J. Trop. Agric. and Fd. Sc. 36(1)(2008): 155–163

Short communication

Isolation and bioprospecting of actinomycetes from Ayer Keroh Recreational Park, Malaysia

(Pemencilan dan bioprospek aktinomiset dari Taman Rekreasi Ayer Keroh, Malaysia)

L.S.H. Jeffrey*, M.R. Azrizal*, A.A.H. Nor Ayshah* and G.H. Tan*

Key words: actinomycetes, bioprospecting, extracellular enzymes and antimicrobial activity

Abstract

A total of 80 isolates of actinomycetes were isolated from soil samples collected at Ayer Keroh Recreational Park. All isolates were tested for their ability for enzymes secretion of cellulase, mannanase, xylanase, lipase and protease. A total of 70 actinomycetes isolates showed positive reaction for the production of lipase followed by 69 isolates for cellulase, 68 for protease, 65 for xylanase and 22 for mannanase. All the 80 isolates were later tested for their antimicrobial reaction towards *Ralstonia solanacearum*, a bacterial wilt causing agent of tomato plants. From this pre-screening, 12 isolates showed inhibition zone formation ranging from 8–20 mm indicating positive result. All the 12 were identified as *Streptomyces* spp. using primers targeting their 16S rRNA sequence.

Introduction

Actinomycetes, a slow growing gram positive bacteria which was found abundantly in soil, has been known for its ability to produce useful secondary metabolites that can be used as antibiotics, herbicides, pesticides and enzymes such as cellulases and xylanases which can be used for waste treatment (Oskay et al. 2004). A lot of studies on the potential use of actinomycetes for either pharmaceutical or agricultural usages had been carried out all around the world. In Malaysia particularly, several research works on the use of actinomycetes for pharmaceutical or agricultural use have been reported by Vikineswary et al. (1997), Lo et al. (2002) and Jeffrey (2006).

The use of beneficial microbes as biocontrol agents have been favoured by consumers globally over agrochemical compounds. More emphasis has been given to biological control of plant diseases due to the increasing concern of human towards their health. Studies have shown that *Streptomyces* spp. gave significant control towards pythium root rot of corn and sugarcane (Johnson 1954) and cotton wilting (Arjunarao 1971). Managing pest and diseases using biological control would give long term advantages, such as cost saving and a sustainable agriculture although the effect is not immediately (Aghighi et al. 2004).

At present, *Streptomyces* strain remains the main producer of antibiotics (Moncheva et al. 2002). Lechevalier (1992) predicted that the search for bioactive compounds produced by actinomycetes will still be the main interest of researchers in the field of

*Strategic Resources Research Centre, MARDI Headquarters, Serdang, P.O. Box 12301, 50774 Kuala Lumpur, Malaysia

Authors' full names: Jeffrey Lim Seng Heng, Azrizal Mohd. Rashdi, Nor Ayshah Ali Ali Hassan and Tan Geok Hun

E-mail: shlim@mardi.gov.my

medicine and agriculture. However, the probability of isolating one strain with the best activity is still low due to the difficulty in the isolation and cultivation methods used. According to Oskay et al. (2004), improved procedure of isolation and characterization of actinomycetes will enhance the discovery of novel antibiotics and species of actinomycetes.

This paper highlights the studies on the isolation and screening of actinomycetes collected at Ayer Keroh Recreational Park soil.

Materials and methods

Sample collection

Soil samples were collected about 15 cm below the soil surface. All the soil samples were randomly collected below the canopy of selected trees. Soil samples were then packed in zip lock bags and stored in a container filled with ice (~ 4 °C) during transportation.

Isolation and enumeration of actinomycetes from soil samples

Soil samples were air dried for 1 week prior to isolation. This helps in decreasing the population of gram negative bacteria. Soil suspension method described by Oskay et al. (2004) was used, where 1 g of the soil sample was taken and mixed with 100 ml of sterile distilled water (sdH_2O). The soil suspension was shaken vigorously under room temperature (25 ± 2 °C) on an orbital shaker at 200 rpm for 1 h. About 200 µl of the soil suspension was pipetted and lawn onto Starch Casien Agar (SCA) (soluble starch 10.0 g, casein hydrolysate 0.3 g, KNO_3 2.0 g, NaCl 2.0 g, K_2HPO_4 2.0 g, $MgSO_4.7H_2O$ 0.05 g, $CaCO_3$ 0.02 g, $FeSO_4.7H_2O$ 0.01 g, agar 18.0 g, distilled water 1,000 ml, cycloheximide 100 µg/ml) at pH 7. All the plates were incubated at 30 °C for 1–2 weeks. Emerging actinomycetes were picked and streaked onto fresh SCA plates and incubated at 30 °C for 1 week. Colony forming unit (CFU) was determined for all the samples collected.

Preliminary testing of extracellular secretion of enzymes

All the 80 isolates were screened for their cellulase, mannanase and xylanase activity using minimal medium agar (MMA) containing AZO-CM-Cellulose, AZO-Carob-Galactomannan or 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. Gelatin hydrolysis assay as described by Frazier (1926) was employed for the screening of protease activity. Lipase activity was screened using method for determination of esterastic activity (Sierra 1957) with modification. Tween 80 used in the esterastic assay test was replaced with Tween 20 for this purpose. Formation of halo zone indicates positive reaction for the entire test conducted. Measurement of the halo zones was taken for 5 days.

Preliminary testing of antimicrobial reaction towards Ralstonia solanacearum

Plate diffusion method (Bauer et al. 1966) with some modification as suggested by Barakate et al. (2002) was used for the antimicrobial testing against *R. solanacearum*. Isolates of actinomycetes were removed from their agar using a sterile cork bore (5 mm in diameter) and placed onto plate lawn with *R. solanacearum.* Formation of halo zone indicates positive reaction. Measurement of the halo zones were taken for 5 days (*Plate 1*).

Antibiotic resistance test

Actinomycetes were grown in nutrient broth for 4 days before being lawned onto SCA plates. Antibiotic discs (by Sigma) were put onto the agar plates and incubated for 5 days with the size of the zone measured at the fifth day (*Plate 2*). Eleven antibiotic discs used were gentamicin, streptomycin, kanamycin, tetracycline, carbenicillin, cefuroxime sodium, ceftriaxone, sulphamethoxazole, amplicillin, nalacidix acid and penicillin G.

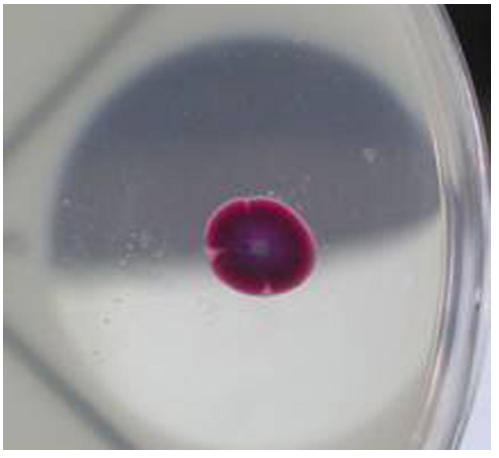

Plate 1. Actinomycetes (AK10) showing positive reaction towards gelatin test

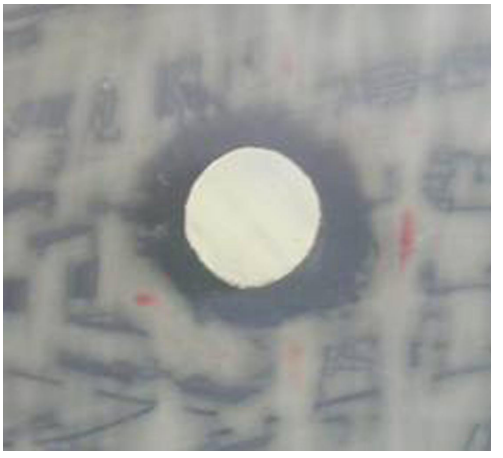

Plate 2. Actinomycetes (AK46) showing positive reaction towards ***Ralstonia solanacearum***

Genomic DNA isolation

Genomic DNA isolation was done using BACTOZOL KIT from Molecular Research Center, Inc. Isolation protocols are according to the manufacturer's instruction (http://www.mrcgene.com/bactozol.htm).

Polymerase Chain Reaction (PCR)

Amplifications were performed in a 25.0 µl mixture containing 16.3 µl sdH_2O, 2.5 µl of 10x PCR buffer (Promega), 1.5 µl of 25 mM $MgCl_2$ (Promega), 0.5 µl of 10 mM dNTP's (Biotools), 0.2 µl of Taq polymerase (Promega), 1.0 µl for both 0.05 mM of Com1 (5'CAGCAGCCGCGGTAATAC3') and 0.05 mM of Com2 (5'CCGTCAATTCCTTTGAGTTT3') primer (Schwieger and Tebbe 1998) respectively, and 2.0 µl of genomic DNA. The reaction tube was then put into MJ Thermalcycler, which had been programmed to preheat at 94 °C for 3 min, followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 60 °C for 30 sec and elongation at 72 °C for 45 sec before a final extension of 72 °C for 10 min. Product size estimated was 408 bp. Sterile distilled water which substituted template DNA was used as a negative control.

Purification of PCR product

PCR product purification was done using *HiYield* Gel/PCR mini Kit supplied by Real Biotech Corporation. Protocol for purification follows as stipulated by the manufacturer (http://www.real-biotech.com).

Sequencing analysis

Purified PCR products were sent to First Base Laboratories Sdn. Bhd. which used ABI PRISM 377 DNA Sequencer (Applied Biosystems) for the sequencing purposes. The obtained 16S rRNA sequences were compared to sequences in the NCBI genebank database with the Basic Alignment Search Tool (BLAST) (Altschul et al. 1990).

Results

Isolation and enumeration of actinomycetes from soil samples

A total of 80 isolates of actinomycetes were isolated from the soil samples collected (*Table 1*). Colony colour of actinomycetes isolates were categorized into dark grey, grey, dark brown, brown, white, reddish-white and yellowish-white. Results showed that 25 isolates (31.25%), 21 isolates (26.25%), 2 isolates (2.50%), 18 isolates (22.50%), 6 isolates (7.50%), 1 isolate (1.25%) and 7 isolates (8.75%) were in dark grey, grey, dark brown, brown, white, reddish-white and yellowish-white

Table 1. Number of isolates, sample origin, colony colour and average colony forming unit (cfu) per 1 g of soil used

Soil sample origin	Average CFU count	Colony colour	Isolate number
Gandis	4.0 X 10^{-6}	Dark grey	AK1, AK4, AK6, AK7, AK8
		Brown	AK2, AK3
		Whitish	AK5
Inggir burung	4.6 X 10^{-6}	Dark grey	AK11
		Grey	AK16
		Dark brown	AK14
		Brown	AK10, AK12, AK15
		Whitish	AK9
		Reddish-white	AK13
		Yellowish-white	AK17, AK18, AK19
Jelutong	2.8 X 10^{-6}	Dark grey	AK23, AK24, AK26
		Grey	AK20, AK28
		Brown	AK21, AK22, AK25
		Whitish	AK27
		Yellowish-white	AK29
Karas	5.1 X 10^{-6}	Dark grey	AK35, AK38
		Grey	AK30, AK31
		Brown	AK33, AK34, AK36, AK37
		Yellowish-white	AK32
Kempas	3.0 X 10^{-6}	Dark grey	AK41, AK42, AK43, AK44
		Grey	AK40
		Yellowish-white	AK39
Keruing gondol	5.6 X 10^{-6}	Dark grey	AK45, AK47, AK53
		Grey	AK46, AK48, AK50, AK51, AK54
		Brown	AK49
		Yellowish-white	AK52
Meranti	3.8 X 10^{-6}	Dark grey	AK56, AK59, AK62
		Grey	AK55, AK58, AK60, AK61, AK63
		Whitish	AK57
Nyatuh	4.5 X 10^{-6}	Dark grey	AK70
		Grey	AK68, AK69
		Brown	AK64, AK66
		Whitish	AK65, AK67
Terap	3.5 X 10^{-6}	Dark grey	AK75, AK77, AK79
		Grey	AK74, AK76, AK78
		Dark brown	AK80
		Brown	AK71, AK72, AK73

respectively. CFU/g obtained from the soil samples showed that soil sample taken beneath the Keruing Gondola gave the highest number of cfu/g (5.6 x 10^6) whereas Jelutong gave the lowest cfu/g count (2.8 x 10^6).

Preliminary testing of extracellular secretion of enzymes

Soil sample collected from Terap area was observed to be the most prominent enzyme producer with a hit of 100% for all the actinomycetes isolated for the activities of cellulase, xylanase, mannanase, lipase and protease as compared to the other samples

collected (*Figure 1*). Mannanase production was observed to be very low as only 27.5% (22/80) gave positive indication. A total of 70 (87.5 %) isolates of actinomycetes showed the ability to produce protease enzyme (*Table 2*).

Preliminary testing of antimicrobial reaction towards Ralstonia solanacearum

Antimicrobial reaction results showed that 12 (15%) of the total isolates produced antimicrobial compound which inhibited the growth of *R. solanacearum.* The isolates were AK14, AK20, AK35, AK39, AK46, AK49, AK51, AK54, AK55, AK58, AK63 and AK68. AK14, AK49, AK51 and AK55 showed a ratio of 4.0 for the halo zone produced while the smallest halo zone produced was 1.6 in ratio by isolate AK46 (*Table 3*).

Antibiotic susceptibility test

All the 12 potential isolates were later tested for their susceptibility against 11 commercial available antibiotics. It was observed that all the tested isolates showed susceptibility to gentamicin, streptomycin, kanamycin and tetracycline.

Interpretation of sequencing data

Based on the BLAST results of the 16S rRNA region obtained from the NCBI genebank, all the 12 isolates belong to the genus *Streptomyces.*

Discussion

Isolation and enumeration of actinomycetes from soil samples

Ndonde and Semu (2000) observed that actinomycetes isolated from the Tanzanian soil can be categorized into six classes, which are cream, blue, grey, pink, red and white. Lo et al. (2002) in their study of actinomycetes isolated from Crocker Range Sabah, indicated that there are three classes of colour observed (grey, white and brown). Grey and white colonies seem to be the most common isolated actinomycetes colonies based on the result obtained from this study, Ndonde and Semu (2000) and

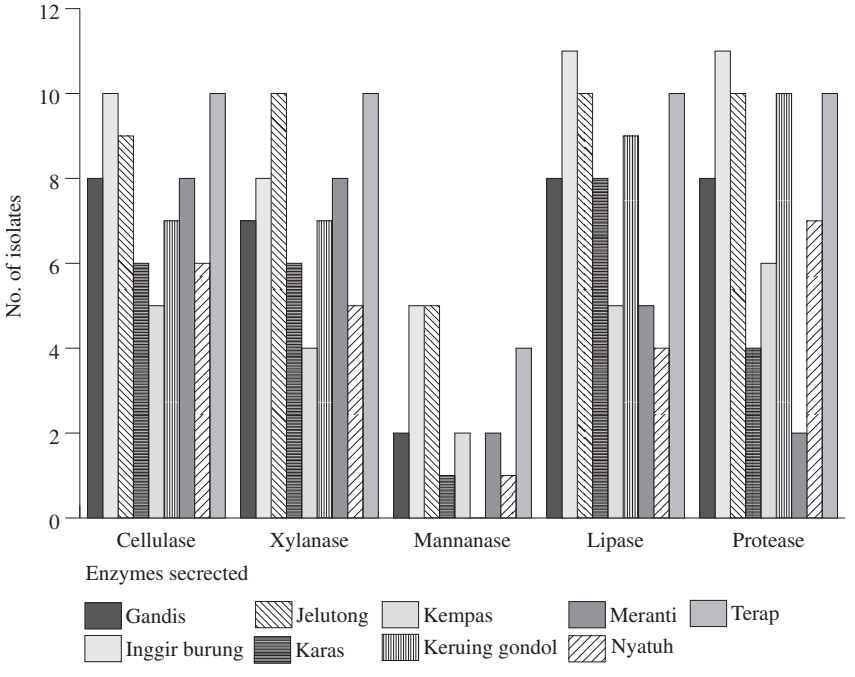

Figure 1. Enzymatic activities produced by actinomycetes of each sample

Table 2. Enzymatic reaction by actinomycetes isolated

Soil sample origin	Percentage of isolates with enzymatic reaction (%)				
	Cellulase	Xylanase	Mannanase	Lipase	Protease
Gandis	100.00 (8/8)	87.50 (7/8)	25.00 (2/8)	100.00 (8/8)	100.00 (8/8)
Inggir burung	90.91 (10/11)	72.73 (8/11)	45.45 (5/11)	100.00 (11/11)	100.00 (11/11)
Jelutong	90.00 (9/10)	100.00 (10/10)	50.00 (5/10)	100.00 (10/10)	100.00 (10/10)
Karas	66.67 (6/9)	66.67 (6/9)	11.11(1/9)	88.89 (8/9)	44.44 (4/9)
Kempas	83.33 (5/6)	66.67 (4/6)	33.33 (2/6)	83.33 (5/6)	100.00 (6/6)
Keruing gondol	70.00 (7/10)	70.00 (7/10)	0.00 (0/10)	90.00 (9/10)	100.00 (10/10)
Meranti	88.89 (8/9)	88.89 (8/9)	22.22 (2/9)	55.56 (5/9)	22.22 (2/9)
Nyatuh	85.71 (6/7)	71.43 (5/7)	14.29 (1/7)	57.14 (4/7)	100.00 (7/7)
Terap	100.00 (10/10)	100.00 (10/10)	40.00 (4/10)	100.00 (10/10)	100.00 (10/10)

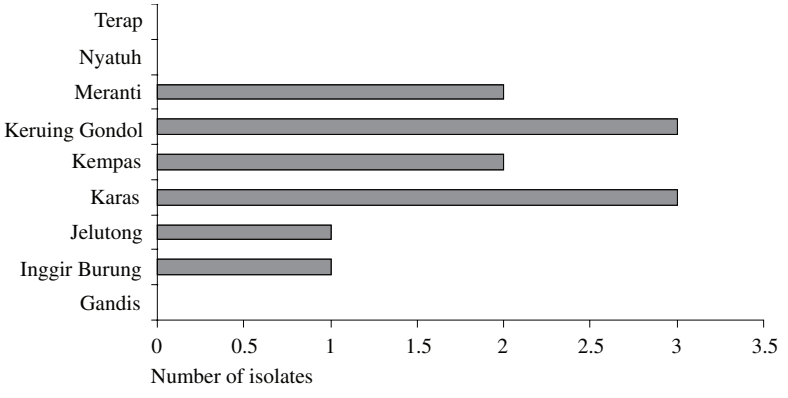

Figure 2. Number of actinomycetes from different source of plant producing antagonistic reaction towards ***Ralstonia solanacearum***

Table 3. Halo zone produce from antagonistic reaction with *Ralstonia solanacearum*

Isolate no.	Ratio of halo zones (mm) to colony diameter (mm)*
AK14	4.0
AK20	3.2
AK35	2.5
AK39	2.0
AK46	1.6
AK49	4.0
AK51	4.0
AK58	3.8
AK55	4.0
AK54	2.2
AK63	2.0
AK68	**3.5**

*Colony diameter = 5 mm

Lo et al. (2002). The variation of colours for the colonies may indicate the diversity of the actinomycetes isolated.

Colony forming unit of actinomycetes per gramme of soil (cfu/g) obtained in this study ($2.8 \times 10^6 - 5.6 \times 10^6$), was higher than the cfu/g for actinomycetes obtained in the previous study conducted by Jeffrey (2006) on garden and agricultural soils ($1.53 \times 10^2 - 1.57 \times 10^3$). The high cfu/g count showed that in an undisturbed soil ecosystem, the activity, density and population of microbes increased. The use of chemical substances on garden and agricultural soil decreased the population of microbes as most of the chemical compounds destroyed the essential components needed for the propagation of beneficial microbes such as actinomycetes. Reganold et al. (1987) stated that the use of

organic substances increases the loss of soil organic matter. In an undisturbed ecosystem such as Ayer Keroh Recreational Park, no chemicals substances were applied. Due to this, the density and population of microbes in particular actinomycetes were increased.

Lee and Hwang (2002), in their study on the diversity of actinomycetes isolated from various vegetative soils in Korea, observed that cfu/g for actinomycetes isolated were $1.17 \times 10^6 - 4.2 \times 10^6$. However this count is still lower compared to the results obtained in this study. The high cfu/g in this area maybe due to the pH, moisture content of the soil, organic matter present and also the type of soil studied. Other than the natural environment factors, agricultural practices may also influence the density and population of soil microbes isolated. Bengtsson et al. (2005) stated that in an organic farming ecosystem, the biodiversity of the area will increase as compared to conventional farming, which maybe the reason for the high population of actinomycetes found in Korean vegetation area.

Screening of actinomycetes for bioactive compounds

Study conducted by Boontim and Lumyong (1999), indicated that only 1 isolate from the 125 isolates of actinomycetes from Chiang Mai soil produces cellulase. This, however, is much lower than the percentage of actinomycetes producing cellulase obtained in this study (86.25 %). These results showed that different sample areas do play a role in the secretion of secondary metabolite by certain microorganisms (in this case actinomycetes).

Study carried out by Jeffrey (2006) on actinomycetes isolated from agriculture and garden soil showed that 6.9% of the actinomycetes produces mannanase while 34.37% produces cellulase and 33.43% produces xylanase. However the result obtained from this study showed a much higher percentage of actinomycetes with the ability to produce cellulase and xylanase but lower in mannanase. This may indicate that actinomycetes isolated from Malaysian soil are not a good producer of mannanase as both studies did not obtain a high mannanase producing actinomycetes.

Many antimicrobial activities had been detected from actinomycetes by researchers all around the world in the past few decades (Moncheva et al. 2002; Aghighi et al. 2004; Oskay et al. 2004; Jeffrey 2006). Most of the studies done were on the medical areas. Little emphasis was given to the use of microorganisms as biological control in agriculture field in Malaysia. Due to the health concern of nowadays consumers on their daily diet, rules and regulations had been imposed onto the amount on chemicals allowed for fruits and vegetables. This has initiated the researchers to not just concentrate on the medical area, but also on the agricultural area as well.

Ndonde and Semu (2000) isolated *Streptomyces* spp. with the ability to inhibit the growth of phytopathogens such as *Clavibacter michiganensis* ssp. *Michiganensis* and *Xanthomonas vascatoria* in vitro with the inhibition zones of more that 30 mm in diameter. Another study done by Sacramento et al. (2004) showed that actinomycetes isolated from the Brazilian soils demonstrated antifungal activities against *Fusarium solani* with the inhibition zone of about 30 mm. This showed that the potential of actinomycetes to produce antimicrobial isolates have been recognized and its usefulness is unlimited.

The ability of actinomycetes to produce antimicrobe isolated from agricultural and garden soil showed only 4% of the isolates were able to secrete antimicrobial compounds towards the phytopathogens tested (Jeffrey 2006). In this study, 15% of the total isolates showed antimicrobial activities towards *R. solanacearum* tested. This proved the idea that novel and better isolates of microbes (actinomycetes) could be isolated from undisturbed regions and this is also the reason why researchers would explore into isolated areas to collect

their samples (Naidenova and Vladimirova 2002). The production on halo zone by actinomycetes in this study needs to be studied more extensively as production of antibiotics is influenced by other chemicals added to the agar media such as salt, carbon and nitrogen sources, temperature and pH (Vasavada et al. 2006). By changing the composition of media used, we may be able to increase the size of the halo zone formed.

Conclusion

From the study, it is observed that most of the actinomycetes with good enzymatic secretion came from soil samples taken under the canopy of Terap. This would suggest that, if exploration of soil microbe with cellulase, xylanase, lipase and protease secretion ability were to be done in the forest ecosystem, soil samples from Terap can be considered as an indicator for the purpose. Although actinomycetes from Terap produces good enzymatic properties, but actinomycetes isolated from Keruing Gondol and Karas are the best antimicrobial producers. This study showed that the most prominent actinomycetes producing bioactive compounds came from the genus *Streptomyces*. Colony forming unit per 1 g soil obtained does not influence the isolation of beneficial microbes in a certain areas as shown in this study.

Acknowledgement

The authors would like to thank MARDI for its support through RM8 and RM9 mechanisms. Thank are also due to Ms Rosnah Hassan and Ms Norzaimawati Aman Nejis for their assistance.

References

Aghighi, S., Shahidi, G.H., Rawashdeh, R., Batayneh, S. and Saadoun, I. (2004). First report of antifungal spectra of activity of Iranian actinomycetes strains against *Alternaria solani, Alternaria alternate, Fusarium solani, Phytophthora megasperma, Verticillium dahliae and Saccharomyces cerevisiae. Asian Journal of Plant Sciences 3:* 463–471

Altschul, S.F., Jenkins, A., Kristiansen, B.E and FrØholm, C.O. (1990). Basic local alignment search tool. *Journal of Molecular Biology 215:* 403–410

Arjunarao, V. (1971). Biological control of cotton wilt III. In vivo effect of antagonists on the pathogens *Fusarium vasiafectum Atk. Proceeding of the Indian Academy of Science B74:* 54–62

Barakate, M., Ouhdouch, Y., Oufdou, K. and Beaulieu, C. (2002). Characterization of rhizospheric soil streptomycetes from Moroccan habitats and their antimicrobial activities. *World Journal of Microbiology and Biotechnology 18:* 49–54

Bauer, A.W., Kirby, W.M., Sherris, J.C. and Turk, M. (1966). Antibiotic susceptibility testing by standard single disk method. *American Journal of Clinical Pathology 45:* 493–496

Bengtsson, J., Ahnström, J. and Weibull, A.C. (2005). The effects of organic agriculture on biodiversity and abundance: a meta--analysis. *Journal of Applied ecology 42:* 261–269

Boontim, N. and Lumyong, S. (1999). Actinomycetes producing chitinase and other polysaccharide hydrolyzing enzymes. *Proceeding of the 5th Asia-Pacific Biochemical Engineering Conference and the 11th Annual Meeting of the Thai Society for Bioctechnology,* Phuket, Thailand

Frazier, W.C. (1926). A method for the detection of changes in gelatin due to bacteria. *Journal of Infectious Diseases 39:* 302–306

Jeffrey, L.S.H. (2006). Screening of locally isolated actinomycetes and endophytic fungi for production of bioactive compounds. Master Thesis, Universiti Putra Malaysia

Johnson, L.F. (1954). Antibiosis in relation to Pythium Root Rot of sugarcane and corn. *Phytopathology 44:* 69–73

Lechevalier, H.A. (1992). Actinomycetes and their products: a look at the future. *World Journal of Microbiology and Biotechnology 8 (Suppl.1):* 72–73

Lee, J.Y. and Hwang, B.K. (2002). Diversity of antifungal actinomycetes in various vegetative soils of Korea. *Review of Canadian Microbiology 48:* 407–417

Lo, C.W., Lai, N.S., Cheah, H.Y, Wong, N.K.I. and Ho, C.C. (2002). Actinomycetes isolated from soil samples from Crcker Range, Sabah. *ASEAN Review on Biodiversity and Environmental Conservation* July–Sept. 2002

Moncheva, P., Tishkov, S., Dimitrova, N., Chipeva, V., Antonova-Nikolava, S. and Bogatzevska, N. (2002). Characteristics of

soil actinomycetes from Antarctica. *Journal of Culture Collections 3:* 3–14

Naidenova, M. and Vladimirova, D. (2002). Isolation and taxonomic investigation of actinomycetes from specific biotopes in Bulgaria. *Journal of Culture Collection 3:* 15–24

Ndonde, M.J.M. and Semu, E. (2001). Preliminary characterization of some Streptomyces species form four Tanzanian soils and their antimicrobial potential against selected plant and animal pathogenic bacteria. *World Journal of Microbiology and Biotechnology 16:* 595–599

Oskay, M., Üsame, A. and Azeri, C. (2004). Antibacterial activity of some actinomycetes isolated from farming soils of Turkey. African Journal of Biotechnology 3: 441–446. Retrieved on 27 Apr. 2005 from http://www.real-biotech.com

Reganold, J.P., Elliott, L.F. and Unger, Y.L. (1987). Long term effects of organic and conventional farming on soil erosion. *Nature 330:* 370–372

Sacramento, D.R., Coelho, R.R.R., Wigg, M.D., Linhares, L.F.T.L., Santos, M.G.M., Semêdo, L.T.A.S. and Silva, A.J.R. (2004). Antimicrobial and antiviral activities of an actinomycete (*Streptomyces* sp.) isolated from a Brazilian tropical forest soil. *World Journal of Microbiology and Biotechnology 20:* 225–229

Schwieger, F. and Tebbe, C.C. (1998). A new approach to utilize PCR-Single-Strand Conformation Polymorphism for 16S rRNA gene-base microbial community analysis. *Applied and Environmental Microbiology 64:* 4870–4876

Sierra, G. (1957). A simple method for the detection of lipolytic activity of microorganisms and some observations on the influence of the contact between cells and fatty substrates. *Antonie van Leeuwenhoek 23:* 15–22

Vasavada, S.H., Thumar, J.T. and Singh, S.P. (2006). Secretion of a potent antibiotic by salt–tolerent and alkaliphilic actinomycete Streptomyces sannanensis strainRJT-1 *Current Science 91:* 1393–1397

Vikineswary, S., Nadaraj, P., Wong, W.H. and Balabaskaran, S. (1997). Actinomycetes from a tropical mangrove ecosystem – antifungal activity of selected strains. *Asia Pacific Journal of Molecular Biology and Biotechnology 5:* 81–86

Abstrak

Sebanyak 80 isolat aktinomiset telah disaringkan daripada sampel tanah Taman Rekreasi Ayer Keroh. Kesemua isolat tersebut diuji untuk menentukan keupayaan isolat tersebut menghasilkan enzim selulase, mannanase, xilanase, lipase dan protease. Sejumlah 70 isolat aktinomiset menunjukkan tindak balas positif untuk lipase diikuti oleh 69 isolat untuk selulase, 68 untuk protease, 65 untuk xilanase dan 22 untuk mannanase. Kesemua 80 isolat itu kemudiannya diuji tindak balas antimikrob terhadap *Ralstonia solanacearum*, penyebab utama penyakit layu bakteria pada tomato. Daripada penyaringan awal ini, 12 isolat menunjukkan terdapatnya zon inhibitasi antara 8.00–20.00 mm yang menunjukkan keputusan yang positif. Kesemua 12 isolat dikenal pasti sebagai *Streptomyces* spp. dengan menggunakan primer yang mensasarkan jujukan 16S rRNA mereka.

Accepted for publication on 30 October 2007