Polish Journal of Microbiology
2008, Vol. 57, No 4, 297–305

ORIGINAL PAPER

Screening for Soil Streptomycetes from North Jordan that Can Produce Herbicidal Compounds

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Received 23 March 2008, revised 15 June 2008, accepted 29 July 2008

Abstract

A total of 231 different soil *Streptomyces* isolates were recovered from 16 different locations in North Jordan. They were assessed for their phytotoxic activity on seeds of cucumber (*Cucumis sativus* L.) and ryegrass (*Lolium perenne* L.) placed adjacent to a 2 cm wide *Streptomyces* culture strips grown at 28C° for 3 weeks on starch casein nitrate (SCN) agar. Phytotoxicity was ascertained on the basis of suppressed seed germination, discoloration of the root tip, reduced root and the shoot growth and eventual death of the root. Twenty one of the isolates exhibited adverse effect against growth of germinated cucumber seeds, germination and growth of ryegrass seeds. Using filter paper bioassay method, culture filtrate from the SCN broth of the isolate R9; identified as *Streptomyces aburaviensis*, significantly inhibited seed germination, radicle and shoot growth of ryegrass, reduced radicle and shoot growth of cucumber and suppressed the shoot growth of milk thistle (*Silybum marianum* L.). Also, culture filtrate from the glucose-peptone-molasses (GPM) broth diluted (1:1) with sterilized distilled water caused complete inhibition of seed germination of redroot pigweed (*Amaranthus retroflexus* L.). Dichloromethane extracted fraction of *S. aburaviensis* (strain R9) culture filtrate from GPM broth completely inhibited seed germination of ryegrass when applied at doses of 3 and 5 mg of dry weight, and the seedling growth of cucumber and milk thistle was severely reduced by the same doses.

Key words: *Streptomyces*, phytotoxin, seeds, weeds

Introduction

Many isolation and screening attempts have been done on streptomycetes to find microbial metabolites with bioherbicidal potentials (Arai *et al*., 1976; Defrank and Putnam, 1985; Li *et al*., 2003; Mallik, 1997; Mishra *et al*., 1987; Murao and Hayashi, 1983; Sekizawa and Takematsu, 1983, Takahashi *et al*., 1995).

Anisomycin, which is produced by *Streptomyces toyocaensis* was the first commercially used phytotoxin (Yamada *et al*., 1972). Bialaphos, which is produced by *Streptomyces hygroscopicus* (Mallik, 2001) and *Streptomyces viridochromogenes* (Charudattan *et al*., 1996), represents the first patented microbial bioherbicide. Arai *et al.* (1976) reported the production of two bioherbicides, herbicidans A and B, by *Streptomyces saganonesis* that are selective against many dicotyledonous plants. Gougerotin is another plant growth inhibitor produced by *Streptomyces* sp. No 179 (Murao and Hayashi, 1983). Babaczinski *et al.* (1991) reported vulgamycin as phytotoxin against dicotyledonous weeds and grasses if applied post-emergence. Phosphenothrixin that is produced by *Saccharothrix* sp. ST-888 inhibits the germination of gramineous and broadleaved weeds (Takahashi *et al*., 1995). Herbimycin represents a potent herbicidal activity when used pre-emergence, against flat-sedge (*Cyperus microiria* Steud.) (Sekizawa and Takematsu, 1983).

All these discovered phytotoxins from streptomycetes represent a wide range of plant inhibitory compounds that are naturally degraded in the environment, which may restrict the regular undesirable consequences of using agrochemicals such as, accumulation, biomagnifications, and excessive persistence (Heisey and Putnam, 1990). The intensive and the arbitrary usage of herbicides lead to the development of resistant weed species to some of those herbicides (Mallik, 2001). Moreover, biotechnological techniques were utilized for testing the possibility of transferring

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the genes of phytotoxin production to a plant pathogen in an attempt to become sufficiently acceptable for control a weed target (Charudattan *et al*., 1996).

There are about 300 common weed species that cause crop losses world wide (Hoagland, 1990). Weed control depends mainly on conventional hand weeding and tillage (Abu-Irmaileh, 2000; Salim and Mokhtar, 2000) and the strategies at this stage are more logical in taking early precautions and avoiding future negative consequences on the environment. Therefore, biological control of weeds represents a logical alternative to the agrochemicals.

In Jordan, weed control is not well managed and implemented. Several studies have already been conducted on soil streptomycetes of Jordan for their potential to produce antibiotics (Saadoun *et al*., 1999; 2008; Saadoun and Gharaibeh, 2002). However, the herbicidal activity of streptomycetes is not studied yet. Therefore, the present investigation was conducted to isolate soil streptomycetes from different locations in North Jordan and screening them for their phytotoxic potential against common broad leaf and grass weeds. The optimal medium suitable for better phytotoxic activity in the culture filtrate and extraction of phytotoxin was, also investigated.

Experimental

Materials and Methods

Location, sampling, treatment of soil samples and isolation technique. Soil samples were collected from 9 different locations in North Jordan representing the most humid and vegetative part of Jordan. One or more soil sample was collected from each location. Enrichment of streptomycetes in the soil samples and isolation of *Streptomyces* spp. were performed as described by Saadoun *et al*. (2008). Dilutions that gave 20–200 colonies were chosen for repeated streaking and selection of pure bacterial colonies showing *Streptomyces*-like characteristics.

Phytotoxic activity assay. Bioassays of the isolated streptomycetes for their phytotoxicity were performed using two indicator plant seeds namely: cucumber (*Cucumis sativus* L.) (UPC, 0-21496-28630-3, Lowes, Texas) and ryegrass seeds (*Lolium perenne* L.) (Local cultivar Beit Alpha) (Mallik, 2001).

Surface sterilization of the indicator plant seeds. Cucumber and ryegrass seeds were surface sterilized by immersing each one of them for 5 min in 2% and 25% solutions of sodium hypochlorite (Clorox commercial containing 6.5% of NaClO), respectively. In both cases, 20 µl of Tween 20 was added to break the water surface tension. Vacuum was applied using vacuum pump to facilitate thorough surface sterilization. Seeds were washed 3 times with sterilized distilled water for 1.5 min at each wash in order to get rid of the residual hypochlorite and Tween 20. Surface sterilized seeds were blotted between double layers of sterilized cheesecloth and transferred to sterilized glass Petri dishes to be used in the bioassay experiments.

Screening of streptomycete isolates for their bioherbicidal activity. The growth of each streptomycete isolate from SCNA plates (28C° for 10 days) was scraped and aseptically transferred into 5 ml vials containing 2 ml of sterilized distilled water and vortexed. Aliquot of 0.4 ml from each isolate cell suspension was placed in the center of SCNA plates, in 3 replicate, and spread using L-shape glass rod over a 2 cm wide strip along the diameter of those plates. Non-inoculated SCNA plates served as controls. After 3 weeks of incubation at 28C° for 3 weeks, 6 sterilized cucumber seeds were placed on one side of the culture strip and 6 sterilized ryegrass seeds were placed on the opposite side of the same culture strip within the same plate. Plates were incubated in dark at 28C° for 4 days. Seed germination was observed and germination percentages were calculated. Average length of radicles and shoots was measured, using Vernier caliper. The experiment was repeated 3 times for the isolates that expressed the phytotoxic activity to confirm observation.

Detecting Phytotoxin(s) in the submerged culture. Primary inoculum of each of the seven most active isolates was prepared by transferring a loop full of inoculum of each isolate into 100 ml Erlenmeyer flask containing 25 ml of SCN broth and incubated for 5 days in orbital shaker incubator (28C° with shaking at 140 rpm). These cultures were used in the inoculation process of larger volumes of SCN broth in a ratio of 1:20 inoculum to broth. In the later process three 250 ml Erlenmeyer flasks containing 60 ml broth for each isolate were inoculated with 3 ml of the primary inoculum and incubated for 7 days in orbital shaker incubator. Culture filtrates were aseptically obtained by vacuum filtration through Whatman No 4 filter paper and stored at 4C° for further phytotoxic bioassay. The experiment was repeated 3 times for each one of the active isolates.

Seed collection of common weeds. Seeds of milk thistle (*Silybum marianum* L.) and wild oat (*Avena fatua* L.) were collected from the site of Jordan University of Science and Technology during the period from April-May 2003. Redroot pigweed (*Amaranthus retroflexus* L.*)* seeds were kindly provided by Dr. Jamal Qasim, Faculty of Agriculture, University of Jordan, Amman-Jordan.

Phytotoxic activity in the culture filtrates. Seeds of two monocotyledonous (ryegrass and wild oat) and two dicotyledonous (cucumber, milk thistle) species were used in the phytotoxicity bioassay in the culture

filtrates. Sterilized Whatman No 4 filter papers were placed inside 9 cm Petri dish and moistened with 2.5 ml of the culture filtrate of each isolate that was prepared as described before. Two controls were prepared in the same way in which uninoculated SCN broth and sterilized distilled water were used separately instead of culture filtrate. Four seeds from each plant species were placed inside each Petri dish, and replicated three times. The plates for each treatment were placed inside plastic bags in order to conserve moisture, and they were incubated in a cooled incubator at 28C° in darkness for 4 days. After that, the length of the radicles and shoots was measured and germination percentages were calculated. The experiment was repeated 3 times for the isolates that expressed phytotoxic activity in submerged culture. In order to determine the appropriate medium composition that support better phytotoxin(s) production and extraction (Halleck *et al*., 1955), an additional broth media of glucose-peptone-molasses (GPM) and peptone-molasses-corn steep (PMC) were used. Furthermore, sterilized Whatman No 4 filter papers were placed inside 9 cm Petri dish and moistened with 2.5 ml of R9 culture filtrate from the GPM submerged culture diluted 1:1 with sterilized distilled water and applied against *Amaranthus retroflexus* seeds (4 seeds per plate).

Extraction of the phytotoxin(s) containing fraction. Extraction of the phytotoxin(s) containing fraction from the most active isolate R9 was performed as described by Mallik (1997). After seven days of R9 incubation in GPM broth (500 ml broth/2 l Erlenmeyer flask) in shaker incubator at 28C° with shaking at 140 rpm, culture filtrate was extracted with dichloromethane (1:3 v/v) and repeated twice with the same solvent. The solvent was evaporated close to dryness using rotary evaporator at 29C°. The residue was reconstituted into 4 ml dichloromethane and transferred into a test tube. The solvent was evaporated using a jet stream of N_2 gas and the weight of the residue was determined by subtraction of the known weight of the test tube. The residue was reconstituted in 10 ml dichloromethane and used as stock concentration that was used to prepare 1.5, 3 and 5 mg crude extract in the bioassay for the phytotoxic activity. These amounts of crude extract were used to be loaded on Whatman No 4 filter papers inside 9 cm glass Petri dishes. Filter papers were air-dried for 15 min to allow solvent evaporation. Three seeds of cucumber, ryegrass and milk thistle were placed over a filter papers moistened with 2.5 ml sterilized distilled water, in three replicate plates. Prepared dishes were placed inside plastic bags to conserve on moisture and incubated inside cooled incubator for 4 days at 28C°. Percent germination, radicle and shoot growth of the germinants were recorded. Uninoculated GPM broth was extracted in the same manner applied with the culture filtrate of the active isolate to serve as a control along with another control of sterilized distilled water.

Characterization of the most active *Streptomyces* isolates. *Streptomyces* isolates that maximally inhibited seed germination, radicle and shoot length were characterized morphologically and physiologically according to the International *Streptomyces* project (ISP) (Shirling and Gottlieb, 1966) and as described by Saadoun *et al*. (2008). The spore surface of five of the active streptomycetes isolates was examined under scanning electron microscope at a magnification of 15000 to 25000 x. The stub that is covered with conductive carbon disk was placed over a 21-day old culture of each isolate grown on oatmeal and glucose asparagin agar. The stubs were placed in a sputter coater (Biorad, Polaron Equipment Ltd. E 6100) for 2–3 min (approximately 150 A° of gold deposited). The gold sputterer was set at 1.2 kv, 40 mA and 10^{-3} mbar. After coating, the specimens were viewed with a FAI Quanta 200 scanning electron microscope with an accelerating voltage of 20 kv. Secondary electron images were recorded with black and white film. The spore surface structures were classified to: smooth (sm), spiny (sp), warty (wa) and hairy (ha) (Nonomura, 1974). Carbon utilization test was performed according to the ISP (Shirling and Gottlieb, 1966) for the most active isolate to be identified to the species level.

Statistical analysis. All experiments were laid out on the basis of completely randomized design (CRD) and generated data was subjected to statistical analysis system (SAS). Means were separated by the least significant differences (LSD) at $\alpha = 0.05$.

Results and Discussion

The present investigation revealed that soils from cultivated fields, forests and barns in North Jordan are rich reservoirs for streptomycetes with phytotoxic activity. The phytotoxicity of these isolates was demonstrated by strip culture as well as the bacterial culture filtrate treatment.

Isolation of *Streptomyces* isolates. By employing enrichment methods, a total of 231 different *Streptomyces* isolates were recovered from 16 most humid and vegetative habitats in Jordan. All of these isolates matched the genus description reported by Shirling and Gottlieb (1966), Nonomura (1974) and Williams *et al*. (1983).

Screening for isolates with bioherbicidal activity. All of the 231 isolates were tested for their phytotoxicity ability towards two indicator plant seeds; cucumber (*Cucumis sativus* L.) (UPC, 0-21496-28630-3, Lowes, Texas) and ryegrass seeds (*Lolium perenne* L.)

Table I
Effect of the most active *Streptomyces* isolates on seed germination, radicle and shoot growth of cucumber and ryegrass assessed by the agar plate screening method

Isolate	Cucumber						Ryegrass					
	Ger[a] %	%Δ G[b]	R.L[c] mm	%Δ R.L	S.L[d] mm	%Δ S.L	Ger. %	%Δ G.L	R.L Mm	%Δ R.L	S.L mm	%Δ S.L
Cont.	100		59.3		27.7		100		10.4		10.3	
R9	100	0	8.9	85.0	2.7	90.3	14.8	85.2	0.2	98.1	0.3	97.1
MB13	100	0	20.4	65.6	4.3	84.5	66.7	33.3	2.5	76.0	1.2	88.3
MS5	100	0	18.5	68.8	4.2	84.8	66.7	33.3	1.7	83.7	0.9	91.3
MS18	100	0	22.7	61.7	5.5	80.1	83.0	17.0	3.0	71.2	2.0	80.6
Is11	100	0	20.0	66.3	3.7	86.6	100	0	6.0	42.3	0.3	97.1
R2	100	0	26.8	54.8	5.1	81.6	66.7	33.3	5.2	50.0	4.2	59.2
MS3	100	0	18.7	68.5	4.2	84.8	77.7	22.3	4.0	61.5	3.0	70.8
J214	100	0	22.1	62.7	3.7	86.6	58.1	41.9	1.9	81.7	0.4	96.1
LSD[e]	**0**		**7.5**		**9.7**		**22.6**		**1.4**		**2.2**	

[a] Ger: Germination; [b] %Δ: Percent decrease over the control; [c] R.L: Radicle length; [d] S.L: Shoot length; [e] LSD: Least significant difference

(Local cultivar Beit Alpha) (Mallik, 2001). As shown in Table I, a considerable proportion of the tested isolates (9.1%) exhibited phytotoxic activity towards the above plant seeds which was indicated as the percentage of seed germination. Other studies (Defrank and Putnam, 1985; Heisey *et al*., 1985) reported a range of 6.7% and 10–12% using same indicator plants of cucumber and barnyard grass, respectively. The isolates MB13, MS5, Is11, R9, and J214 (Table I) inflicted significant phytotoxic effect on cucumber and ryegrass seeds under culture strips treatment. The isolate R9 caused 85.2% inhibition of ryegrass seed germination and germinated seeds showed 98.1% and 97.1% reduction in their radicle and shoot growth, respectively. However, R9 did not affect cucumber seed germination, although it caused 85% and 90.3% reduction in their radicle and shoot growth, respectively. The isolate J214 caused 41.9% inhibition in seed germination of ryegrass in contrast with the control. The germinated seeds showed 81.7% reduction in growth of their radicle and 96.1% reduction in their shoot growth. J214 caused reduction in the cucumber radicle and shoot growth by 62.7% and 86.6%, respectively. The isolates MB13 and MS5 had almost similar phytotoxic activity against both cucumber and ryegrass seeds. Those isolates caused 33.3% inhibition in seed germination of ryegrass compared with the control. The germinated seeds of both plant species showed more than 76% reduction in growth of their radicle and more than 88% reduction in growth of their shoot. Though MB13 and MS5 had no adverse effect on cucumber seed germination, but they caused more than 65% reduction in growth of the radicle and about 84% reduction in growth of shoot in both plant species. The isolate Is 11 had no adverse effect on ryegrass and cucumber seed germination in contrast with the control, but it caused more than 40% reduction in both plant species radicle growth and more than 86% reduction in the growth of their shoot.

The phytotoxicity symptoms observed in this investigation were represented by discoloration and death of the root tips, suggesting that the phytotoxic effect is a cytotoxic one working on the meristematic cells. This confirms that the mechanism of action of such bacteria is by the production of some extra cellular agent (s) or toxin (s) that affects the meristematic cells. Such activity was more profoundly inflicted by the isolates; R9, MB13, MS5 and J214.

***Streptomyces* culture filtrates from SCN broth.** The culture filtrate of R9 caused 33.3% inhibition in the seed germination of ryegrass compared to the SCN broth control (Fig. 1). R9 caused more than 67% reduction in the growth of the radicle for cucumber and ryegrass and significantly reduced the growth of the shoot for cucumber, ryegrass and milk thistle. The culture filtrate of MB13 caused more than 16% inhibition of seed germination for cucumber and ryegrass in contrast with the control. However, germinated cucumber and ryegrass seeds showed reduced radicle growth, whereas germinated milk thistle seeds showed reduced shoot. The culture filtrate of MS5 inhibited the germination of ryegrass and wild oat seeds by 33.3% and 88.9% respectively, compared to the control. Germinated cucumber, ryegrass and milk thistle seeds showed reduced radicle growth. Germinated ryegrass and milk thistle showed reduced shoot growth, while a complete inhibition for wild oat shoot growth was observed. The culture filtrate of MS18 inhibited seed germination of ryegrass and wild oat. Whereas germinated cucumber and ryegrass seeds showed reduced radicle growth, but germinated ryegrass and wild oat seeds showed reduced shoot growth. The culture filtrate of Is11 inhibited 33.3% seed germination of ryegrass. Germinated cucumber

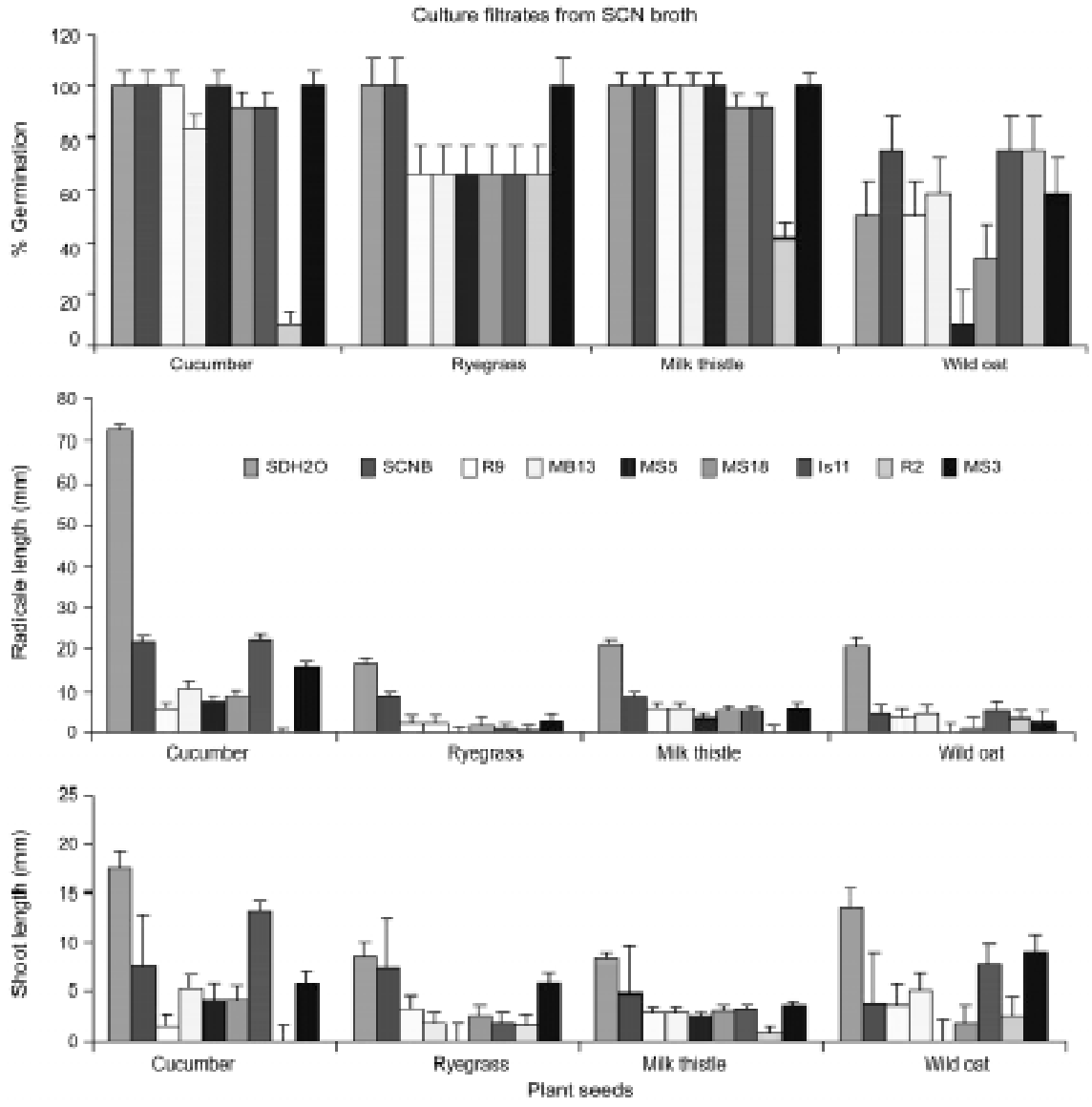

Fig. 1. Effect of culture filtrates of the isolates R9, R2, MB13, MS5, MS18, MS3 and Is11 from their SCN broth cultures compared to the controls (SD H_2O and uninoculated SCN broth) on germination, radicle and shoot growth of cucumber, ryegrass and milk thistle. Bars represent the standard errors for means of a plant species at $\alpha = 0.05$.

and ryegrass seeds showed reduced radicle growth while germinated ryegrass and milk thistle showed reduced shoot growth compared to the control. R2 culture filtrate caused 91% inhibition for seed germination of cucumber and inhibited more than 33% seed germination of ryegrass and milk thistle compared to the control. Germinated cucumber, ryegrass and milk thistle seeds showed about 90% reduction in the growth of the radicle. Germinated ryegrass and milk thistle showed about 80% reduction in the growth of the shoot with complete inhibition of the growth of the shoot. The culture filtrate of MS3 caused inhibition in the seed germination of cucumber and ryegrass by 27.8% and 66.7%, respectively compared to the control.

Most of the active isolates were active in the agar plate screening method and the activity was evident in their culture filtrates as well, a criterion that indicates the feasibility of gross fermentation, and extraction of significant amounts of the active component. The extracellular metabolite activity of the actinomycetes was demonstrated in number of ways such as the strip culture technique on agar, broth extraction (Mallik, 1997), and the possible volatile constituent of this product (El-Trabily *et al*., 1997).

***Streptomyces* culture filtrates from GPM broth.** The GPM broth composition was found to support profuse phytotoxin production. The phytotoxic activity of R9 culture filtrate from its GPM broth culture presents a broad spectrum phytotoxin activity. This activity affects both dicotyledonous and monocotyledonous plant seeds such as cucumber, ryegrass, milk thistle, and redroot pigweed in pre-emergence

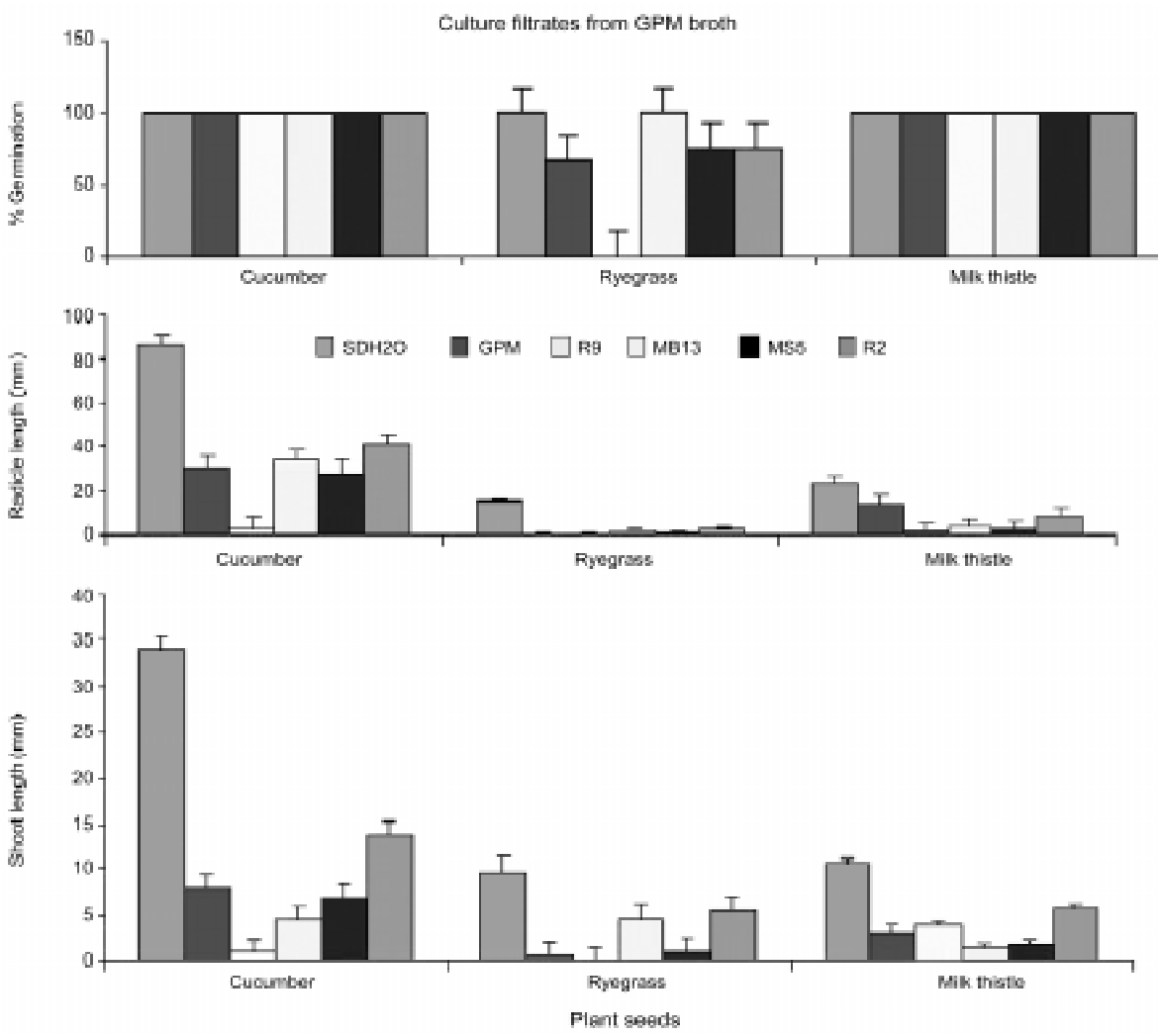

Fig. 2. Effect of culture filtrates of the isolates R9, R2, MB13 and MS5 from their GPM broth cultures compared to the controls (SD H_2O and uninoculated GPM broth) on germination, radicle and shoot growth of cucumber, ryegrass and milk thistle. Bars represent the standard errors for means of a plant species at $\alpha = 0.05$.

applications. R9 culture filtrate caused complete inhibition in seed germination of ryegrass (Fig. 2). Germinated cucumber showed more than 87% reduction in the growth of the radicle and shoot while germinated milk thistle showed 89.1% reduction in the growth of the radicle compared to the GPM broth control. The culture filtrate of R9 caused significant phytotoxic effect against *Amaranthus retroflexus* seeds indicated by complete inhibition for seed germination when applied in 1:1 dilution with sterilized distilled water (Fig. 3). The effect of the R9 culture filtrate on ryegrass substantiates the finding reported by Mallik (1997). A complete inhibition of the redroot pigweed germination caused by R9 culture filtrate was more pronounced than what was previously reported by Heisey and Putnam (1990). Through their study they reported the inhibition for radicle elongation of redroot pigweed caused by geldanamycin and nigericin rather than the complete inhibition of the germination.

The culture filtrate of MB13 showed reduction in growth of the radicle and shoot of milk thistle by 75.3% and 54.5%, respectively in contrast with the control. The culture filtrate of MS5 showed reduction in the growth of the radicle and shoot of milk thistle by 82.6% and 42.4%, respectively compared to the control. R2 culture filtrate reduced 42% growth of the radicle in milk thistle compared with the control.

***Streptomyces* culture filtrates from peptone-molasses-corn steep (PMC) broth.** R9 culture filtrate showed more than 74% reduction in growth of the radicle and shoot of cucumber whereas, the culture filtrates of the isolates MB13, MS5 and R2 showed no adverse effect on the seed germination or the growth of the radicle and shoot of the tested plant species compared to the PMC broth control.

Extraction of the phytotoxin(s). The R9 culture filtrate was the only one to be extracted with dichloromethane. Dichloromethane extracted fraction of the R9 culture filtrate (50 mg) from GPM broth caused

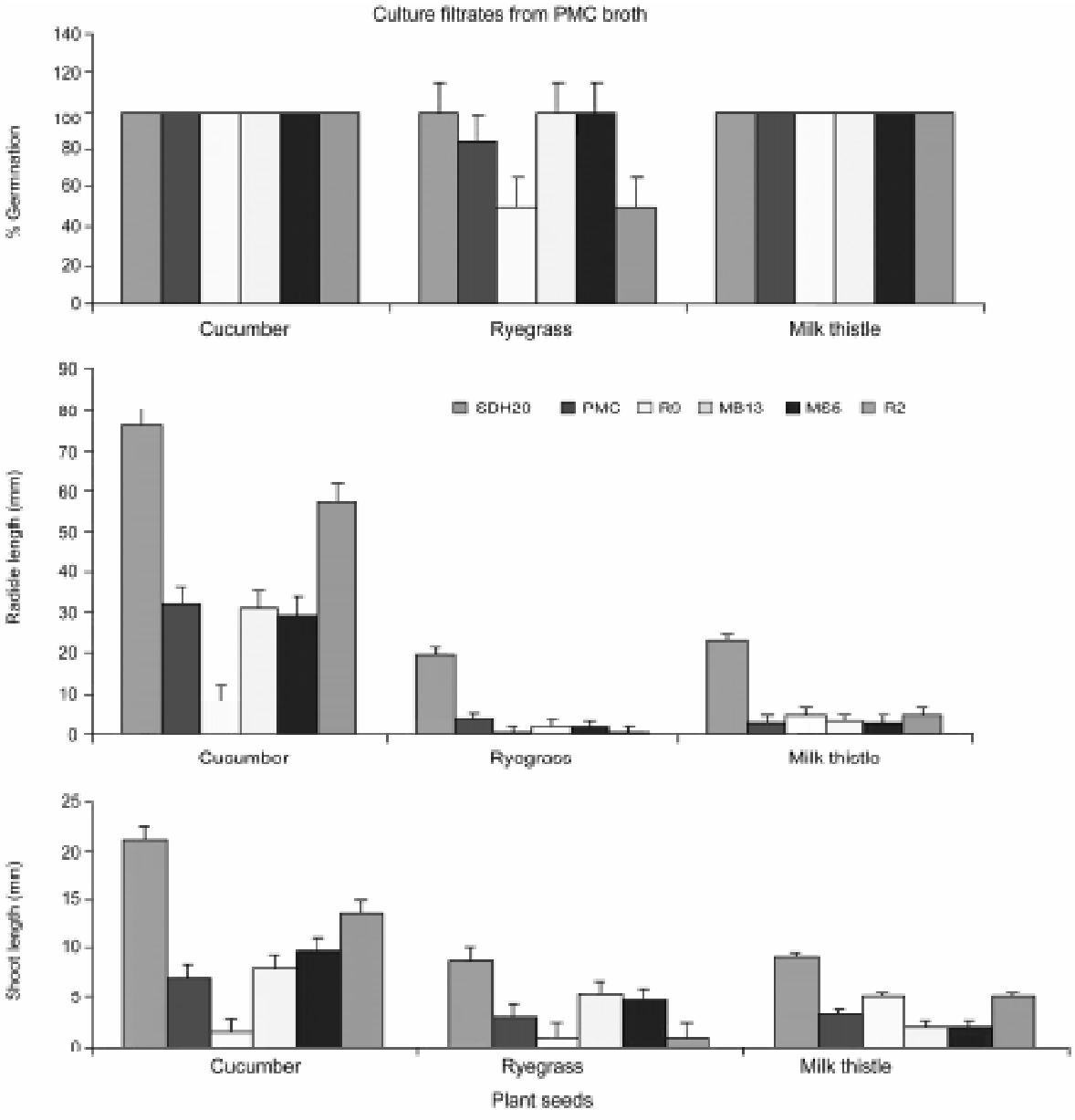

Fig. 3. Effect of culture filtrates of the isolates R9, R2, MB13 and MS5 from their PMC broth cultures compared to the controls (SD H_2O and uninoculated PMC broth) on germination, radicle and shoot growth of cucumber, ryegrass and milk thistle. Bars represent the standard errors for means of a plant species at $\alpha = 0.05$.

complete inhibition of ryegrass seed germination at 3 and 5 mg crude extract (Table II). However, at 1.5 mg, there was no inhibition of seed germination, but the growth of the radicle and shoot were reduced by 97.5% and 90.4%, respectively, compared to water control. In case of cucumber, there was no inhibition of seed germination, but the growth of the radicle was reduced by 95.6%, 98.1% and 99.4% at 1.5, 3 and 5 mg, respectively, compared with its growth under sterilized distilled water control. The growth of the cucumber shoot was completely suppressed at 3 and 5 mg and reduced by 97.7% at 1.5 mg, compared with the sterilized distilled water control. The seed germination of milk thistle was inhibited by 55.7% at 5 mg compared to the sterilized distilled water control with no significant inhibition at 1.5 and 3 mg. Radicle growth of milk thistle was reduced by 66.5%, 93.2% and 96.6% at 1.5, 3 and 5 mg respectively compared to the sterilized distilled water control. Whereas the growth of milk thistle shoots was completely suppressed at 5 mg, and reduced by 73% at 3 mg, but there was no significant reduction at 1.5 mg compared with the sterilized distilled water control.

Further testing revealed that other isolates are important to be handled in similar manner such as J214, MB13 and MS5, which was kept for future investigation. The phytotoxic potential of R9 crude extract against the growth of cucumber, ryegrass, and

Table II
Responses of cucumber, ryegrass and milk thistle on filter paper moistened with solvent extract of fresh R9 culture filtrate (CF) and uninoculated broth (UB)

Concentration of extract (mg/ml)	Radicle length (mm)		Shoot length (mm)		Germination (%)	
	UB	CF	UB	CF	UB	CF
Cucumber						
1.5	75.0	3.7*	28.8	0.7*	100.0	100.0
3.0	73.0	1.6*	21.4	0.0*	100.0	89.0
5.0	55.8	0.5*	11.6	0.0*	100.0	89.0
control (S.D.H_2O) [a]	85.0		30.4		100.0	
LSD [b]		2.7		6.0		25.4
Ryegrass						
1.5	17.7	0.4*	17.7	1.2*	100.0	55.7
3.0	14.8	0.0*	10.5	0.0*	100.0	0.0*
5.0	7.0	0.0*	2.4	0.0*	100.0	0.0*
control (S.D.H_2O)	16.3		12.5		100.0	
LSD		1.9		4.9		48.0
Milk thistle						
1.5	31.5	7.9*	9.7	7.5	100.0	100.0
3.0	21.5	1.6*	7.1	2.4	100.0	66.7
5.0	20.0	0.8*	5.7	0.0*	100.0	44.3*
control (S.D.H_2O)	23.6		8.9		100.0	
LSD		4.7		3.5		36.6

* Significantly different from corresponding uninoculated broth at $\alpha = 0.05$
[a] S.D.H_2O: Sterile distilled water; [b] LSD: Least significant difference

Table III
Morphological and physiological characterization of streptomycetes active isolates

Isolate	Characterization														
	Morphological						Physiological								
	Macroscopic						Microscopic				Sugar utilization[d]*				
	Aerial mass color	Reverse side color	Diffusible pigment[a]	Melanin Production	Spore chain morphology[b]	Spore Surface[c]	D-Glucose	L-Arabinose	D-xylose	D-Fructose	Sucrose	I-inositol	Rhamnose	Raffinose	D-mannitol
R9	Gray	Distinctive	0	–	RF	Sm	+	–	+	+	–	–	–	–	–
R2	White	Distinctive	1	–	RF	Sm									
MS5	White	Distinctive	0	–	RF	Sm									
MB13	White	Distinctive	0	–	RA	Sm									
MS18	White	Distinctive	0	–	RF	Sm									
MS3	Gray	Distinctive	0	–	RF	Sm									
Is11	White	Distinctive	0	–	RF	Sm									

[a] Diffusible pigment: 1 – Black, 0 – none; [b] RF: *Rectiflexibiles*, RA: *Retinaculiaperti*; [c] sm: smooth,
[d]* Sugar utilization test was done only for R9

milk thistle at the higher two concentrations suggests a promising broad spectrum phytotoxin. This activity on cucumber seeds coincide with the phytotoxic activity on cucumber apical growth done by Mishra *et al.* (1987).

The phytotoxic activity observed here indicates that it may be a function of concentration coupled with the degree of susceptibility of each crop to the active ingredient produced. The phytotoxic effect of R9 crude extract suggested a potential phytotoxin that may adversely affect the embryos and hinder the seed germination of weeds. The extracted compound(s) would be a promising phytotoxin that may be mass produced, purified, formulated and commercialized.

Characterization of the most active isolates. Macroscopic and microscopic characterization of five of the most active isolates was shown in Table III. Based on spore surface, carbon utilization test and the other cultural properties, stain R9 was identified as *Streptomyces aburaviensis*.

Acknowledgements

Deanship of Scientific Research at Jordan University of Science and Technology funded this research (Grant No. 135/03).

The authors are grateful to Prof. M.A.B. Mallik (Emeritus Research Professor, Langston University, Oklahoma) for his guidance and valuable suggestions through out the present work.

Literature

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