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Isolation and screening of actinomycetes from Malaysian soil for their enzymatic and antimicrobial activities

(Pemencilan dan penyaringan aktinomiset dari tanah di Malaysia untuk aktiviti enzim dan anti-mikrob)

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Key words: actinomycetes, biodegradation agent, biocontrol agent, bioactive compounds

Abstract

Actinomycetes, a slow growing gram positive bacteria, are known as an organism that is useful in the search for bioactive compounds. In this study, 212 isolates of actinomycetes were isolated from soil samples collected in the area of Serdang, Bangi, Petaling Jaya and Putrajaya. From the total of 212 isolates, 91 showed the ability to degrade cellulose; 16 for mannan and 90 for xylan. The 212 isolates were then subjected to anti-microbial testing, where they were tested for their ability to produce anti-microbial activity against selected phytopathogens. From the test, only two strains of isolates (strain 161 and 176) showed positive result towards *Xanthomonas campestris.* These two isolates were then identified using research microscope.

Introduction

The role of microorganisms, especially soil microbes as degradation and biocontrol agents, has been widely known and studied. One of these well-known soil microbes is actinomycetes. There are about 100 genera of actinomycetes in the soil (Lo et al. 2002).

As degradation agents, actinomycetes are important in the degradation of soil organic materials into humus. Some actinomycetes secrete a range of enzymes that can completely degrade all the components of lignocellulose (lignin, hemicellulose and cellulose), while others may secrete a narrower range of enzymes that can only partially achieve this degradation (Mason et al. 2001). With their ability to secrete these enzymes, they are effective at attacking tough raw plant tissues and softening them for other microbes.

The use of chemicals to control plant disease pathogens may be harmful for both human and environment. Many researchers are working towards isolating actinomycetes which have the ability to degrade harmful chemicals and also those with ability to act as biocontrol agents.

In a study done by Moncheva et al. (2002), actinomycetes were tested for their antimicrobial activities towards *Erwinia chryzanthemii, Erwinia amylovora* and *Pseudomonas syringae.* Another study by Aghighi et al. (2004), actinomycetes were isolated with antifungal activities towards *Fusarium solani* and *Phytophthora megasperma.*

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Plant-actinomycetes interaction has been extensively studied (Valois et al. 1996). Actinomycetes have the ability to secrete herbicidal compounds (Tanaka and Omura 1993), nitrogen fixation agent (Benson and Silvester 1993) and exudates to protect roots from fungal infections (Weller 1988).

The purpose of this preliminary study was to isolate and screen actinomycetes that have the ability to act as biodegradation agents or biocontrol agents.

Materials and methods

Sample collection

Soil samples were collected 10 cm below the soil surface of ornamental plant soil in Serdang, Bangi and Petaling Jaya. Soil samples from Putrajaya were collected from herbal plant area. All the samples were collected from November 2002 to January 2003.

Isolation

Soil samples collected were pretreated by drying them in open air for 2 days. Samples of 1 g each were mixed with 10 ml of sterile distilled water and incubated at room temperature (25 ± 2 °C) for 1 h on orbital shaker with vigorous shaking. Soil suspension was then pipetted and spread onto Humic acid B-Vitamin (HV) agar (Hayakawa et al. 2004) and incubated at 30 °C for 7 days. Colonies of actinomycetes were picked up using sterile toothpicks and placed onto starch casein agar (SCA). These SCA plates were then incubated for 7 days. Pure colonies of actinomycetes were then subcultured onto SCA slants and incubated for 7 days at 30 °C.

Enzymatic screening

Actinomycetes that were grown on SCA were transferred to the minimal medium (MM) agar with mannan, MM agar with xylan and MM agar with cellulose and were incubated at 30 °C for 7 days. The formation of halo indicated positive result *(Plate 1).*

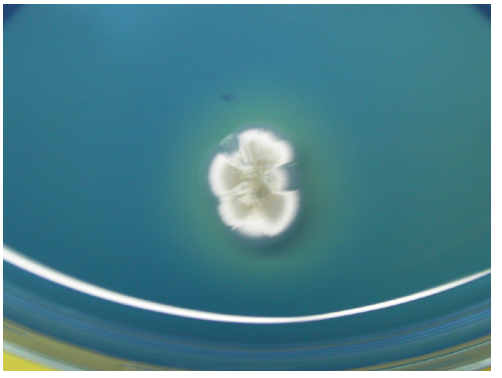

Plate 1. Halo zone formed as the result of actinomycetes hydrolysing xylan

Antimicrobial screening

Assay plates were prepared using different agar media for each test strain. Corn meal agar was used for *Phytophthora* spp., potato semi-synthetic agar was prepared for *Xanthomonas campestris,* casamino-peptone-glucose agar for *Rostalnia solanacearum* and potato dextrose agar for *Fusarium* spp.

Actinomycetes were grown in a 5 ml starch casein broth (SCB) at 30 °C for 4 days. For the extraction of actinomycetes, isopropanol extraction method was used (Cheah 2001). In this method, equal volume of isopropanol was added into the culture broth and was vortexed vigorously for 10 min. The culture broth was then left to settle for about 30 min. Twenty microliter of the supernatant was pipetted and applied to each paper disc. Paper disc that was dipped into sterile distilled water (sdH_2O) was used as the negative control in this test. All the paper discs were then left to dry in the laminar airflow for 60 min.

Paper discs were put onto each assay plates and were incubated at 30 °C. Plates were checked everyday for 4–7 days. Plate with clear zone around the paper disc indicated positive result and the diameters of the zones were recorded.

Morphology identification

Actinomycetes were streaked onto SCA. Kawato and Shinobu (1959), cover slip method was employed for microscopic

purposes where the cover slip was stabbed onto the agar at the angle of 45° and incubated at 30 °C for 6 days. After 6 days of growth, the actinomycetes were examined. Cover slips were then taken out from the agar and put onto the prepared slides. Crystal Violet staining dye was used for this purpose (Sahilah 1991). Slides were then viewed using a research microscope. Identification of actinomycetes to genus level was then carried out based on The Bergey's Manual of Determinative Bacteriology, 9th edition (Zenova et al. 2000; Pandey et al. 2002).

Table 1. Number of actinomycetes isolated from different soil types and areas

Area of collection	Colony forming unit g^{-1} of soil	Number of isolates
Serdang	6.50×10^2	62
Putrajaya	1.57×10^3	113
Bangi	3.03×10^2	16
Petaling Jaya	4.40×10^2	21
Total		212

Results

Isolation

From the soil samples collected, 212 isolates of actinomycetes were isolated. *Table 1* shows the CFU g^{-1} and number of isolates, isolated from different areas. Colony colour of actinomycetes strains isolated were categorised into dark grey, greyish white, whitish and brownish *(Plate 2)*. Results showed 45.3% of total isolates in greyish white, 43.4% in dark grey, 9.9% in whitish and 1.4% in brownish *(Figure 1)*.

Enzymatic screening

In vitro test of actinomycetes for enzymatic reaction showed 42.9% of total isolates were able to hydrolyse cellulose, 42.4% hydrolysed xylan and only 7.5% hydrolysed mannan *(Table 2)*.

Antimicrobial screening

All the 212 isolates of actinomycetes were then tested for antagonist reaction with the phytopathogens selected. Only two strains (A161 and A176) or 0.9% of the isolates showed positive reaction towards *Xanthomonas campestris.*

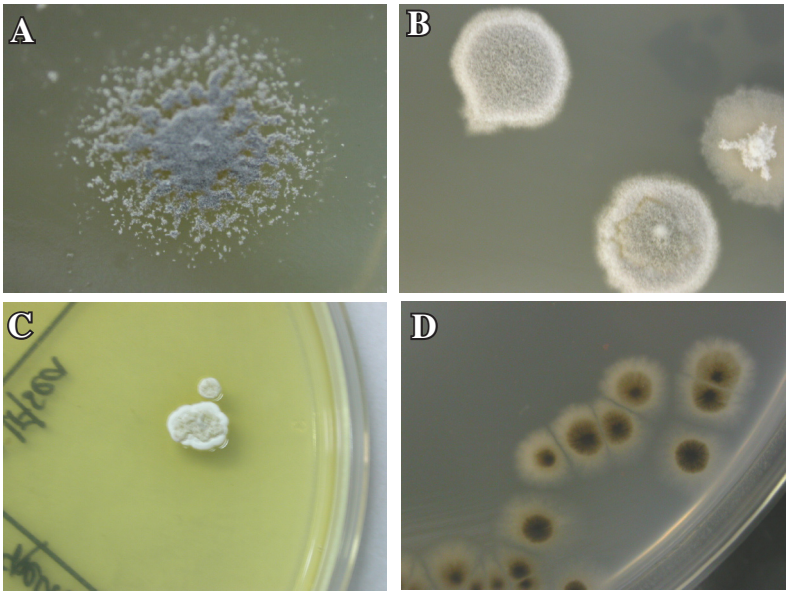

Plate 2. Colony colour of isolated actinomycetes, A) dark grey, B) greyish white, C) whitish and D) brownish

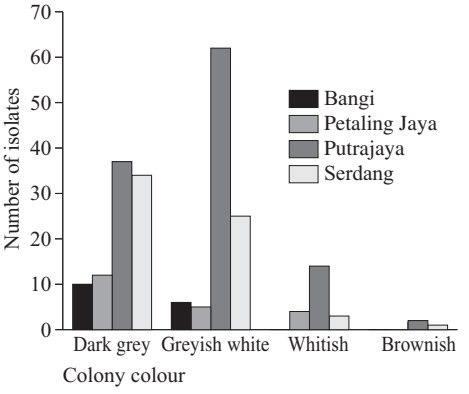

Figure 1. Colony colour of actinomycetes isolated observed on SCA media

Table 2. Number of actinomycetes isolates that were able to hydrolyse cellulose, mannan and xylan

Areas of collection	Number of actinomycetes that are able to hydrolyse		
	Cellulose	Mannan	Xylan
Serdang	14	6	13
Putrajaya	68	10	65
Bangi	3	0	5
Petaling Jaya	6	0	7
Total	91	16	90

Morphology identification

Isolates no 161 and 176 were observed to be from the genus *Streptomyces (Plates 3–4)* according to the spore chain arrangement. At 400X magnification, spore chains were observed to be spiral with different turning number (Bergey's Manual of Determinative Bacteriology, 9th edition, 1994).

Discussion

Isolation

The number of actinomycetes isolated was varied in the four sampling areas, with Putrajaya soil being the highest (113 isolates) and Bangi soil the lowest (16 isolates). This could be due to the differences in the chemical composition of the soil (Tian et al. 2004). Another reason for the variation in the number of actinomycetes from Putrajaya compared to the other three areas was that Putrajaya soil was collected

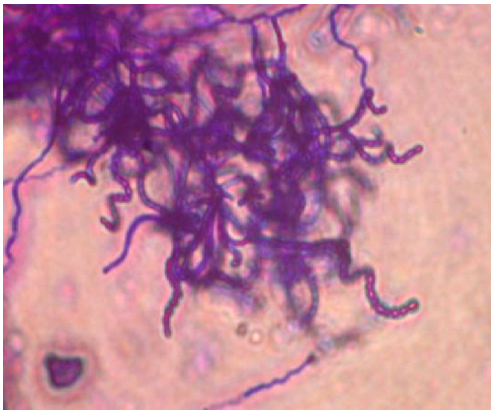

Plate 3. Spores arrangement of actinomycetes strain A161 viewed at 400X

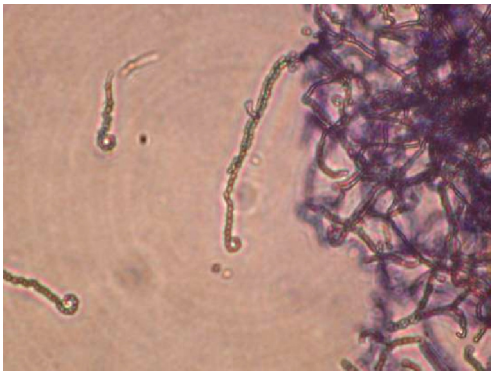

Plate 4. Spores arrangement of actinomycetes strain A176 viewed at 400X

from herbal plants area while the other samples were from ornamental plants.

Study done by Lo et al. (2002) suggested that the actinomycetes diversity might be influenced by the diversity of the plant species where the soil samples were collected. Apart from these, the usage of pesticides and fertilizers will also affect the distribution of these microorganisms. This may be true as the herbal plant was not sprayed with any fertilizers or pesticides while the ornamental plants are scheduled for fertilizers and pesticides from time to time.

Result obtained for colony colour *(Plate 2)*, showed that the actinomycetes isolated could be grouped into four colours (dark grey, greyish white, whitish and brownish). This suggested that there might

be four different genus of actinomycetes but according to study done by Lo et al. (2002), actinomycetes may belong to the same genus although their colours might be different because they give a broad colour group diversity.

Enzymatic screening

Most of actinomycetes isolated from Serdang, Bangi, Petaling Jaya and Putrajaya have the ability to hydrolyse cellulose and xylan compared to mannan. Montiel et al. (1999) stated that mannanase activity was not widely spread among actinomycetes. The ability of actinomycetes to degrade cellulose, mannan and xylan, make actinomycetes an important agent in composting (Lacey 1997), due to its ability to tolerate higher temperature and pH than fungi (Tuomela et al. 2000). The use of microbes, which are able to degrade cellulose and hemicellulose, are important because of high percentage of these cellulose and hemicellulose contents in plant biomass.

Antimicrobial screening

The ability of actinomycetes to produce antibiotic is often associated with its ability to be a biocontrol agent (Crawford et al. 1993). In this study, only two strains of actinomycetes were observed to show antimicrobial activity towards *Xanthomonas campestris.* Both isolates 161 and 176 also produced enzyme activities against cellulose, mannan and xylan and mannan and xylan respectively. Study done by Valois et al. (1996) stated that there are no correlation between the ability to secrete hydrolytic enzymes and the ability of actinomycetes as biocontrol agent. This is justified by our study, where both producers of antimicrobial compound for *Xanthomonas campestris* do not give the same enzymatic activity. Test conducted on *Ralstonia solanacearum, Phytophthora* spp. and *Fusarium* spp. showed negative result. All the test strains that did not produce positive result in this study might give positive results if other pathogens were used. These actinomycetes were kept and preserved for future use.

Conclusion

In this study, 212 isolates of actinomycetes were studied. Actinomycetes isolated from Selangor and Kuala Lumpur areas have more potential in hydrolysing cellulose and xylan than mannan. Only two (161 and 176) isolates or 0.94% of the total isolates secreted antimicrobial compound towards *Xanthomonas campestris.* Both of the isolates were identified as *Streptomyces* spp. using Bergey's Manual based on their spores arrangement. Further studies need to be done before they can be used as biodegraders and biocontrol agents in the agriculture practices.

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Abstrak

Aktinomiset merupakan bakteria gram positif yang berguna untuk penemuan bahan bioaktif. Sebanyak 212 pencilan aktinomiset telah dipencilkan dari sampel tanah di kawasan Serdang, Bangi, Petaling Jaya dan Putrajaya. Daripada 212 pencilan ini, 91 pencilan memberikan keputusan positif terhadap selulase, 16 untuk mannan dan 90 untuk xylan. Kesemua 212 pencilan ini kemudiannya diuji untuk keupayaan menghasilkan anti-mikrob terhadap beberapa penyakit tumbuhan yang terpilih. Daripada ujian itu, hanya dua pencilan (strain 161 dan 176) yang menunjukkan keputusan positif terhadap *Xanthomonas campestris.* Kedua-dua pencilan ini kemudiannya dikenal pasti menggunakan mikroskop.

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