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Identification for *Streptomyces padanus* Strain PMS-702 as A Biopesticide Agent

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ABSTRACT

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A *Streptomyces* PMS-702 strain was isolated from farmland soil collected from Taiwan. Antimicrobial activities of PMS-702 strain against phytopathogenic fungi and bacteria showed differential inhibitory effects on the growth of fungal pathogens, but no effect for *Sclerotium rolfsii* and nine plant pathogenic bacteria. According to the results of morphological, physiological and biochemistry characteristics, and 16S rRNA gene sequence, the PMS-702 strain was identified as *Streptomyces padanus*. Biomass of *S. padanus* PMS-702 strain varied among different nutrient media for growing. Soybean Meal-Glucose Broth (SMG) medium was the best for enhancing the growth of *S. padanus* strain PMS-702 and its crude culture filtrate diluted to 10 fold completely suppressed spore germination of *Fusarium oxysporum* f. sp. *cubens*e, which was used as an indicator for assessing antimicrobial activities. In bioassay experiment, the culture filtrates of *S. padanus* from SMG cultures fermented at 30 degrees Centigrade for 4 days could control effectively a broad range of plant diseases including lettuce brown spot, mango anthracnose, Chinese cabbage anthracnose, peach fruit rot, and orange green mold. These results suggested that *S. padanus* strain PMS-702 is potential to be used as a biological control agent to suppress fungal crop diseases.

Keywords: *Streptomyces padanus*; identification; biopesticide agent

INTRODUCTION

At the beginning of the 21st century, to have a high quality of life and a friendly environmental ecosystem are highly desired. The safety and security of food supply has become a particular focus for concern. In recent years, countries all over the world have begun to promote organic farming, the main tenet of which is the avoidance of using chemical fertilizers and pesticides. Integrated pest management (IPM), involving the use of conservation tillage, organic additives and biocontrol of plant diseases, has become the aim for long-term control of diseases and pests. It is believed that these technologies offer safe methods of crop production and contribute to sustainable agriculture.

Baker [4] reported that biological control is an important part of a strategy for maintaining a balance with pests, diseases and natural enemies of agriculture crops. The definition of biological control is: reducing the damage to crops by disease-causing fungi by suppressing their population, vigor or ability to infect crops through the use of one or several antagonistic micro-organisms in a natural or artificial environment [2, 3, 5, 8, 20]. At the end of the twentieth century, scientists carried out a wide range of researches into biocontrol, mostly using fungi, nematodes, viruses, bacteria or actinomycetes as the biological agents. Many biological products resulted from this work [7, 10, 14, 22, 30, 34]. By the end of 1998, 175 active components and 700 derivative products had been developed [29, 36]. These included: AQ10® (*Ampelomyces quisqualis*), Fusaclean® (*Fusarium oxysporum*), SoilGard® (*Gliocladium virens* GL-21), T-22G® (*Trichoderma harzianum*), Kodiak® (*Bacillus subtilis*), BioJect Spot-Less® (*Pseudomonas aureofaciens*), Stealth® (*Steinernema feltiae*), CYD-X® (Heliothis Nucleopolyhedrosis Virus; NPV), Actinovate® (*Streptomyces lydicus* WYEC 108), Mycostop® (*S. griseoviridis* K61) [8, 22].

For this research, we isolated 200 strains of actinomycetes from field soil, compost and agricultural media in Taiwan. Ninety eight percent of these strains were identified as *Streptomyces* species; the other strains were identified as *Actinomadura*, *Herbidospora*, *Microbispora* and *Streptosporangium* species. It is known that *Streptomyces* spp. have wide-ranging antagonistic effects against plant diseases caused by fungi [13, 30]. Therefore, the objectives of this study were to characterize the taxonomic identification of PMS-702, and assay the ability of antagonistic effects and evaluate the potential of PMS-702 strain for plant disease control.

MATERIALS AND METHODS

Organisms, nutrient media and plant materials

Antimicrobial activities were tested *in vitro* against phytopathogenic fungi and bacteria listed in Table 1 and Table 2, respectively. The following six nutrient media were used in this study: Glucose-Molasses medium(GMM), Glucose-Soybean meal-Glycerol Broth (GSG), Modified Chitin Broth (MCB), Soybean meal-Fish meal-Chitin Broth (SFC), Soybean meal-Glucose Broth (SMG), and Tryptone-Yeast extract-Glucose Broth (TYG). All media were prepared as described in Ronald [31] and Tsao *et al.* [35] and adjusted to pH 8.0. Seeds and fruits of tested plants were obtained from commercial companies and traditional market in Taiwan. They were lettuce (*Lactuca scariola* var. *sativa* Bisch.), Chinese cabbage (*Brasica rapa* L. Chinese Group), mango (*Mangifera indica* L.), peach (*Prunus persica* L. Batsch) and orange (*Citrus reticulate* Blanco).

Isolation of actinomycetes

Soils and composts from different fields of Taiwan were brought to the laboratory. The PMS-702 strain from soil had been isolated by pour plate technique on Nutrient agar, Chitin agar and Humic acid-vitamin agar (HV) [16] after serial dilution in distilled water. Dry colonies of actinomycetes were selected and isolated. These isolated colonies were then preserved in glycerol based media and stored at -20^{o}C for further analysis and test.

Antimicrobial activities of PMS-702 strain against phytopathogenic fungi and bacteria

In vitro antifungal and antibacterial activity tests were performed using plate assay technique. *Streptomyces* strain PMS-702 was inoculated by streaking on PDA at 1.5 cm from the edge of the Petri dish. The PDA plate was further inoculated with mycelial mat plugs (0.8 cm in diameter) of 31 isolates of phytopathogenic fungi and 9 strains of phytopathogenic bacteria at 5 cm from *Streptomyces* strain PMS-702. The inoculated plates were placed in an incubator at 24°C for 7 days. Antagonism was measured by the determination of the size of the inhibition zone. There were 3 replicates for each treatment. This experiment was repeated for 3 times.

Identification of PMS-702 strain

PMS-702 strain was characterized by morphological, physiological and biochemical methods, and 16S rRNA gene sequence. The medium used for morphological studies was International *Streptomyces* Project (ISP) media 2, 3, and 4 and the incubation time of the pure culture was 7 days at 28°C [27]. Morphological methods consist of light microscopic and scanning electron microscopic methods. The microscopic characterization was done by cover slide culture. The mycelial structure, color and arrangement of conidiospore and arthrospore on the mycelium were observed through the oil immersion. The characteristic of mycelial structure was compared to the description of Bergey's manual of Determinative Bacteriology [37]. Various physiological and biochemical tests performed for the identification of PMS-702 strain were as follows: utilization of different carbon and nitrogen sources, production of chitinase and β-1,3-glucanase, lysozyme resistance, melanin and nitrate reductase [32]. Chromosomal DNA was isolated as a template for 16S rRNA gene sequencing, which was completed using the MicroSeq 16S rRNA gene kit (Perkin-Elmer Applied Biosystems Division [PE-ABD], Foster City, USA). Cycle sequencing was performed by the Applied Biosysterms DNA sequencer (model ABI 310). The sequences of amplified 16S rRNA genes were compared with existing sequences in GenBank using the NCBI- BLAST (Basic Local Alignment Search Tool) program [1, 15].

Biomass of *S. padanus* PMS-702 in different nutrient media

Spores of *S. padanus* PMS-702 were collected from 7-day-old cultures on ISP4 at 30°C, suspended in sterile distilled water and adjusted to 10^8 spores/ml. Aliquots (1 ml) of spore suspensions was inoculated into 250 ml shake flasks containing 150 ml of GMM, GSG, MCB, SFC, SMG and TYG media respectively. Cultures were incubated for 7 or 14 days with shaking at 120 rpm at 25°C. After incubation, mycelia were harvested by centrifugation (12000 rpm for 15 min at 4°C), washed once with distilled water and air-dried. The dry weight of mycelia was used as a measure of growth. There were three replicates for each treatment. This experiment was repeated 3 times.

Effect of *S. padanus* PMS-702 crude culture filtrate on spore germination of *F. oxysporum* f. sp. *cubense*

The effect of crude culture filtrates of *S. padanus* PMS-702 from GMM, GSG, MCB, SFC, SMG and TYG broth cultures, prepared as described above, on spore germination of *F. oxysporum* f. sp. *cubense* was studied in cavity slide (Assistent, Sondheim, Germany). Aliquots (20 μl) of the culture filtrate diluted to 5 fold for each media were mixed with 20 μl of a conidial suspension (2 x 10^4 spores/ml) of *F. oxysporum* f. sp. *cubense* and pipetted into each cavity slide. Control treatments included uninoculated GMM, GSG, MCB, SFC, SMG and TYG broth cultures alone and inoculated sterile distilled water. The cavity slide was placed in glass Petri dish and then incubated at 24°C for 16 hrs in the dark. After incubation, the percentage of spore germination was determined by microscopic examination for 100 conidia per replicate and compared with those of the control. There were three replicates for each treatment. This experiment was repeated 3 times.

Table 1. Origin of phytopathogenic fungi used in this study

Plant pathogen	Strain	Host	Collector[a]
Acremonium diospyri	A-5012	Cineraria	1
A. lactucum	Al-0818	Lettuce	1
Alternaria brassicicola	ABA-31	Chinese Cabbage	1
Athelia rolfsii	AR-0102	Lily	1
Botrytis cinerea	BC-01	Rose	3
B. elliptica	B-066	Lily	3
Colletotrichum dematium	MP-3	Lettuce	1
C. higginsianum	PA-19	Chinese Cabbage	1
C. gloeosporioides	COL-05	Mango	1
Fusarium oxysporum f. sp. *cubense*	BFT-0403	Banana	1
F. oxysporum f. sp. *conglutinans*	FOC-03	Cabbage	1
F. oxysporum f. sp. *lactucae*	LFO-03	Lettuce	1
F. oxysporum f. sp. *lilii*	G-16	Lily	1
F. oxysporum f. sp. *niveum*	FNH-0103	Watermelon	1
F. oxysporum f. sp. *raphani*	FOR-4566	Radish	1
F. moniliforme	FM-0024	Lily	1
F. proliferatum	FOL-02	Lily	1
F. solani	FS-05	Peanut	1
Mycosphaerella pinodes	MP-72	Garden Pea	1
Phellinus noxius	PN-20	Palm	1
Penicillium digitatum	PG-1	Orange	1
Pestalotiopsis eriobotryfolia	PST-03	Loquat	1
Phytophthora capsici	PH-5	Pepper	1
Ph. citrophthora	PH-1	Peach	2
Ph. infestans	PI-109	Tomato	2
Ph. palmivora	PH-2	Papaya	2
Ph. parasitica	PH-3	Lily	2
Pythium aphanidermatum	PA-105	Cucumber	1
P. myriotylum	PY-01	Tomato	1
Rhizoctonia solani AG 4	RST-04	Cruciferous crop	1

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Fermentation

For large-scale production of culture filtrates, fermentation was carried out in a 5-L fermentor (BTF-600; Bio-TOP, Taiwan). *S. padanus* PMS-702 was grown in 5-L fermentor containing 3 L of SMG. Temperature, aeration and agitation were kept at 30°C, 3 vvm and 120 rpm, respectively. pH was adjusted to 7.5 to 8.3. The 4-day-old cultures were filtered under vacuum, and the culture filtrates were lyophilized and stored at 4°C in the dark until used for the control of plant diseases.

Application of *S. padanus* PMS-702 for control of plant diseases

In bioassay experiments, the whole plant, detached leaf and fruit tissues were used to assess disease severity. All plants and fruits tested including 28-day-old lettuce plants, 21-day-old Chinese cabbage plants, young leaves of mango plants, peach fruits, and orange fruits were spray-inoculated respectively to runoff with each spore suspension of *Acremonium lactucum* (2 x 10^5 spores/ml), *Colletotrichum higginsianum* (2 x 10^5 spores/ml), *C. gloeosporioides* (1 x 10^5 spores/ml), *Phytophthora citrophthora* (10^5 zoospores/ml), *Penicillium digitatum* (1 x 10^6 spores/ml). Immediately following inoculation of crude culture filtrates of *S. padanus* PMS-702 fermented in SMG cultures at 30°C for 4 days diluted to 100- to 300-fold were done, the plants were kept in the greenhouse and detached leaves and fruits were placed in an incubator. The control plants were sprayed with distilled water. After incubation for 2 to 10 days depending on different crops, disease was expressed as affected, which was determined by percent severity. Disease severity was recorded using a scale of 0 (no symptoms) to 4 (more than 50% of whole leaves or fruits were necrotic) modified from James [17]. This experiment was repeated twice.

Data analysis

Data of bioassay experiments of *S. padanus* PMS-702 were analyzed by SAS/STAT software (SAS Institute, Cary, NC). Means of treatments for each treatment were compared using Student's t-test.

Table 2. Origin of phytopathogenic bacteria used in this study

Plant pathogen	Strain	Host	Collector [a]
Acidovorax avenae subsp. citrulli	AAC-21	Watermelon	1
Agrobacterium tumefaciens	RO-19	Rose	1
A. tumefaciens	RS-3	Rose	1
Burkholderia caryophylli	CO-16	Carnation	1
B. caryophylli	GO-8	Carnation	1
Erwinia carotovora subsp. *carotovora*	ZL-1	Calla lily	1
E. chrysanthemi	CAS-7	Calla lily	1
Xanthomonas axonopodis pv. *citri*	XW-38	Citrus	1
X. axonopodis pv. *vesicatoria*	T-40	Tomato	1
X. campestris pv. *campestris*	XCC-70	Chinese cabbage	1
Ralstonia solanacearum	PS-21	Tomato	1

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RESULTS

Antimicrobial activities of PMS-702 strain against phytopathogenic fungi and bacteria

Results of the dual cultures showed that the strain PMS-702 suppressed significantly the growth of various fungal phytopathogens, except for *Sclerotium rolfsii*. The inhibition zone of foliar plant pathogens (*Alternaria brassicicola*, *Botrytis cinerea*, *B. elliptica*, *Colletotrichum gloeosporioides*, *Mycosphaerella pinodes* and *Pestalotiopsis eriobotryfolia*) and soil-borne plant pathogens (*Fusarium oxysporum* f. sp. *niveum*, *F. moniliforme*, *F. proliferatum* and *F. solani*) was over 2.0 cm when they were dually cultured with PMS-702 for 7 days at 24°C. However, the PMS-702 strain had no obvious effects on inhibiting the growth of bacterial phytopathogens (Table 3, Table 4).

Table 3. Inhibitory effect of *Streptomyces padanus* PMS-702 on mycelial growth of plant fungal pathogens in PDA medium

Plant pathogen	Inhibition effect
Acremonium diospyri	++[a]
A. lactucum	++
Alternaria brassicicola	+++
Botrytis cinerea	+++
B. elliptica	+++
Colletotrichum dematium	++
C. higginsianum	++
C. gloeosporioides	+++
Fusarium oxysporum f. sp. *cubense*	++
F. oxysporum f. sp. *conglutinans*	++
F. oxysporum f. sp. *lactucum*	++
F. oxysporum f. sp. *lilii*	++
F. oxysporum f. sp. *niveum*	+++
F. oxysporum f. sp. *raphani*	++
F. moniliforme	+++
F. proliferatum	+++
F. solani	+++
Mycosphaerella pinodes	+++
Phellinus noxius	++
Penicillium digitatum	++
Pestalotiopsis eriobotryfolia	+++
Phytophthora capsici	++
Ph. citrophthora	++
Ph. infestans[b]	++
Ph. palmivora	++
Ph. parasitica	++
Pythium aphanidermatum	++
P. myriotylum	++
Rhizoctonia solani	+
Sclerotium rolfsii (*Athelia rolfsii*)	−

[a, b] in Table 4 footnote

Table 4. Inhibitory effect of *Streptomyces padanus* PMS-702 on growth of plant bacterial pathogens in the PDA medium

Plant pathogen	Inhibition effect
Acidovorax avenae subsp. citrulli	−
Agrobacterium tumefaciens [c]	−
Burkholderia caryophylli	−
Erwinia carotovora subsp. *carotovora* [c]	−
Erwinia chrysanthemi [c]	−
Ralstonia solanacearum [c]	−
Xanthomonas axonopodis pv. *citri*	−
Xanthomonas axonopodis pv. *vesicatoria*	−
Xanthomonas campestris pv. *campestris*	−

[a] Ratings: +++, r＞2.0 cm; ++,2.0cm＞r＞1.0 cm; +, 1.0cm＞r＞0.5 cm; –, r＜0.5 cm
[b] On rye agar
[c] On Nutrient agar

Identification of PMS-702 strain

Morphological observation of the 7-day-old culture of strain PMS-702 grown on ISP2, 3, and 4 media revealed that both aerial and vegetative hyphae were abundant. Aerial mycelium varied from grayish yellowish brown to medium gray on different test media. The substrate hyphae were from grayish yellow to orange yellow. Spore chains with more than 20 were spiral, smooth and ovoid in sharp. Diffusible yellow pigments were produced on ISP2 and ISP4 media, and melanin was not produced. The cell-wall peptidoglycan of strain PMS-702 contained LL-diaminopimelic acid, glucose and ribose, indicating that strain PMS-702 had chemotype cell wall type I [(21)]. The utilization of carbohydrates and nitrogen sources of growth characteristics and other characteristics were summarized in Table 5.

Table 5. Comparison of cultural characteristics among 3 strains of *Streptomyces* determined on ISP media 2,3, 4, and 5

Medium	Cultural characteristics								
	PMS-702			CCRC-12168			CCRC-12166		
	Substrate color	Aerial mycelia	Soluble pigments	Substrate color	Aerial mycelia	Soluble pigments	Substrate color	Aerial mycelia	Soluble pigments
Yeast extract-malt extract agar (ISP2)	Light yellow	Grayish yellowish brown	Strong yellow	Deep yellowish brown	Grayish yellowish brown	Yellow	Strong yellowish brown	Yellowish white	yellow
Oatmeal agar (ISP3)	Dark grayish yellow	Grayish yellowish brown	Dark yellow	Dark yellowish brown	Grayish yellowish brown	Dark yellow	Deep yellow	Medium gray	Strong yellow
Inorganic salts starch agar (ISP4)	Moderate yellow	Grayish yellowish brown	Medium yellow	Dark grayish yellow	Grayish yellowish brown	Medium yellow	Strong yellow	Light yellow	Strong yellow
Glycerol asparagine agar (ISP5)	Vivid yellow	Grayish yellowish brown	Strong yellow	Medium yellowish brown	Grayish yellowish brown	Yellow	Light olive brown	Grayish yellow	yellow to yellow green

The full 16S rDNA sequence of strain PMS-702 was compared to that of *Streptomyces* species in the GenBank database. The strain PMS-702 was identical to *S. padanus* CCRC-12168 deposited previously in GenBank (accession number: AF455813), sharing 100% similarity. Based on the combination of phenotypic, physiological and biochemistry (Table 6) and genotypic data, we propose strain PMS-702 as a strain of *Streptomyces padanus* [6, 15, 39].

Table 6. Comparison of physiology and biochemistry of *Streptomyces* sp. strain PMS-702, *S. padanus* strain CCRC-12168 and *S. galbus* strain CCRC-12166

Substrate	Cultural characteristics		
	PMS-702	CCRC-12168	CCRC-12166
L-arabinose	−*	−	−
Cellulose	−	−	−
Esculin	+	+	+
D-fructose	+	−	+
D-glucose	+	+	+
i-inositol	−	−	+
D-mannitol	+	+	+
Potassium nitrate	−	−	−
Raffinose	−	−	−
Rhamnose	−	−	−
Salicin	−	−	−
Sucrose	−	−	−
Urea	−	−	+
Xylose	+	+	+
Decomposition of:			
Adenine	+	ND	ND
Casein	+	ND	ND
Hippurate	−	ND	ND
Hypoxanthine	+	ND	ND
Starch	−	ND	ND
Tyrosine	−	ND	ND
Xanthine	−	ND	ND
Production of:			
Chitinase	+/−	ND	ND
β-1,3-glucanase	+	ND	ND
Lysozyme resistance	−	−	ND
Melanin	−	−	+
Nitrate reductase	−	−	ND

*+: positive; −: negative; +/−: indeterminate reaction; ND: not detected

Biomass of *S. padanus* PMS-702 in different nutrient media

The six media were selected for growth of *S. padanus* PMS-702. Biomass of *S. padanus* PMS-702 strain varied among different nutrient media. After incubation for 7 days, biomass of *S. padanus* PMS-702 in SMG cultures significantly higher than those in the other media. When the culture period were prolonged to 14 days, the growth of *S. padanus* PMS-702 in GMM cultures were the highest, with 12 mg per milliliter, compared to SMG cultures.

Effect of *S. padanus* PMS-702 crude culture filtrate on spore germination of *F. oxysporum* f. sp. *cubense*

The antifungal activity of crude culture filtrate of S. padanus PMS-702 from SMG liquid cultures was evaluated using *F. oxysporum* f. sp. *cubense*. The cavity slide assay method indicated that 7- and 14-day-old SMG filtrates (ten times dilute) were most effective inhibiting the spore germination of *F. oxysporum* f. sp. *cubense* by 100% and 68.62%, respectively. However, MCB filtrates regardless of incubation days did not inhibit the spore germination of *F. oxysporum* f. sp. *cubense* (Fig. 1).

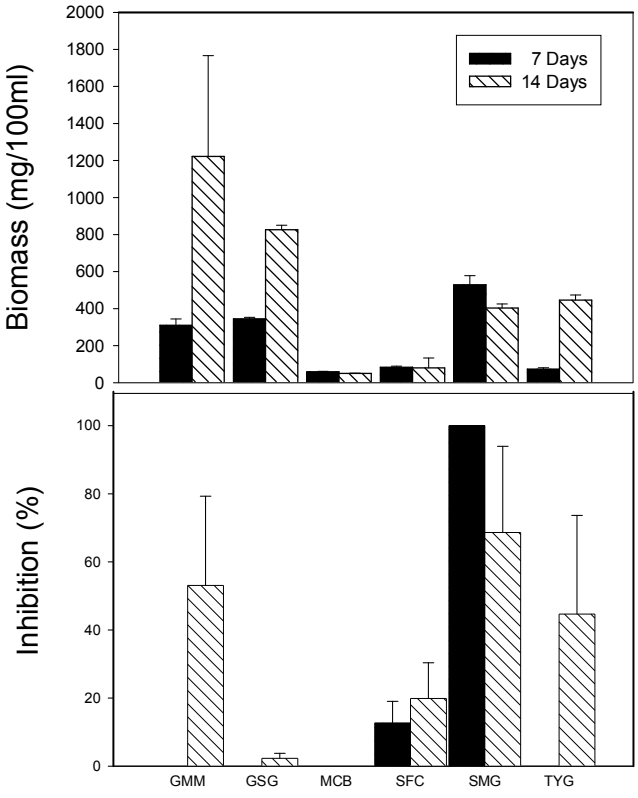

Fig. 1. Inhibitory effect of the diluted culture filtrate of *Streptomyces padanus* PMS-702 on spore germination in *Fusarium oxysporum* f. sp. *cubense*, grown in six different media for 7 and 14 days. (Note: GMM, Glucose-Molasses medium; GSG, Glucose-Soybean meal-Glycerol Broth; MCB, Modified Chitin Broth; SFC, Soybean meal-Fish meal-Chitin Broth; SMG, Soybean meal-Glucose Broth; TYG, Tryptone-Yeast extract-Glucose Broth.

Application of *S. padanus* PMS-702 for control of plant diseases

Results of bioassay experiments in the greenhouse and laboratory tests showed that the severities of lettuce brown spot, mango anthracnose, Chinese cabbage anthracnose, peach fruit rot and orange green mold were significantly reduced by the culture filtrate of *S. padanus* PMS-702 fermented in SMG cultures at 100- to 300-fold dilution. The severity of lettuce brown spot, mango anthracnose, Chinese cabbage anthracnose, peach fruit rot and orange green mold was reduced respectively to 12.5-18.8%, 12.5-16.7%, 25%, 5.6%-11.1% and 19-20% by spray-inoculated treatment with culture filtrate of *S. padanus* PMS-702 compared to 100% of the control (Fig. 2, Table 7).

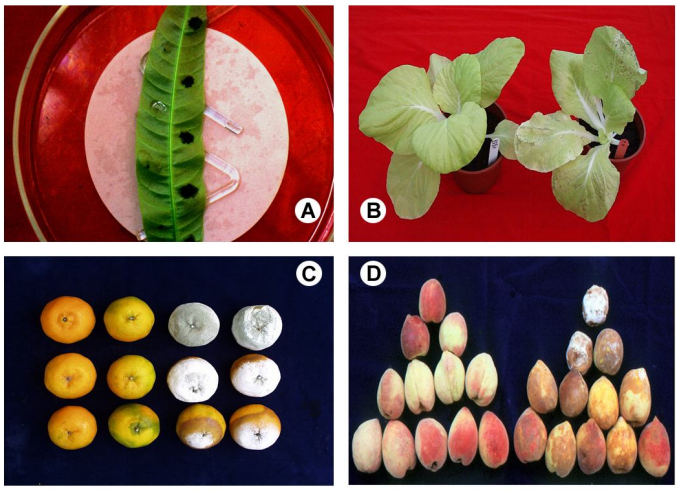

Fig. 2. Effect of the culture broth of *Streptomyces padanus* PMS-702 grown in soybean meal glucose broth for 7 days on control of four plant diseases *in vivo*. A, Treatment (left): Inhibition of mango anthracnose with 100-fold dilution of PMS-702 culture broth after inoculation with *Colletotrichum gloeosporioides*, Control (right): no treatment. B, Treatment (left): control of Chinese cabbage anthracnose with 300-fold dilution of PMS-702 broth after inoculation with *C. higginsianum*, Control (right): no treatment. C and D, Treatment (left): Inhibition of citrus green mold and peach fruit rot of harvest practices applied with 300-fold dilution of PMS-702 culture broth before inoculation with *Penicillium digitatum* and *Phytophthora citrophthora*, respectively, Control (right): no treatment.

Table 7. Effect of cultural filtrate of *Streptomyces padanus* PMS-702 on control of plant diseases *in vivo*

Plant pathogens	Host	Disease severity (%)			
		Test I		Test II	
		PMS-702	Check	PMS-702	Check
Acremonium lactucum	Lettuce	12.5A[1]	100.0B	18.8a	100.0b
Colletotrichum higginsianum	Chinese cabbage	25.0A	91.7B	25.0a	91.7b
Colletotrichum gloeosporioides	Mango	16.7A	100.0B	12.5a	100.0b
Phytophthora citrophthora	Peach fruit	11.1A	100.0B	5.6a	100.0b
Penicillium digitatum	Orange fruit	20.0A	100.0B	19.4a	100.0b

[1]Means (n=3) in each raw followed by the same letter are not significantly different at P= 0.05 based on Student's t-test.

DISCUSSION

Actinomycetes are a widespread group of microorganisms [38]. Some species are capable of infecting plants and animals and causing disease in humans, livestock and crops. However, they are also the main producers of industrial and medical antibiotics [12]. Actinomycetes display radial growth on culture media. They are able to develop mycelia, spores, sporangia and sporangiospores in the same way as fungi. Because of these characteristics, actinomycetes are also known as ray fungi, and have been classified by some as fungi. By the mid twentieth century it was known that they were Gram positive, had no nuclear membrane and were prokaryotic. Classic actinomycetes have substrate mycelia, and develop aerial mycelia on the vegetative mycelia, develop spores directly (*Micromonospora*), or develop sporangia (*Actinoplanes*). Single spores (*Thermoactinomyces*), bispores (*Microbispora*) or multiple spores in spiral chains (*Streptomyces*) can develop on aerial mycelia. There is also one genus of actinomycetes of which the mycelia can break off and form spheroidal or rod-shaped clusters, e.g. *Nocardia*, *Mycobacterium,* and *Rhodococcus.* The evolution of this genus is similar to that of fungi, and they are known as preactinomycetes [21, 32].

In the 1950s and 60s actinomycetes were classified primarily according to their shape, physiological and biochemical characteristics. In the 1970s chemotaxonomy and numerical taxonomy were used to reclassify the group and to establish new genera. There are now over 60 genera which have been published in the literature, but only 50 or so of these had been officially recognized in by 1989. The Society for Actinomycetes Japan [26] has defined 8 groups based on the fragmentation of mycelia and the different forms of spore development. In recent years, with the development of molecular biology, DNA-DNA hybridization, DNA-rRNA hybridization and 16S rDNA base sequencing have become important techniques in the classification of actinomycetes.

The cell walls of PMS-702 contained L-diaminopimelic acid (L-DAP); cells contained no unusual sugars; the chemotype was IC. This showed that PMS-702 was a *Streptomyces*. It flourished and developed spores on International *Streptomyces* Project Media (ISP) 2, 3 and 4. On ISP2 and ISP4 media, it produced a yellow pigment, but no melanin. Actinomycetes DNA has a G+C content of over 50 mol%, and their 16S rRNA are phylogenically very similar. Some scholars believe that organisms whose phenotype, DNA or 16S rRNA are more than 95% similar should be classed as a single species [18]. The PMS-702 strain had a 100% match to *S. padanus* CCRC-12168 deposited in GenBank when a 1520bp 16S rRNA comparison was carried out. Meanwhile, its physiological and biochemical characteristics were closer to those of *S. padanus*. Therefore, PMS-702 was confirmed as *Streptomyces padanus* Baldacci. *et al* [33].

Microbiological agents and additives can have an effect on the lifespan and disease resistance of other microorganisms, and can also affect the development of crops [11]. Adjusting the use of biological agents to meet the requirements of the specific time and place of usage, and integrating biological agents into a complete disease biocontrol system, is a crucial stage in the development of biological agents for agricultural use [9]. In order to effectively use *S. padanus* strain PMS-702 as a biological disease control agent, it is necessary to discuss what substrates are suitable for its growth. To this end, the research for this paper included the culturing of PMS-702 in 6 different media broths. SMG, based on soybean flour, was the best cultures for the growth of *S. padanus* PMS-702. Using *F. oxysporum* f.sp. *cubense* to assess the inhibitory effectiveness of PMS-702, result showed that the filtrate of 7- to 14-day-old cultures of PMS-702 in SMG cultures was the most effective against spore germination of the pathogen.

Many of the metabolic by-products of actinomycetes have antibiotic properties [38]. The complete spectrum of the *S. padanus* PMS-702 fermentation contains OD_{320}, OD_{337}, OD_{340} and OD_{357} in the invisible part and OD_{440} in the visible portion of the spectrum. This suggests that it may contain macrolide antibiotics [28]. Spores of *F. oxysporum* f.sp. *cubense* examined under the microscope were swollen or burst after being treated with the filtrate from PMS-702 fermentation. Results indicated that filtrate contained some element capable of rupturing spores. Most polyene macrolide fermentations require a high concentration of glucose (7-9.5%) as the carbon source [23]. However, in experiments were too high a concentration of glucose used, the production of candidin was actually inhibited [19]. Glucose and polyene macrolide production are linked in carbon source metabolic control. Actinomycin is only produced after glucose has been absorbed by *Streptomyces*, so a low glucose concentration (5-15 mg/ml) stimulated the production of this polyene macrolide. However, when the glucose has been completely used up, ceases are produced[23]. Soybean flour is easily available and is a rich source of nutrition, and so is commonly used in microbiological fermentation [24]. The soybean flour used in this experiment was full fat soybean flour, containing 42.68% crude protein, 20% fats (including unsaturated fats such as omega oils), 5.32% fiber, 5.0% crude ash and 27% carbohydrate. It provides a conducive environment for bacteria during the growth phase and the antibiotic production phase with its high nutritional content and its slow rate of dissolution in water. This makes it an excellent nitrogen substrate for use in the production of polyene macrolides [19]. Half a century ago McCarthy [25] and others had noted that the addition of soya oil increased the production of fungichromin. The SMG medium used in this research contained an appropriate balance of soybean flour and glucose to promote the production of metabolic by-products by PMS-702.

SMG was used to ferment PMS-702 on a large scale. The resulting fermentation was conducted for antagonism against several plant pathogens. In addition, the results of disease control assays showed that *S. padanus* PMS-702 was effective in inhibiting foliar diseases development caused by *Acremonium lactucum, Colletotrichum gloeosporioides, C. higginsianum* and protected peaches and mandarins from infection by *Penicillium digitatum* and *Phytophthora citrophthora*. The results of this study suggested that *S. padanus* PMS-702 could be developed into a bioprotectant for control of fungal crop diseases.

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摘 要

石信德[1,5]、鍾文全[2]、黃鴻章[1,3]、曾敏[4]、黃振文[5,6]. 2013. 鑑定稠李鏈黴菌 PMS-702 作為生物農藥的菌種. 植病會刊 22:145-158. ([1] 臺中市 行政院農業委員會農業試驗所; [2] 臺中市 行政院農業委員會種苗改良繁殖場; [3] 加拿大農業部萊斯布來奇研究所; [4] 新竹市 財團法人食品工業發展研究所; [5] 臺中市 國立中興大學植物病理學系; 6 聯絡作者，電子郵件：jwhuang@dragon.nchu.edu.tw; 傳真: +886-422851676)

放線菌 PMS-702 菌株係由台灣農田中分離所獲得，其對於各種供試植物病原真菌具有不同程度的拮抗能力，對於白絹病菌 (*Sclerotium rolfsii*) 與 9 株植物病原細菌則均不具有拮抗的效果。分析 PMS-702 菌株之形態、生理、生化特徵及 16S rRNA 基因序列後，確定 PMS-702 菌株為稠李鏈黴菌 (*Streptomyces padanus*)。PMS-702 菌株在不同營養配方培養具有差異化，以黃豆粉葡萄糖培養基 (SMG) 培養 PMS-702 具有最佳的生質量，其 10 倍稀釋培養粗濾液具有抑制香蕉黃葉病菌 (*Fusarium oxysporum* f. sp. *cubense*) 孢子發芽的效果。PMS-702 菌株在 SMG 培養基於 30°C 培養 4 天後，利用生物檢定分析法測試其對於數種植物病害的影響，結果發現 PMS-702 菌株培養濾液對於萵苣褐斑病、芒果炭疽病、白菜炭疽病、甜桃果實疫病及椪柑果實綠黴病均具有防治功效，這些結果顯示稠李鏈黴菌 PMS-702 具有研發成為生物製劑，用於防治作物真菌性病害的潛力。

關鍵詞：*Streptomyces padanus*、鑑定、生物農藥、生物製劑