anti-malarial activities isolated from Thai medicinal plants *World Journal of Microbiology and Biotechnology 20:* 272–278

Woropong, J., Strobel, G.A., Ford, E J., Li, J.Y., Baird, G. and Hess, W.M. (2001). *Muscodor albus* anam. nov., an endophyte from *Cinnamomum zeylanicum*. *Mycotaxon 79:* 67–79

Zikmundová, M., Drandarov, K., Bigler, L., Hesse, M. and Werner, C. (2002). Biotransformation of 2-Benzoxazolinone and 2-Hydroxy-1,4-Benzoxazin-3-one ny endophytic fungi isolated from *Aphelandra tetragona*. *Applied and Environmental Microbiology 68:* 4863–4870

Abstrak

Sejumlah 100 isolat fungi endofit telah dipencilkan daripada 19 jenis spesies tumbuhan herba yang dikumpulkan dari Stesen MARDI Sessang, Sarawak. Sejumlah 55% daripada fungi endofit yang dipencilkan adalah daripada bahagian daun manakala 45% daripada bahagian ranting. Penyaringan untuk penghasilan enzim mendapati 15 , 28 dan 12 isolat masing-masing menghidrolisiskan selulos, xilan dan mannan. Kesemua 100 isolat juga disaring untuk keupayaan menghasilkan aktiviti antimikrob terhadap beberapa mikrob yang berpatogen terhadap tumbuhan dan manusia. Daripada penyaringan ini didapati hanya satu isolat saja yang menunjukkan keputusan positif apabila diuji dengan *Xanthomonas campestris*. Keputusan yang diperoleh menyatakan bahawa fungi endofit yang dipencilkan daripada tumbuhan herba di Stesen MARDI Sessang, Sarawak berpotensi untuk diekploitasikan bagi bioaktivitinya.

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