Identification and characterization of actinomycetes for biological control of bacterial wilt of *Ralstonia solanacearum* isolated from tomato

(Pengenalpastian dan pencirian aktinomiset untuk pengawalan biologi layu bakteria *Ralstonia solanacearum* yang dipencilkan daripada tomato)

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Abstract

Five actinomycetes which showed antimicrobial activity towards *Ralstonia solanacearum* were identified using specific polymerase chain reaction (PCR) of 16S rDNA gene. Strain C1 and Strain G10 were identified as *Streptomyces aureofaciens* and *S. roseoflavus* respectively. All actinomycetes were then characterized using antimicrobial and extracellular enzyme activity, metabolic and restriction fragment length polymorphism (RFLP) profiles. Strain A3 showed positive reaction to three bacteria namely *Xanthomonas campestris*, *Staphylococcus aureus* and *Listeria monocytogenes*. Strain C1 and Strain I15 showed positive reaction towards *S. aureus* and *X. campestris* respectively. Strains A3, C1 and I15 were able to metabolize xylan and cellulose, while Strain G10 and Strain L8 were able to use all substrates (xylan, mannan and cellulose) as carbon sources. All the *Streptomyces* strains were positive towards more than 25 carbon sources and can be differentiated into five distinct strains. These results were consistent and confirmed with DNA analysis of RFLP profiles. The specific amplification of 16S rDNA PCR restriction profiles for the strains using three restriction endonucleases, resulted two restriction profiles produced from the digested 16S rDNA product using *Hae*III (H1–H2) and *Hinf*I (Hf1–Hf2), while *Pst*I produced three restriction profiles (P1–P3). No profiles were produced from restriction endonuclases of *Xba*I, *Spe*I and *BamH*I.

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Introduction

Malaysia is one of the richest biodiversity in the world that possesses vast unexplored flora and fauna as well as rich with microorganisms like actinomycetes species that are yet to be discovered (Lo et al. 2002). Actinomycetes is a gram-positive, aerobic, high GC-content and 0.5–1.0 μm in size. They are filamentous, sporulating colonies and recognized as a transition group between primitive bacteria and fungi. Actinomycetes were also known as slow growing bacteria and micro goldmine with useful secondary metabolites. They are sources of approximately 70% antibiotics, extracellular enzymes and other commercially important bioactive compounds (O'Donnell 1988).

Among the actinomycetes groups, *Streptomyces* are the most popular and found worldwide in soil, and important in soil ecology. They belong to the order Actinomycetales. *Streptomyces* are metabolically diverse and can utilise almost anything as carbon source due to its ability to produce extracellular hydrolytic enzymes, including sugars, alcohols, amino acids, organic acids, aromatic compounds and other complex substrate such as cellulose, mannan and xylan. They are also well known for their abilities to produce antibiotics and other secondary metabolites (Willey et al. 2008). Thus, these microorganisms have been implicated in the antagonism of a wide variety of plant pathogenic bacteria, fungi and nematodes for their potential use as biological disease control agents (Crawford et al. 1993; Dicklow et al. 1993; Hodges et al. 1993).

The advantages of the wide range of substrate that can be metabolized by *Streptomyces* are attractive to be applied in oil palm compost to accelerate the degradation process, and also as biological control. Malaysia produces 90 million tonnes of renewable lignocellulosic annually including empty fruit bunches (EFB), mesocarp fibres, shells, oil palm trunks (OPT) and oil palm fronds (OPF) from over 300 oil mill locations (Chew et al. 2002).

Biological control is an alternative to synthetic pesticide in the future of Malaysian agriculture. Previous studies had proved the potential of actinomycetes as effective biocontrol for *Ralstonia solanacearum* and some were also able to promote growth of tomato plant with larger leaves and greater biomass (Moura et al. 2004). *Ralstonia solanacearum* is a soil-borne gram-negative bacterium that causes bacterial wilt disease in over 450 plant species, including many economically important crops such as potato, chilli, tobacco, tomato, eggplant, bell pepper, peanut and banana (Hayward 1991).

In Malaysia, the incidence of *R. solanacearum* was reported in tomato, ginger rhizomes, groundnut, pepper and indigenous vegetable such as *Cantella asiatica*. Malaysian losses caused by the disease are not known (not documented).

This study identified and characterized the *actinomycetes* strains which have potential in controlling *R. solanacearum* from tomato. The purpose of screening extracellular enzyme activity was to explore *Streptomyces* that have the ability to degrade raw material of EFB, can survive and act as biocontrol agent when applied in the field.

Materials and methods

Actinomycetes were obtained from Sahilah et al. (2006).

Screening for biological control agent and other bacteria

Screening for antimicrobial activity was conducted using plate diffusion method (Barakate et al. 2002). The formation of halo or clearing zone indicated positive reaction to bacteria tested. Test strains for *R. solanacearum* and other plant pathogenic bacteria (*Xanthomonas campestris*, *Erwinia chrysanthemi* and *Pantoe stewartii*) were obtained from MARDI. *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella typhimurium* and *Vibrio parahaemolyticus*

were obtained from Universiti Putra Malaysia, Serdang.

Prior to the antimicrobial activity screening, the actinomycetes were grown onto Starch Casein Nitrate Agar (SCN) (Kuster and Williams 1964) for 3 days at 30 °C. The grown actinomycetes on SCN medium then, were cut (5 mm in diameter) and placed onto *R. solanacearum* lawn onto Casamino Peptone Glucose Agar (CPG) (10 g glucose, 10 g peptone, 1 g casein hydrolysate, 15 g agar per litre of distilled water).

For *X. campestris*, *E. chrysanthemi* and *P. stewartii*, the actinomycetes were placed onto nutrient agar (NA) (20 g NA (Merck) per litre of distilled water). For *V. parahaemolyticus*, the actinomycetes were placed onto bacteria lawn on Lauria Bertani (LB) agar with 3% NaCl (10 g tryptone, 5 g yeast extract, 30 g NaCl, 20 g agar per litre of distilled water).

For other foodborne bacteria (*S. aureus, L. monocytogenes*, *S. typhimurium*), the study was conducted by putting the actinomycetes onto lawn bacteria on Muller Hinton (MH) plates (38 g MH agar per litre of distilled water).

Morphological characteristic

Actinomycete cells morphology was examined using Holt et al. (1994) methods. Genus identification was based on the Bergey's Manual of Determinative Bacteriology (Holt et al. 1994).

Actinomycetes growth and DNA preparation

All actinomycete strains were grown in nutrient broth (NB) (8 g NB per litre of distilled water) and incubated in reciprocal-shaker (200 rpm) for 4 days at 30 °C. Prior to amplification by 16S rDNA PCR, total genomic DNA of the actinomycetes were extracted by the conventional phenol-chloroform-isoamyl method as described by Lesley (2002).

16S rDNA Gene Specific PCR

Specific Polymerase Chain Reaction (PCR) of 16S rDNA gene was conducted as described by Paolo et al. (2002). The primers used during the PCR amplification were primer 243F (5'-GGATGAGCCCGCGGCCTA-3') and primer A3R (5'-CCAGCCCCACCTTCGAC-3'), with the expected PCR amplified fragment size of 1.25 kb.

For the specific amplification of 16S rDNA fragments of actinomycetes, the reaction mixture (50 μl) was as follows: 2 μl of template DNA (ca. 20 pg), 3 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 0.2 mM deoxynucleoside triphosphates, 250 nM of each primer (243F and A3R), 10x Denhardt's reagent and 0.2 μl (5 U/μl) *Taq* Polymerase (Promega, Madison, Wis.).

PCR amplification was performed in a thermal cycler (MJ Research Inc., Waltham, MA,) with a temperature programme consisting the initial denaturation at 95 ºC for 5 min followed by 30 cycles of denaturation at 95 °C for 2 min, annealing for 1 min at 35 °C and polymerization at 72 °C for 2 min. Final extension was at 72 °C for 10 min. This hot start was necessary to prevent non-specific annealing of the primers to non-target DNA.

The amplification product were analysed by electrophoresis on 1.5% agarose gel for 40 min at 100 V with 1X TBE buffer against 1 Kb DNA ladder (Promega, Madison, Wis.) of nucleic acid markers. The nucleic acids on gel were visualized using UV transilluminator (Biorad) and gel documentation system (Gel Doc software) after staining with edithium bromide (1 μg/μl) for 3 min and destaining in sterile distilled water for 15 min.

Sequencing

Specific PCR of 16S rDNA gene products were conducted on 1.5% agarose gel and the DNA bands were excised with sharp

sterile scalpel. The agarose containing PCR products were then extracted and PCR products purified using the QIAEX II Gel Extraction Kit (Qiagen Inc., Valencia, CA) according to the manufacturer's instructions.

Nucleotide sequence analysis of purified PCR products were sequenced by a commercial sequencing company, 1st Base Company (www.base-asia.com/my). DNA sequencing was performed by the method outlined in the Prism Ready Reaction Dye Deoxy Terminator Cycle Sequencing Kit (Applied Biosystems) on an Applied Biosystems Model 373A DNA Sequencing System.

Sequence similarities were determined by comparing to 16S rDNA sequences available in the nucleotide databases of the GenBank (http://www.ncbi.nlm.nih.gov/BLAST), using the Basic Local Alignment Search Tool (BLAST) program (Altsuchul et al. 1997) from for genus identification.

Restriction fragment length polymorphism (RFLP) analysis
Six types of restriction endonuclases of *Hae*III, *Hinf*I, *Pst*I, *Xba*I, *Spe*I and *BamH*I (New England BioLabs) were chosen to cut the 16S rDNA amplification PCR products. Digestions with *Hae*III, *Hinf*I, *Pst*I, *Xba*I, *Spe*I and *BamH*I enzymes were performed in a total volume of 20 μl containing 10 μl amplified DNA, 5 U enzyme, 2 μl 1X digestion buffer and 0.2 μl 100 μg/ml bovine serum albumin (BSA).

The digestion mixture was incubated for 4 h at 37 °C in water bath shaker. The digestion products were fractionated by electrophoresis using 2% agarose gel in 1X TBE buffer (0.1 M Tris, 0.1 M boric acid, 0.2 mM EDTA), detected by staining with ethidium bromide and photographed under UV translumination (BioRad USA). The 100 bp DNA ladder (Promega, USA) was used as a DNA size marker.

Extracellular enzymes activity
The actinomycetes colonies grown on Starch Casein Nitrate agar (SCN) plate for 3 days at 30 °C were transferred to the Minimal Medium (MM) (1 g peptone, 1 yeast extract, 0.2 KH_2PO_2, 1 $(NH_4)2HPO_4$, 0.5 $MgSO_4.7H_2O$, 15 agar and 1 g of substrate per litre of distilled water) agar supplied either mannan, xylan or cellulose (MegaZyme) respectively and incubated for 7–14 days at 30 °C. The MM agar supplied with substrate (mannan, xylan or cellulose) showed blue colour due to indicator added in the products (MegaZyme). Thus, strains of actinomycete which have an ability to degrade the substrate will show a clear zone around the colony and scored as a positive result.

Metabolic profiles using Biolog System
Actinomycetes were grown on Starch Casein Nitrate agar (SCN) for 3 days at 30 °C. Spores of actinomycetes were then scrub off from the plate and immersed into SP-F2 inoculating fluid purchased from Biolog Inc. (USA). Transmission for the fluid of 60% was needed for the purpose. Inoculation fluid was then poured into a reservoir and 150 μl of the inoculating fluid was pipetted out using a multi-channel pipetter into the well on the microplate. SP-F2 microplates were then incubated at 30 °C and read using Biolog System (USA) reader everyday for 7 days.

Results
Morphological characteristics
Preliminary study of 227 actinomycetes isolated (tropical rain forest of Taman Negara, Pahang) and screened with plant pathogenic of *R. solanacearum*, 87 actinomycetes showed antagonism activity towards these bacteria (Sahilah et al. 2006). Five strains (Strain A3, C1, G10, I15 and L8) that showed most clearing zone towards *R. solanacearum* were chosen and their colonies and cells morphological characteristic were studied.

Actinomycetes Strain A3 and L8 were white colonies, while actinomycetes Strain C1 and I15 were grey and G10 was yellow colony (*Plates 1a–5a*). All actinomycetes
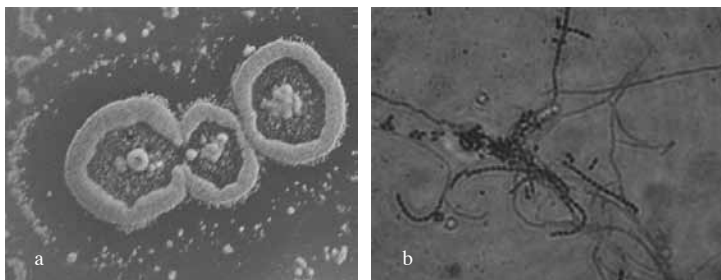
*Plate 1. Colonies (a) and cells morphology (1000x magnification) (b) of **Streptomyces** Strain A3 after 7 days of incubation on Starch Casein Nitrate (SCN) medium*
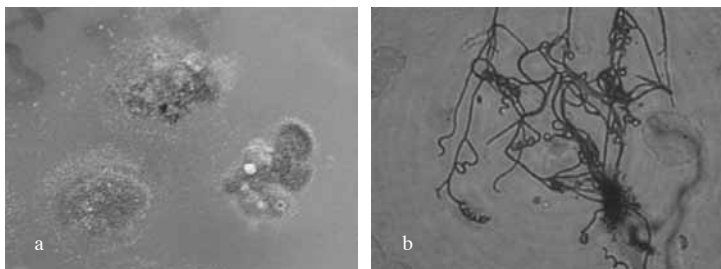

*Plate 2. Colonies (a) and cells morphology (1000x magnification) (b) of **Streptomyces aureofaciens** Strain C1 after 7 days of incubation on Starch Casein Nitrate (SCN) medium*
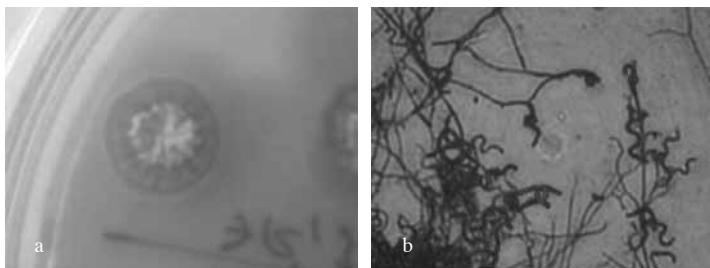

*Plate 3. Colonies (a) and cells morphology (1000x magnification) (b) of **Streptomyces roseoflavus** Strain G10 after 7 days of incubation on Starch Casein Nitrate (SCN) medium*
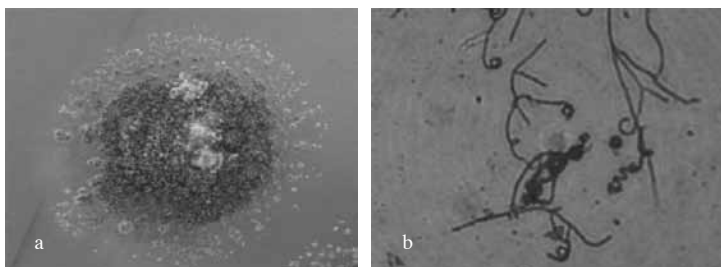

*Plate 4. Colonies (a) and cells morphology (1000x magnification) (b) of **Streptomyces** Strain I15 after 7 days of incubation on Starch Casein Nitrate (SCN) medium*
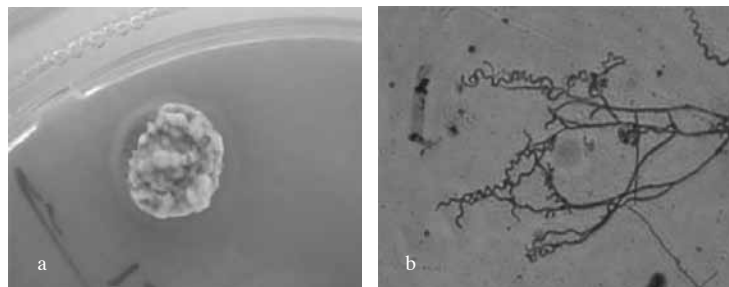
*Plate 5. Colonies morphology (a) and cells morphology (1000x magnification) (b) of **Streptomyces** Strain L8 after 7 days of incubation on Starch Casein Nitrate (SCN) medium*

formed aerial spores with white to whitish-grey in colour. Two strains (Strain G10 and I15) were able to form yellow and red pigment on SCN medium. Whereas, none for the other strains (A3, C1 and L8).

Observation under microscope showed that the five actinomycetes strains represented members of genus *Streptomyces* (*Plates 1b–5 b*). The five actinomycetes possessed either straight or spiral types of spore chain morphology. Of the five strains, only *Streptomyces* Strain A3 (*Plate 1*) was characterized by long chains of straight conidia. Whereas, the others were spirals at the tip of the non-fragmented hyphae (*Plates 2b–5b*). These results also were consistent with results obtained for 16S rDNA gene sequences DNA, indicated that all actinomycetes were represented as *Streptomyces* species.

Identification of actinomycetes using 16S rDNA partial secquences DNA

All actinomycetes were examined for the specific amplification of 16S rDNA gene secquences (Paolo et al. 2002). As indicated in *Table 1*, all five actinomycetes were identified as *Streptomyces* species with more 85% sequence similarity. Strain C1 and strain G10 were identified up to species level, namely *Streptomyces aureofaciens* and *S. roseoflavus* respectively.

Antimicrobial and extracellular enzymes activity

All *Streptomyces* strains showed antimicrobial activity towards *R. solanacearum* (Sahilah et al. 2006). The Strain A3 showed positive antimicrobial activity towards three other bacteria (*Xanthomonas campestris*, *Staphylococcus aureus* and *Listeria monocytogenes*). Strain C1 and I15 showed positive antimicrobial activity towards *S. aureus* and *X. campestris* respectively.

In extracellular enzyme activity, three strains were able to metabolize xylan and cellulose (*Streptomyces* Strain A3, C1 and I15). While, *S. roseoflavus* Strain G10 and *Streptomyces* Strain L8 were able to metabolize all substrates (xylan, mannan and cellulose) as their carbon sources (*Table 2*). These advantages characteristic will help the five actinomycetes (Strain A3, C1, G10, I15 and L8) survive in complex substrate such as empty fruit bunch (EFB) compost which can be applied into soil as organic fertiliser and at the same time may possess biological control value.

Metabolic profiles using Biolog System

All *Streptomyces* were examined using Biolog System to obtain their metabolic profiles or biotyping. Biolog System analysis is based on carbon (C) utilization patterns of the streptomycetes towards different carbon source. The ability to use wide range of carbon source may indicate that the *Streptomyces* were able to survive in

Table 1. Blast result of partial 16S rDNA gene sequence of 5 actinomycetes which are positive towards *Ralstonia solanacearum*

Actinomycete isolates	Closest BLAST match	Accession no.	Sequence similarity (%)
A3	*Streptomyces* sp. LK1334.5	AY465336	98
C1	*Streptomyces aureofaciens* Strain IMET 43577	AY289116	87
G10	*Streptomyces roseoflavus* Strain Men-myco-93-63	AY370677	97
I15	*Streptomyces* sp. 434D03 gene	AB123523	97
L8	*Streptomyces* sp. s14	AY436315	98

Table 2. Potential *Streptomyces* strains for controlling *Ralstonia solanacearum* and other bacteria and their extracellular enzyme activity

Actinomycete isolates	Antimicrobial activity against plant and food pathogenic bacteria							Extracellular enzyme activity		
	RS	XC	PS	SA	LM	ST	VP	X	M	C
A3	+	+[c]	–[d]	++	++	–	–	+	–	++
C1	++	–	–	+	–	–	–	+	–	+
G10	+	–	–	–	–	–	–	++	+	+++
I15	+	+	–	–	–	–	+	++	–	+
L8	+	–	–	–	–	–	–	+++	+	+

Bacteria: RS = *Ralstonia solanacearum*; XC = *Xanthomonas campestris*; PS = *Pantoe sterwatii*;
SA = *Staphylococcus aureus*; LM = *Listeria monocytogenes*; ST = *Salmonella typhimurium*;
VP = *Vibrio parahaemolyticus*
 X = Xylan; M = Mannan; C = Cellulose
 + = Good enzyme activity; ++ = Better enzyme activity; +++ = Best enzyme activity
 – = None

Table 3. Characterization of *Streptomyces* strains using metabolic profiles

Carbon grouping	C sources	*Streptomyces* strains				
		A3	C1	G10	I15	L8
Polymers (n = 7)	α-Cyclodextrin	+	–	+	–	–
	β-Cyclodextrin	+	–	+	–	–
	Dextrin	+	+	+	+	+
	Glycogen	–	–	–	–	+
	mannan	–	+	+	–	+
	Tween 40	+	+	+	+	+
	Tween 80	+	+	+	+	+
Sugar and sugar derivatives (n = 35)	Amygdalin	–	+	–	–	+
	N-acetyl-D-glucosamine	+	+	+	+	+
	L-Arabinose	–	–	+	–	–
	D-Arabitol	–	–	+	–	+
	Arbutin	+	–	+	–	+
	D-Cellobiose	+	+	+	+	+
	D-Fructose	+	–	+	–	+
	D-Galactose	–	+	+	+	+
	D-Galactoronic Acid	–	–	+	–	+
	Gentiobiose	–	+	+	+	+

(cont.)

Table 3. *(cont.)*

Carbon grouping	C sources	*Streptomyces* strains				
		A3	C1	G10	I15	L8
	D-Gluconic Acid	+	+	+	+	−
	α-D-glucose	+	−	+	+	+
	m-inositol	−	−	−	−	+
	α-D-lactose	+	−	+	+	+
	Lactulose	−	−	−	−	+
	Maltose	+	+	+	+	+
	Maltotriose	+	+	+	+	+
	D-Mannitol	−	−	+	+	+
	D-Mannose	+	+	+	−	+
	D-Melezitose	−	−	+	−	−
	D-Melibiose	−	−	+	−	+
	α-Methyl-D-galactoside	+	−	−	−	+
	β-Methyl-D-galactoside	−	−	−	−	+
	palatinose	−	−	−	−	+
	D-Raffinose	−	−	+	−	+
	L-Rhamnose	−	−	+	−	+
	D-Ribose	−	+	+	+	+
	Salicin	−	−	+	+	+
	D-sorbitol	+	−	−	−	−
	Stachyose	−	−	+	+	−
	Sucrose	−	+	+	+	−
	D-Trehalose	−	+	+	+	+
	Turanose	−	−	−	−	+
	Xylitol	−	−	+	−	−
	D-Xylose	−	−	+	−	−
Carboxylic acids and methyl esters (n = 4)	Acetic acid	−	−	−	+	−
	γ-Hydroxybutyric acid	−	−	+	−	−
	Lactamide	−	−	+	−	−
	D-Malic acid	−	+	+	−	−
Carboxylic acids and methyl esters (n = 14)	L-Malic acid	−	+	+	+	+
	Methyl Pyruvate	−	+	−	−	−
	Mono-methyl succinate	−	−	+	−	−
	Pyruvic acid	−	+	+	−	+
	Succinamic acid	−	−	−	−	+
	L-Alaninamide	+	+	+	+	−
	L-Alanine	+	−	+	+	−
	L-Alanyl-glycine	+	+	+	−	+
	L-Asparagine	+	+	−	+	+
	L-Glutamic acid	+	−	+	−	+
	Glycyl-L-glutamic acid	+	−	+	−	+
	L-Pyroglutamic acid	−	+	+	+	+
	L-Serine	−	+	+	−	−
	Putrescine	+	+	+	−	−
Alcohols (n = 1)	Gylcerol	−	+	+	+	+
Nucleosides and nucleotides (n = 7)	Adenosine	−	+	+	+	−
	2'-deoxy adenosine	+	+	−	−	+
	Inosine	−	+	+	+	−
	Thymidine	+	−	−	−	−

(cont.)

Table 3. *(cont.)*

Carbon grouping	C sources	Streptomyces strains				
		A3	C1	G10	I15	L8
	Uridine	–	–	+	–	–
	Adenosine-5'-mono-phosphate	–	+	+	+	–
	Uridine-5'-Mono-phosphate	–	+	+	+	–
Sugar phosphates (n = 4)	Fructose-6-phosphate	–	–	–	–	+
	Glucose-1-phosphate	–	–	–	–	+
	Glucose-6-phosphate	–	–	–	–	+
Total C sources	72	26	32	53	29	45

different environment in nature. Metabolic profiles resulted from Biolog System analysis indicated the five *Streptomyces* were differentiated into different strains.

As shown in *Table 3*, the five *Streptomyces* (Strain A3, C1, G10, I15 and L8) have different capability to metabolize 95 carbon sources from SP-F2 microplates. The 95 carbon sources are categorized as polymers, sugar and sugar derivatives, carboxylic acids and methyl esters, carboxylic acids and methyl esters, alcohol, nucleosides and nucleotides and sugar phosphates. Of the 95 carbon sources, only 72 can be utilized by the five strains. Strain G10 was one from the five colonies, showing significantly higher in carbon sources activity with 53 followed by Strain L8, C1, I15 and A3 with 45, 32, 29 and 26 respectively.

Restriction fragment length polymorphism (RFLP) analysis

Of the six restriction endonucleases *Hae*III, *Hinf*I, *Pst*I, *Xba*I, *Spe*I and *Bam*HI, only three enzymes (*Hae*III, *Hinf*I and *Pst*I), showed restriction profiles. The 16S rDNA PCR restriction profiles for the five *Streptomyces* strains with the three restriction endonuclases are shown in *Figure 6* and *Table 4*. Two restriction profiles were produced from the digested 16S rDNA product using *Hae*III (H1–H2) and *Hinf*I (Hf1–Hf2), while *Pst*I produced three restriction profiles (P1–P3).

Discussion and conclusion

Identification through morphological colonies and cells indicated that all the actinomycetes were in the genus of *Streptomyces*. This was confirmed using 16S rDNA gene sequence analysis. Two strains were identified to species level, *Streptomyces aureofaciens* (Strain C1) and *S. roseoflavus* (Strain G10).

Besides having antagonistic activity towards one or more pathogen bacteria, the five *Streptomyces* also have the ability to produce extracellular enzymes of complex substrates such as cellulose, xylan and mannan. This suggested that the five strains can be applied in usable raw materials such as palm oil empty fruit bunch (EFB) since the major components of this agriculture waste are consisted of 45–50% cellulose, 25–35% hemicellulose and lignin (equal amount) (Deraman 1993).

Table 2 shows that all the actinomycetes have the ability to produces extracellular enzymes. Hence, these strains (Strain A3, C1, G10, I15 and L8) have the potential to be used as microbial consortia with two advantages as biocontrol and EFB decomposer However, further studies for those *Streptomyces* are advisable to assure that the actinomycetes are not harmful to humans, non-target organisms or the environment.

Results obtained from Biolog System showed that the *Streptomyces* strains were also able to degrade different carbon sources. The ability to utilize
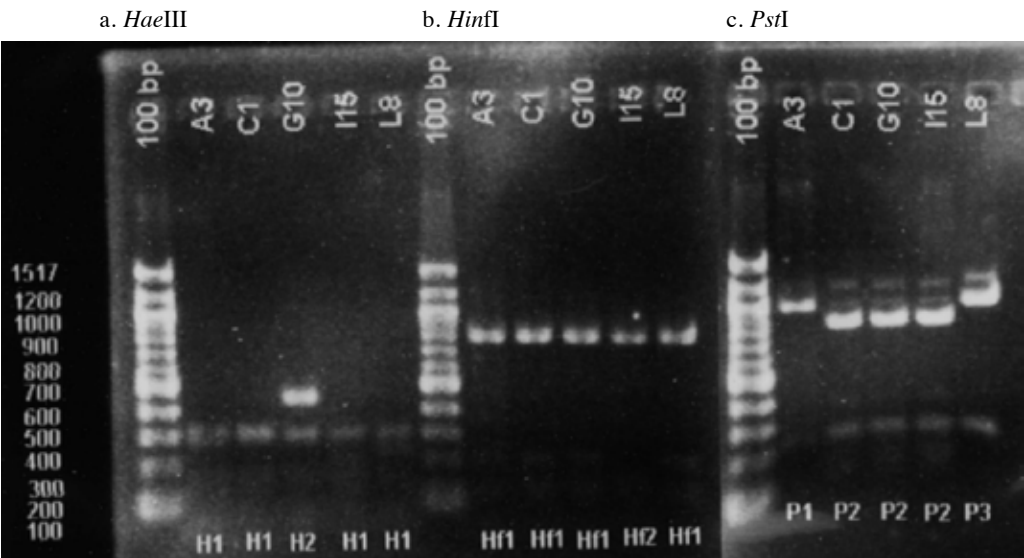
Plate 6. RFLP=PCR profiles of streptomyces using restriction endonucleases of, *a. JaeIII (H1-H2) b. HinfI (Hf1-Hf2) and c. PstI (P2-P3)*

Table 4. The RFLP-PCR profiles of *Hae*III, *Hinf*I and *Pst*I

Strains	Restriction endonucleases			Genome types
	*Hae*III	*Hinf*I	*Pst*I	
A3	H1	Hf1	P1	G1
C1	H1	Hf1	P2	G2
G10	H2	Hf1	P2	G3
I15	H1	Hf2	P2	G4
L8	H1	Hf1	P3	G5

those substrates indicated that the five *Streptomyces* strains have wide range ability in decomposition of different carbon sources. The Biolog System results also can be used as biotyping which indicated that the five *Streptomyces* strains were of different species. This was further supported using the RFLP analysis (*Plate 6)* where they were differentiated into five distinct strains. In this study, the Biolog System analysis not only determined the strains differentiation but also the ability of all *Streptomyces* to metabolize the carbon sources available in the microplate analysed.

In conclusion, the five *Streptomyces* have potential value as decomposer and for biocontrol agent purposes. Metabolic and

RFLP profiles were able to differentiate the *Streptomyces* into five distinct species. They are different species of *Streptomyces* rather than a single species.

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Abstrak

Lima aktinomiset yang menunjukkan aktiviti antimikrob terhadap *Ralstonia solanacearum* telah dikenal pasti dengan menggunakan gen khusus PCR 16S rDNA. Strain C1 dan Strain G10 telah dikenal pasti masing-masing sebagai *Streptomyces aureofaciens* dan *S. roseoflavus*. Kesemua aktinomiset dicirikan dengan menggunakan aktiviti antimikrob dan enzim luar sel, profil metabolik dan polimofisma panjang pemotongan cebisan (RFLP). Strain A3 menunjukkan aktiviti antimikrob terhadap tiga bakteria iaitu *Xanthomonas campestris*, *Staphylococcus aureus* dan *Listeria monocytogenes*. Strain C1 dan strain I15 menunjukkan aktitiviti positif masing-masing terhadap *S. aureus* dan *X. campestris*. Strain A3, C1 dan I15 boleh memetabolismekan xilan dan selulosa, manakala Strain G10 dan Strain L8 boleh menggunakan kesemua substrat (xilan, manan and selulosa) sebagai sumber karbon. Kelima-lima strain *Streptomyces* positif terhadap lebih daripada 25 sumber karbon dan boleh dibezakan kepada lima strain yang berbeza. Keputusan ini seragam dan disahkan dengan analisis DNA profil RFLP. Amplifikasi spesifik profil 16S rDNA PCR menggunakan tiga pemotongan endonukleas, menunjukkan dua profil pemotongan terhasil daripada penghadaman 16S rDNA dengan menggunakan *Hae*III (H1–H2) dan *Hinf*I (Hf1–Hf2), manakala *Pst*I menghasilkan tiga profil pemotongan (P1–P3). Tiada profil terhasil daripada pemotongan endonukleas untuk enzim *Xba*I, *Spe*I dan *Bam*HI.