Malaysian Journal of Science **23** (2) : 37 - 47 (2004)

Characterization of Selected Isolates of Indigenous *Streptomyces* Species and Evaluation of their Antifungal Activity against Selected Plant Pathogenic Fungi

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Received 26 March 2004, accepted in revised form 27 October 2004

ABSTRACT Twenty-five representative isolates from a collection of 54 indigenous actinomycetes isolated from coastal resources, were evaluated for inhibition against plant pathogenic fungi *in vitro*. *Fusarium oxysporum* f.sp. *cubense* (Foc) race 4 and *Rhizoctonia solani* exhibited higher resistance to the antifungal effects of the isolates when compared to the other test fungi. Seven isolates with potential pathogen-inhibitory capabilities belonged to the gray colour class of *Streptomyces*. Isolate g10 with a wide and strong antifungal spectrum against a variety of pathogenic fungi belonged to the *Streptomyces violaceusniger* cluster. This isolate could be a potential biological control agent.

ABSTRAK Sebanyak dua puluh lima pencilan yang mewakili satu koleksi pencilan dari berbagai sampel pinggir pantai, telah dikaji untuk aktiviti antikulat terhadap patogen tumbuhan. *Fusarium oxysporum* f.sp. *cubense* (Foc) race 4 dan *Rhizoctonia solani* adalah paling rintang terhadap aktiviti antikulat pencilan-pencilan tersebut. Tujuh pencilan aktinomiset yang paling aktif adalah daripada genus *Streptomyces* yang mempunyai miselium udara berwarna kelabu. Pencilan g10 yang mempunyai aktiviti antikulatspektra luas yang tinggi serta terhadap pelbagai kulat patogenik terletak di dalam kluster *Streptomyces violaceusniger*. Pencilan ini berpotensi sebagai agen pengawalan biologi.

(marine-derived actinomycetes, streptomycetes, *Streptomyces violaceusniger*, antagonistic actinomycetes, biological control)

INTRODUCTION

In many studies, the genus *Streptomyces* had been shown to produce antifungal compounds that inhibit *in vitro* growth of a variety of plant pathogenic fungi [1]. This fact led to the speculation that antifungal compounds may be formed in soil and may play an active role in fungal antagonism [2]. Antibiotics produced on or near the root surfaces (the rhizoplane/rhizosphere region) decrease the competition for scarce food reserves by killing or inhibiting fungal growth. Therefore, streptomycetes were targeted and selectively isolated as biological control agents to suppress fungal plant diseases in greenhouse and field experiments [3].

In Malaysia, studies had shown that the actinomycetes isolated from coastal mangrove resources including mangrove mud, mangrove rhizosphere and rhizoplane soils can be potential agrochemical producers [4, 5]. Environmental conditions of the coastal mangrove forests are extremely different from natural terrestrial conditions and the microorganisms of these habitats may have different characteristics from known terrestrial microorganisms [4, 5]. However, there not many investigations on the antifungal activity or biocontrol potentials of indigenous actinomycetes from other marine-

derived resources. Further, to our knowledge, there is a paucity of information on the isolation of biocontrol agents of the genus *Streptomyces* spp. from coastal environments in Malaysia.

The aims of this investigation were to
(a) Isolate actinomycetes from samples from a selected coastal area (a sandbar)
(b) To assess selected isolates for *in vitro* antagonism towards several important soil and seed-borne plant pathogenic fungi and
(c) To characterize the potential antagonistic isolate/s.

MATERIALS AND METHODS

Sampling site and collection of samples
The sampling site, a coastal sandbar with some vegetation above the high-water line and bordered by mangrove trees [*Rhizophora apiculata* (Blume)] was about 35 meters from the shoreline of the seventh kilometer stretch of beach, Port Dickson, West Malaysia. Samples were collected from intertidal zones, from the low water mark along the seafront. Mud samples were also collected at low tide from the mangrove stands bordering the sandbar. Fine leaf litter samples beneath the degrading litter debris were collected from near-shore vegetation on the sandbar.

Isolation of marine-derived actinomycetes
The samples were air-dried at 25 ± 2°C for two days followed by moist-heat treatment [6]. The samples were then plated on starch-casein agar [7] incorporated with filter-sterilized cycloheximide and nystatin, each at 50 mg/l, to inhibit growth of fungal contaminants. Inoculated plates were incubated at 28 ± 2°C and observed up to four weeks. Putative isolates of actinomycetes were purified and maintained on inorganic salts-starch (ISP 4) agar and yeast extract-malt extract (ISP 2) agar [8].

Determination of macroscopic characteristics and colour groups of actinomycetes
Putative actinomycetes were grouped based on macroscopic features. Isolates with aerial mycelium were streaked on ISP 4 agar and incubated at 28 ± 2°C for 14 days. Colours of mature sporulating aerial growth, substrate mycelium and soluble pigment, if any, were determined based on the colour charts in Methuen Handbook of Colour [9].

In vitro antagonism assay in plate studies
Representative isolates of actinomycetes were screened for antagonistic properties against selected fungal plant pathogens using modified 'cross-plug' method [6].
The fungal strains studied were: *Pyricularia oryzae* MPO 293 (provided by the Malaysian Agricultural Research and Development Institute, Seberang Prai); *Fusarium oxysporum* f.sp. *cubense* (Foc) race 4, *Rhizoctonia solani* R1 and *Phytophthora palmivora* P250 (provided by Syngenta Crop Protection R&D Station, Rembau, Malaysia). All cultures were maintained on potato dextrose agar (PDA) at 27± 2°C. Triplicate plates were set up for each isolate tested.

Antifungal activity of antagonistic actinomycetes in submerged culture
The antifungal activities of seven potential isolates were further tested in submerged cultures. Aerial growth of the isolates from seven day-old cultures on ISP 4 agar was suspended in five ml of sterile distilled water and inoculated into 50 ml of modified ISP 2 medium [6]. Duplicate flasks were set up for each isolate studied. After five days of incubation at 28 ± 2°C and 160 rpm, fermentation broths were lyophilized and extracted with a 100 ml mixture of dichloromethane:methanol (1:1, v/v) [6]. The antifungal activity in the crude extract against *Pyr. oryzae* was quantified using the paper-disc assay [10]. For each actinomycete tested, 20 µl of the crude extract (0.5 mg) was applied to sterile paper discs and the diameter of inhibition zone was measured after 48 hours of incubation at 27 ± 2°C.

Chemotaxonomic and micromorphological characteristics of antagonistic actinomycetes
Isomers of 2, 6-diaminopimelic acid (DAP) in cell walls of the seven potential antagonistic actinomycetes were determined by thin-layer chromatography [11]. For micro morphological observations, the isolates were streaked on ISP 4 agar and sterile 12 mm diameter cover slips were inserted at 45° to the lawned culture [12]. After 14 d of incubation at 28 ± 2°C, cover slips with attached growth were carefully removed and fixed overnight in 2 % (w/v) aqueous osmium tetroxide vapour at 4°C. The cover slips were then gold coated using a Cool E5100 diode sputter coater (Biorad, England), mounted onto aluminum specimen stubs using carbon adhesive cement and viewed in a Phillips SEM model 515.

The SEM preparations were examined for spore chain type, spore-surface ornamentation, presence of sclerotia and substrate spores, and fragmentation of the substrate mycelium.

Phenotypic characteristics of the selected strain g10

Isolate g10 that exhibited potential antagonism towards plant pathogens tested and produced active antifungal ingredients *in vitro* was characterized further. The taxonomic keys of Bergey's Manual of Systematic Bacteriology [13], numerical classification of Williams *et al.* [14] and International *Streptomyces* Projects [15] were used to compare morphological and cultural characteristics of isolate g10 with those of known species of *Streptomyces*. Physiological characteristics such as degradation of L-tyrosine, xylan, casein and starch, the pH and temperature range for growth of isolate g10 were determined by methods of Williams *et al.* [14]. Tolerance to sodium chloride [16], susceptibility to different antibiotics [17] and growth on different carbon sources [8] were also examined.

RESULTS AND DISCUSSION

Isolation and macromorphological characterization of marine-derived actinomycetes

54 putative isolates of actinomycetes, including 43 isolates with aerial mycelium and 11 with only substrate mycelium, were selected as morphological representatives of the actinomycete population from the samples plated. Isolates presumptively assigned to the *Streptomyces*-like group were distinguished from other colonies on the isolation plates by presence of abundant aerial mycelium with powdery spore mass. Isolates classified in the *Micromonospora*-like group had only substrate mycelium and lacked aerial mycelium. Further, the colonies were orange to orange-brown mycelium and the spores, when present, were brown to black [18]. A higher proportion of streptomycete-like isolates were obtained from the intertidal sediments (51%) and the mangrove mud samples (42%), compared to the leaf litter samples (7%). In contrast, 82% of the isolates from the leaf litter sample were Micromonospora-like.

The representatives of presumptive streptomycete-like isolates growing on ISP 4 agar were assigned to four aerial mycelium colour classes: gray, white, red and pink. 63% of the isolates belonged to the gray colour class (Table 1). These isolates showed colour integrades within the gray spore-colour and also within the substrate mycelium colour. The largest group of 15 isolates formed grayish-white aerial spore mass and lacked characteristic colours in the vegetative (substrate) mycelium and soluble pigments.

Table 1. Colour-name designations for the aerial and substrate mycelium of presumptive *Streptomyces*-like isolates included in each spore-colour class, and their frequency of occurrence in the marine-derived samples

Aerial mycelium and substrate mycelium colours*	Number of isolates			Total isolates	
	Sources of samples†			No.	%
	SD	MM	LF		
Gray / Gray	1	-	-	1	
Gray / Gray-brown	-	2	-	2	
Gray / Green (+)	-	1	-	1	
Gray-white / Gray-white	7	6	2	15	
Gray-white / Gray-yellow	1	2	-	3	
Gray-white / Yellow (+)	-	1	-	1	
Dark gray / Dark gray	4	-	-	4	
Total	13	12	2	27	62.8
White / Yellow-brown	-	-	1	1	
White / White	3	1	-	4	
Total	3	1	1	5	11.6
Red-gray / Red (+)	-	1	-	1	
Red-gray / Red	-	4	-	4	
Total	-	5	-	5	11.6
Pink-white / Pink	6	-	-	6	
Total	6	-	-	6	14.0
Total isolates	**22**	**18**	**3**	**43**	**100**

* Aerial mycelium colour / substrate mycelium colour (+, presence of soluble pigment) observed after 14 days of incubation at 28 ± 2°C on ISP 4 agar.

† SD, near-shore sediment; MM, mangrove mud; LF, leaf litter.

In vitro antagonism assay in plate studies

Of the 25 isolates screened, 11 (44%) of the isolates were antagonistic activity against one or more fungi tested (Table 2). Twelve percent of the isolates showed strong to very strong antagonism towards *Phy. palmivora*, while *Fus. oxysporum* f.sp. *cubense* (Foc) was strongly inhibited by isolate g10 only. *Rhizoctonia solani* was only moderately inhibited by 28% of the tested isolates. The antagonism of some isolates towards the test fungi in cross-plug assay plate is shown in Figure 1.

Further, it was noted that of the 25 isolates screened, 60% of the gray colour class and 50% of the white colour class isolates had antagonistic activity against one or more fungi. However, isolates belonging to the red and pink colour classes did not exhibit antagonistic activity against the fungi tested. Seven out of the 11 antagonistic isolates (28%) were inhibitory at varying degrees against all four pathogens. Six out of these potential fungus-antagonistic isolates (g10, g35, g39, g40, g48 and g49) belonged to the gray colour class and one isolate, g51, belonged to the white colour class. Isolate g10 that showed strong to very strong antagonism towards three out of four of the fungi tested, could be a promising biological control agent.

As all seven potential isolates were highly inhibitory to the growth of the rice blast pathogen, *Pyr. oryzae* (Table 2), their antifungal activity in submerged culture was evaluated quantitatively.

Antifungal activity of antagonistic actinomycetes in submerged culture

Antifungal activities of the seven potential isolates in submerged culture are shown in Table 3. Isolate g10 showed the highest activity with a clear inhibition zone of 20 mm. Crude extracts of the other isolates produced either smaller or hazy inhibition zones. The crude extract of isolates g48 and g51 (each at 0.5mg/disc) did not inhibit the growth of *Pyr. oryzae*. When tested at a higher concentration of 2.5 mg/disc, the crude extract of isolate g48 was inhibitory. Isolate g51, however, did not show any antifungal activity even at the higher extract concentration tested.

Cell wall diaminopimelic acid (DAP) type and micromorphology of antagonistic actinomycetes

All seven potential isolates had the LL-DAP in their cell walls, and thus had cell wall chemotype I [19]. Based on the cell wall composition, the isolates were assigned to the genus *Streptomyces*. Isolate g51 from the white colour class had spore chains of the "retinaculum-apertum" (RA) or hooks to open loops type [8] with smooth surfaced spores (Figure 2). The other six isolates had spirals or "spira" (S) sporophores with rugose spores (Figure 2) [20]. All seven isolates did not form sclerotia and there was no sporulation on or fragmentation of substrate mycelium.

Morphological, cultural and physiological characteristics of selected strain g10

Sporophores of isolates g10 were both the compact spirals (five to six turns) and the extended long and open spirals (Figure 2). The cylindrical to barrel-shaped spores were 0.7 - 0.8 × 1.0 - 1.1 μm. The initial white aerial mycelium of g10 turned to gray nearing 14 days of incubation on ISP 4 agar. Substrate mycelium varied from pale yellow, grayish yellow to grayish yellow-brown. Melanin pigments were not produced. The morphological and cultural characteristics of g10 were compared with closely related species of *Streptomyces*. Isolate g10 was then assigned to *Streptomyces violaceoniger* cluster 32 in the numerical phenetic survey of Williams *et al.* [154]. The physiological characteristics of strain g10 are summarized in Table 4.

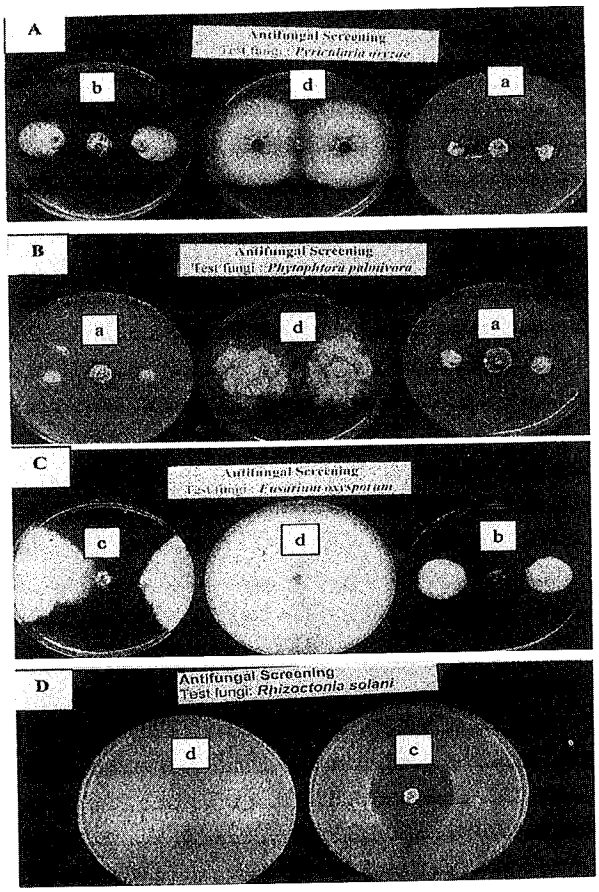

Figure 1. Cross-plug assay to test the *in vitro* antagonism of actinomycete strains against
A: *Pyricularia oryzae*;
B: *Phytophthora palmivora*;
C: *Fusarium oxysporum* f.sp. *cubense*;
D: *Rhizoctonia solani* (a – very strong inhibition, b – strong inhibition, c – moderate inhibition, d – control)

Table 2. Antagonistic activity of marine-derived actinomycetes against different plant pathogenic fungi tested by cross-plug method

Isolates (spore colour class)	Antagonism* observed after 96 h against			
	Rs	Foc	Pp	Po
g2 (gray)	0	0	0	2
g3 (gray)	1	1	0	2
g10 (gray)	2	3	4	4
g35 (gray)	2	2	4	4
g37 (gray)	1	1	2	2
g39 (gray)	2	2	2	3
g40 (gray)	2	2	4	4
g43 (gray)	0	0	0	1
g48 (gray)	2	2	2	4
g49 (gray)	2	2	2	4
g5 (white)	1	0	2	2
g41 (white)	1	0	1	1
g51 (white)	2	2	2	4

* 4 - very strong inhibition, with total inhibition of growth all around the fungal plug
3 - strong inhibition, with hyphal growth strongly inhibited on the area facing the actinomycete colony
2 - moderate inhibition, with obvious zone of inhibition near the actinomycete colony
1 - weak inhibition, with hyphal growth slightly retarded; 0 - no inhibition (growth of fungus comparable to control)
Test fungi: Rs, Rhizoctonia solani; *Foc,* Fusarium oxysporum *f.sp.* cubense; *Pp,* Phytophtora palmivora; *Po,* Pyricularia oryzae

Table 3. Antifungal activity of selected actinomycetes in submerged culture assayed against *Pyricularia oryzae* by paper-disc method

Actinomycete isolates	Mean diameter of inhibition zone (mm)*
g10	19.5
g35	13.2
g39	14.5
g40	13.0
g48	0 (17.5)
g49	14.0
g51	0 (0)

*: Crude extract of each isolate was tested at a concentration of 0.5 mg/disc, unless stated otherwise (2.5 mg/disc).

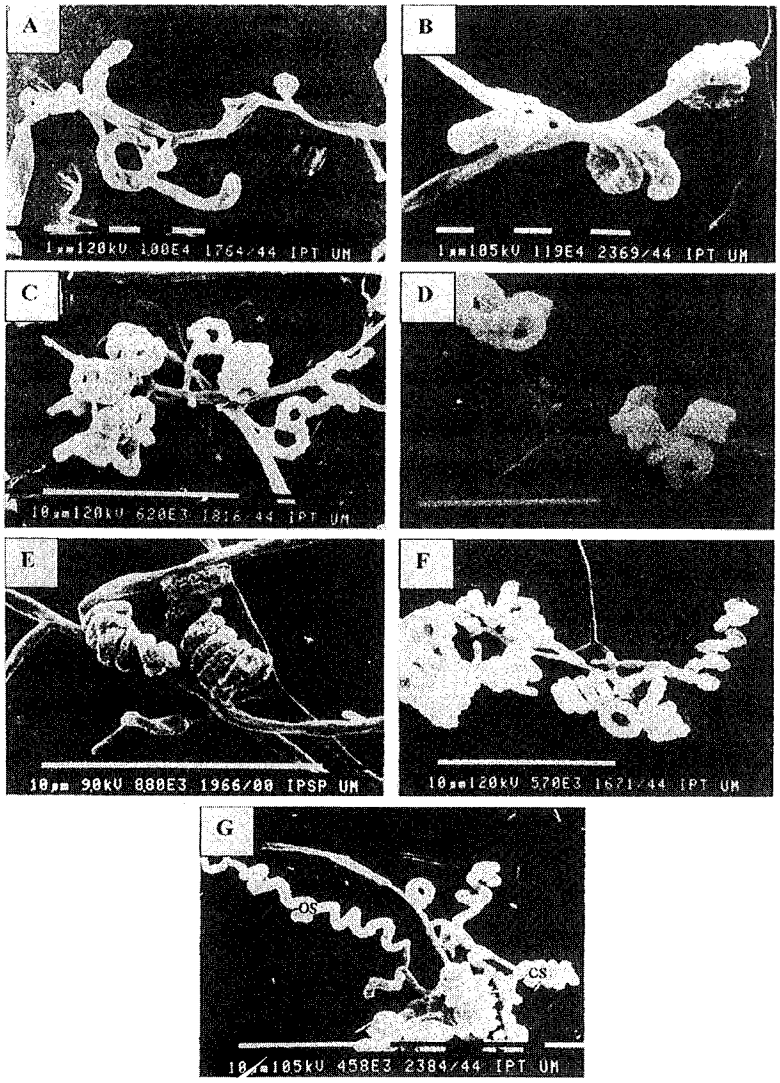

Figure 2. Scanning electron micrographs of spore chain types of potential antagonistic marine-derived streptomycetes (14-day-old culture on ISP 4 agar)
A: strain g51 (bar = 1 μm);
B: strain g35 (bar = 1 μm);
C: strain g48 (bar = 10 μm);
D: strain g49 (bar = 10 μm);
E: strain g39 (bar = 10 μm);
F: strain g40 (bar = 10 μm);
G: strain g10 (bar = 10 μm; cs-compact spiral; os-open spiral)

79% of the marine-derived isolates strains isolated on starch-casein-seawater agar were streptomycete-like. Numerous studies had shown that when starch-casein (SC) agar was the isolation medium the *Streptomyces* were not only very frequently but in large numbers, too [21]. The fast growing streptomycetes may have retarded the growth of slower growing actinomycetes, such as *Micromonospora* and related genera [4]. This could be one of the factors that contributed to a low percentage (21%) of *Micromonospora*-like isolates recovered in the current study. However, the incorporation of antibacterial antibiotics into isolation media could enhance the isolation of different actinomycete genera. The incorporation of selective agent such as novobiocin [22] had been recommended for the selective isolation of *Micromonospora* spp.

Streptomycetes dominated in many actinomycete populations, including those in marine sediments [23], coastal mangrove mud [4] and seaweeds [24]. Variations in the colours of the aerial and substrate mycelium of the isolates and in the soluble pigments they produced may be an indication of the diversity and variability of the isolated streptomycete-like isolates [25]. Isolates from the gray colour class were dominant (63%) in the samples of the present study. Similarly, a high percentage of streptomycetes with gray aerial mycelium was previously isolated from coastal mangrove mud [4] and mangrove rhizosphere soil [26] samples of Malaysia.

Many attempts had been made to correlate the efficiency of potential biocontrol agents with some specific trait(s) that was easy to quantify *in vitro*. It has generally argued that there is no relationship between the *in vitro* antagonism of biocontrol candidates and disease suppression *in vivo*. However, potential biocontrol *Streptomyces* spp. are exceptions to this view [26, 27]. *In vitro* assays can be useful in identifying the antagonists that may function in soil [28]. Therefore, streptomycete-like isolates were evaluated for antifungal activity in *in vitro* antagonism assays. The strategy adopted in this study was to screen and select isolates which may have the greatest potential as biocontrol agents by producing extracellular phytopathogen-inhibiting metabolites against a variety of fungal pathogens.

In the present study, 44% of the selected streptomycete-like isolates had varying degrees of antagonism against one or more test fungi. The sample suspensions were moist-heat pretreated prior to plating on isolation media to limit the growth of contaminating microorganisms. However, in studies where the coatal mangrove samples were only air dried prior to plating, 33% of the *Streptomyces* isolates had antagonistic activity towards fungal pathogens [4]. In another study, a selection for chitinolytic isolates segregated a group of marine-derived actinomycetes of which 47% were antagonistic towards fungi [29]. This number far exceeded the number of fungus-antagonistic isolates when chitinolyis was not used as a selective criterion. Therefore, the use of more selective isolation procedures could enhance the isolation of actinomycetes, particularly species with antimicrobial capabilities.

Earlier workers had reported that the occurrence of antagonistic streptomycetes from terrestrial samples were from as low as 10% to as high as 47% of the total isolates screened [30]. The potential application of these antifungal streptomycetes for the biocontrol of soil-borne fungi was demonstrated successfully in many studies [27, 2]. The high proportion of antagonistic streptomycete-like isolates obtained in the present study indicated that the indigenous coastal resources may be an alternative gene pool largely unexplored in screening programmes for biological control agents.

The test fungi in this study differed from each other in their sensitivity to the antifungal effects of the actinomycetes (Table 2). *Rhizoctonia solani* was particularly resistant because none of the isolates tested very strongly or strongly inhibited it. However, 28% of isolates were moderately active against *Rhi. solani*. *Fusarium oxysporum* f.sp. *cubense* (Foc) also showed a relatively high resistance, whereas *Phy. palmivora* was moderately sensitivite towards the antifungal effects of the isolates. On the other hand, the highest percentage of active isolates was against *Pyr. oryzae* (44% showed moderate to very strong inhibition). Since *Rhi. solani* and Foc proved to be relatively resistant against the antibiotic effects of the actinomycetes, it may be expected that isolates that can inhibit these two fungi may also be highly effective against the remaining test fungi. This, indeed, is seen in Table 2. Moreover, g10, the single isolate that strongly inhibited Foc was also very strongly effective against *Pyr. oryzae* and *Phy. palmivora*.

Its effect, however, was distinctly less pronounced against *Rhi. solani*. Fungal plant pathogens that are known to have active saprophytic life in soil, or suspected to survive in soil, were more tolerant to antagonistic actinomycetes. Their tolerance to antagonism may be considered as one of the factors contributing towards their survival in soil [31]. In the absence of the host plant, different formae specials of *Fus. oxysporum* are able to survive in soil by formation of chlamydospores [32]. *Rhizoctonia solani*, the rice sheath blight pathogen, is also known to produce sclerotia that can survive in soil [33].

The isolate g10 in submerged cultures produced antifungal substance/s that inhibited fungal growth and relatively large zones of inhibition of at least one of the pathogens was observed. The strong activity indicated that g10 produce water-soluble antimicrobial metabolites which may play an important role of antibiosis in the biocontrol of plant [34]. The remaining isolates tested varied in their ability to produce antifungal substances, as seen in the sizes of the inhibition zones formed on assay plates. The inhibition of fungal growth by these isolates could be attributed to the synthesis of very low concentration of antibiotics [1].

In the present study, it was interesting to note that six out of the seven streptomycetes tested that inhibited *in vitro* growth of all tested fungi were from the gray colour class of aerial mycelium. These six isolates (g10, g35, g39, g40, g48 and g49) shared some morphological characteristics. All had the spiral spore chain with rugose spore surface ornamentation. In the numerical phenetic survey of Williams *et al.* [14], test strains that produced gray-coloured aerial spore mass and rugose spores in spiral chains were assigned to a single taxon, *Streptomyces violaceoniger* cluster 32 (*S. violaceusniger*). By comparing the morphological, cultural and physiological properties with other *Streptomyces* species in the literature, strain g10 is considered to belong to the *S. violaceusniger* cluster. Members of this group are particularly known for their antagonistic activities towards root-infecting fungi both *in vitro* [35, 36] and *in vivo* [3].

Yuan & Crawford [35] reported that *Streptomyces lydicus* WYEC108 was a potent biocontrol agent for controlling seed and root rot and the strain was selected for its strong *in vitro* activity against *Pythium ultimum*. In this study, strain g10 was selected based on its high degree of antagonism towards various fungal plant pathogens and production of active antifungal antibiotics *in vitro*. Furthermore, it was the only isolate that strongly inhibited the *Fusarium* wilt pathogen of banana.

The good growth isolate g10 observed at pH 5.0 to 6.0 (Table 4) could be advantageous. As fungi typically prefer acidic environments, the choice of the more acidophilic actinomycetes as antagonists whenever possible may be advantageous [28]. Isolate g10 was isolated from coastal mangrove habitats that are known to be areas highly influenced by terrestrial runoff [18] g10 with a 4% salt tolerance may have adapted to the salinity of seawater and sediments. However, the high salt tolerance should not affect its growth when applied as a fungal antagonist to soil because isolate g10 grew well even in the absence of salt (Table 4). Further work is warranted to evaluate the *in vivo* biocontrol efficacy of strain g10 against *Fusarium* wilt pathogen of banana including the conditions for the establishment and colonization on/in roots of banana plantlets.

Table 4. Physiological characteristics and growth response on sole carbon sources of strain g10

Characteristics	Results	Sole carbon sources	Growth*
Degradation tests †:		At 1%, w/v:	
Starch (1.0%, w/v)	+	D-Glucose	+
Casein (1.0%, w/v)	+	D-Galactose	+
Xylan (0.4%, w/v)	+	D-Fructose	+
L-Tyrosine (0.5%, w/v)	+	Sucrose	+
		meso-Inositol	+
Growth at †:		D-Xylose	+
pH 4.0	-	L-Arabinose	+
pH 5.0 - 10.0	+	D-Mannitol	+
4°C	-	L-Rhamnose	+
10°C	-	Xylitol	-
45°C	-	D-Raffinose	+
		D-Melezitose	+
Growth in the presence of ‡:		Cellobiose	+
Sodium chloride (0 - 3%, w/v)	++	D-Melibiose	+
Sodium chloride (4%, w/v)	+		
Sodium chloride (5 - 10%, w/v)	-	At 0.1%, w/v:	
		Sodium propionate	-
Susceptibility to antibiotics (µg per disc) §:			
Kanamycin sulphate (30)	++ (47)**		
Gentamicin sulphate (10)	++ (49)		
Neomycin sulphate (30)	++ (37)		
Erythromycin (15)	± (17)		
Streptomycin sulphate (10)	± (12)		
Chloramphenicol (30)	± (11)		
Novobiocin sulphate (30)	-		
Tetracycline hydrochloride (30)	-		
Ampicillin (10)	-		

† +: positive reaction; - : negative reaction.

‡ ++: good growth; + : moderate growth; - : no growth.

§ ++: extremely susceptible (diameter inhibition zone 31 - 50 mm); + : fairly susceptible (21 - 30 mm); ± : a little susceptible (10 - 20 mm); - : resistant.

* +: good growth; - : no growth.

** Numbers in parentheses indicate the diameter of the inhibition zone in mm.

Acknowledgements Authors would like to thank University Malaya and the Ministry of Science, Technology and the Environment for the (IRPA 08-02-03-0144) for this study. We also thank Mrs. Vijaya Lakshmi for technical assistance with the SEM.

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