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Isolation, Characterization and Screening for Fiber Hydrolytic Enzymes-Producing Streptomycetes of Jordanian Forest Soils

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Abstract: There is considerable interest in different fiber hydrolytic enzymes such as cellulases, xylanases and pectinases for their potential applications in the paper, food and feed industries. Therefore, this study aimed to screen for soil streptomycetes producing such enzymes and characterize them. A total of 110 isolates of streptomycetes were recovered from 15 soil samples collected from three regions of Ajlun forests in northern Jordan then screened for their ability to produce cellulase, pectinase and xylanase enzymes by observing the clear zones around the colonies on cellulose, pectin and xylan agar media, respectively. Data showed that 79% of the isolates were able to produce at least one of the enzymes with cellulase being the highest (71.8%). Thirty five isolates (32%) were able to produce the three enzymes with 24 of them were characterized morphologically and physiologically as *Streptomyces*. RAPD DNA analysis has been used to determine the relatedness of 11 active isolates based on their RAPD-PCR fingerprints. Genetic polymorphisms between these active isolates showed two common bands of 500 and 800 pb shared by approximately (90%) of them.

Key words: Hydrolytic, enzymes, RAPD, *Streptomyces*

INTRODUCTION

Streptomyces species have been always a source of thousands of bioactive compounds that attracted the researchers many years ago. Enzymes are one of the important products of this unique group of bacteria and among these are the fiber hydrolytic enzymes that are essential for the turn over and recycling of the carbon locked in plant polymers. For this reason, actinomycetes and particularly *Streptomyces* species are considered as important decomposers of plant and plant components (Blanchette *et al*., 1981; King and Eggins, 1977; Tsujibo *et al*., 1990).

Streptomyces play an important role in soil as decomposers of plant and other materials, thus participating in the turnover of relatively complex and recalcitrant polymers (Daffonchio *et al*., 1996). The production of wood hydrolytic enzymes, such as cellulases and hemicellulases (xylanases) by several *Streptomyces* species has been demonstrated by many workers (Beg *et al*., 2000; Crawford and Sutherland, 1979; Elegir *et al*., 1994; Gilbert *et al*., 1995; Sutherland *et al*., 1979).

Cellulases, xylanases and other hemicellulolytic enzymes have received attention worldwide due to their potential applications especially in the biodegradation of agricultural wastes as tomato pomace, olive mill wastes (Squires *et al*., 1991) in addition to their applications in the processing and clarification of fruit juices and wine (Coughlan and Hazlewood, 1993). Also, the digestibility of plant materials has been increased by applying these enzymes, thus the animal feed industry has developed (Blanco *et al*., 1995). Xylanases have an important industrial applications in processing and bleaching of pulp and paper (Elegir *et al*., 1994; Niehaus *et al*., 1999).

In this study, streptomycetes isolated from forest soils in Jordan were screened for their potential to produce fiber hydrolytic enzymes (cellulases, pectinases and xylanases). These isolates were characterized by conventional and molecular methods.

MATERIALS AND METHODS

Study area: Ajlun as the highest forest cover in Jordan is located in the northern part of the country, 75 km north of Amman. The area is mountainous ranging from 500 to 1250 m above sea level, with steep slopes, valleys and numerous springs. In the indigenous forest where *Pinus* sp., *Quercus* sp. and *Ceratonia* sp. predominate, wild relatives of pistachio, apricot and almond are still

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found. The area has a sub-humid Mediterranean climate, situated within Jordan's highest rainfall region (300-600 mm). Highest temperatures occur in August (mean maximum 34°C) and lowest temperatures in January (mean minimum 4.2°C) (Al-Eisawi *et al*., 2000).

Collection of soil samples: Five soil samples were collected from each location; Ibbin, Rasun and Ishtafayna during Fall season of 2000. These locations were chosen to represent the Ajlun forest in north of Jordan. The soil samples (rich in lignocellulosic materials) were collected with an auger, (down to 10 cm depth) after removing approximately 5 cm litter of fallen leaves and fruits. Samples were placed in polyethylene bags and stored at 4°C until processed (Katsifas *et al*., 1999).

Sample processing: Each soil sample was air dried at room temperature then mixed thoroughly and sieved through a 2 mm pore size sieve (Retsch, Haan, Germany) to get rid of large debris. The sieved soil was used for the isolation purposes.

Enrichment of *Streptomyces*: One gram of sieved soil samples was placed in a crucible dish and heated in an oven (Supertek, India) at 45°C for 12 h (Williams *et al*., 1972). After drying, soil samples were mixed with 0.1 g of $CaCO_3$ then incubated at 28°C for 7 days in a water bath (GFL, Germany) (El-nakeeb and Lechevalier, 1963; Tsao *et al*., 1960).

Isolation: Treated soil samples of 1 g were suspended in 100 mL sterile distilled water then incubated in an orbital shaker incubator (TEQ, Portugal) at 28°C with shaking at 140 rev/min for 30 min. Mixtures were allowed to settle, then serial dilutions up to 10^{-4} were prepared. From each dilution, 0.1 mL was taken and spread evenly over the surface of starch casein nitrate agar (SCNA) (El-nakeeb and Lechevalier 1963; Kuster and Williams, 1964) plates (supplemented with cycloheximide 50 μg mL^{-1}) (in triplicate) with sterile L-shaped glass rod, then incubated at 28°C for 10 days. Dilutions that gave 20-200 colonies were chosen for further isolation.

Screening for xylanase-producing *Streptomyces*: Pure isolates of *Streptomyces* were cultured on xylan agar (Per 1 L: yeast extract:1 g; xylan: 10 g; KH_2PO_4: 4 g; NaCl: 2 g; $MgSO_4.7H_2O$: 1 g; $MnSO_4$: 0.05 g; $FeSO_4.7H_2O$: 0.05 g; $CaCl_2.2H_2O$: 2 g; NH_4Cl: 2 g and agar: 15 g; pH 7-7.2) plates (Nanmori *et al*., 1990) and incubated at 28°C for 4 days. The plates were then flooded with absolute ethanol (99%) and left for 16 h at room temperature. Colonies producing xylanase enzyme showed clear zones against an opaque colour of non-hydrolyzed media. Positive isolates were tested again for confirmation.

Screening for cellulase-producing *Streptomyces*: Pure isolates of *Streptomyces* were cultured on cellulose agar (Per 1 L: yeast extract:1 g; CMC: 10 g; KH_2PO_4: 4 g; NaCl: 2 g; $MgSO_4.7H_2O$: 1 g; $MnSO_4$: 0.05 g; $FeSO_4.7H_2O$: 0.05 g; $CaCl_2.2H_2O$: 2 g; NH_4Cl: 2 g and agar: 15 g; pH 7-7.4) plates then incubated at 28°C for 4 days. The plates were then flooded with 0.1% Congo Red and left for 15-20 min, washed with 1 mL NaCl (1 M) and incubated in the referegerator at 8°C for over night. Bacterial colonies producing cellulase will show clear zones against red colour of non-hydrolyzed. Positive isolates were tested again for confirmation (Carder, 1986).

Screening for pectinase-producing *Streptomyces*: Pure isolates of *Streptomyces* were cultured on pectin agar (Per 1 L: yeast extract:1 g; pectin: 5 g; KH_2PO_4: 4 g; NaCl: 2 g; $MgSO_4.7H_2O$: 1 g; $MnSO_4$: 0.05 g; $FeSO_4.7H_2O$: 0.05 g; $CaCl_2.2H_2O$: 2 g; NH_4Cl: 2 g and agar: 15 g; pH 7-7.4) plates, then incubated for 4 days at 28°C. Plates were then flooded with a 1% solution of polysaccharide precipitant cetavlon (acetyl trimethyl ammonium bromide), dissolved in a 15% alcoholic solution and then used to detect pectinase production. After 30-60 min exposure, colonies producing pectinase showed a clear zone against an opaque colour of the non-hydrolyzed medium.

Characterization of the isolates: Only *Streptomyces* isolates that gave a positive result for any of the three above enzymes, were characterized morphologically and physiologically following the directions given by the International *Streptomyces* Project (ISP) according to Shirling and Gottlieb (1966).

General morphology was determined on oatmeal agar plates, incubated in the dark at 28°C for 21 days, by direct light microscopy examination of the surface of crosshatched cultures. Colours were determined according to the scale adopted by Prauser (1964) and isolates were grouped into separate colour series according to the system proposed by Nonomura (1974). Melanin reactions were detected by growing the isolates on at least one of the ISP media (No. 6 and No. 7) (Shirling and Gottlieb, 1966).

The spore chain morphology was determined by direct microscopic examination using the 21-day-old oatmeal cultures by observing the crosshatched cultures under a compound light microscope (Nikon, Japan) using 100X magnification power. The species involved in the genus *Streptomyces* were divided into three arrangements: Rectiflexibiles (RF), Retinaculiaperti (RA) and Spirales (S) (Nonomura, 1974).

Growth conditions: All *Streptomyces* isolates that showed unusual fiber hydrolytic enzyme profile were cultured on tryptic soy broth (TSB) (30 g L^{-1}) (Hopwood *et al*., 1985) at 28°C with shaking at 140 rev/min for 48 h. Purity of the cultures was confirmed by plating 0.1 mL from the broth on starch casein nitrate agar (SCNA) (Kuster and William, 1964) plates and incubated at 28°C for 72 h.

Extraction of genomic DNA from pure *Streptomyces* isolates: Genomic DNA extraction was conducted using Wizard Genomic DNA Purification Kit (Promega, USA) according to the manufacturer instructions. All DNA manipulation, handling and PCR work was conducted using DNase, RNase-Free barrier tips (Promega, USA).

Estimation of the purity and quantity of the extracted DNA: The isolated DNA was checked for purity and quantity by a spectrophotometric method (Sambrook *et al*., 1989).

Random amplified polymorphic DNA (RAPD) study: Two random primers (Operon Technologies, USA), 10-mer long each, were used separately in the RAPD study. These primers were OPA9: 5`-GGGTAACGCC-3` and OPO20: 5`-GTGATCGCAG-3`. In order to determine the typeability, reproducibility and discrimination of the each primer, separate amplifications of each primer were conducted (three trials for each primer). The output of each experiment was compared to the previous one (Arbeit, 1994). Amplification reactions were performed according to Williams *et al*. (1990) in volumes of 25 μL containing 0.5 μmol L^{-1} primer, 1X PCR Buffer ($MgCl_2$ free) (Promega, USA), 2 mmol L^{-1} $MgCl_2$ (Promega, USA), 100 μmol L^{-1} of each dNTP (Promega, USA), 0.5 U Taq DNA polymerase (Promega, USA), 0.3 mg template DNA. Nucleases free water (Promega, USA) was used to bring the reaction volume to 25 μL.

PCR amplification was carried out in 0.2 mL thin walled, nucleases free PCR tubes (Treff Lab, Switzerland) using iCycler thermocycler (BIO-RAD, USA) programmed as follows: Initial denaturating step at 95°C for 3 min, followed by 40 cycles of 95°C for 30 sec, annealing at 36°C for 30 sec, extinction at 72°C for 1 min. Finally, extension at 72°C for 7 min, after that tubes were held at 4°C for direct use, or stored at 20°C until needed.

Electrophoresis and photography: Gels were viewed using Fotodyne U.V. illuminator (Fotodyne Inc., USA) and photographed using Polaroid MP4 + Instant Camera System (Polaroid Corp., USA).

Data analysis of RAPD profiles: The RAPD photographs were analyzed under a magnifying lens over an engineer's disk supplied with horizontal and vertical rulers. The fingerprints were recorded in the binary form i.e., 1 in case of presence of a band and 0 when there is no band, to generate a binary matrix (Demeke and Adams, 1994; Sneath and Sokal, 1973) for each primer. These binary matrices were used to calculate the similarities and the differences between the isolates by the SPSS software, using the simple matching coefficient (Sneath and Sokal, 1973). Dendrograms were generated using the Unweighted Pair Group Method (UPGMA) (average linkage) and Nearest Neighbor and Sneath and Sokal 1, (single average) (Demeke and Adams, 1994; Sneath and Sokal, 1973; Mangin *et al*., 1999).

RESULTS

Isolation of *Streptomyces* isolates: After enrichment of soil samples, a total of 110 different actinomycetes (*Streptomyces*-like colonies) were isolated (data not shown). All of these isolates were selected based on their colony morphology, resembling that of *Streptomyces* species.

Streptomyces isolates were screened for detection of cellulase, pectinase and xylanase hydrolytic enzymes. Only 11 isolates were able to produce the three enzymes (cellulases, pectinases and xylanases) and they were subjected to further characterization by conventional and molecular methods.

Screening for the fiber hydrolytic enzymes production by streptomycetes: A total of 110 isolates were tested for the hydrolytic enzyme production (cellulases, pectinases and xylanases) with 78.9% of them were able to produce one, two or the three hydrolytic enzymes. Data revealed that of the enzyme-producing isolates, 19.3% were able to produce at least one enzyme, 27.8% produced two different enzymes and 31.8% of the 110 isolates were able to produce the three enzymes. However, 21.1% of the 110 isolates were not able to produce any of these.

Results indicate that 71.8% of the isolates were able to produce CMCase enzyme, whereas 54.5% produced xylanase and only 43.6% produced pectinase. The relative activities of the isolates to produce the enzymes ranged from weak, moderate to strong depending on the diameter of the clear zone formed in the screening process (Table 1 and Fig. 1).

Isolates (35 isolates) which were able to produce the three fiber-hydrolytic enzymes were further characterized morphologically and physiologically.

Table 1: Screening for hydrolytic enzymes produced by different active *Streptomyces* isolates

	Activity of the different *Streptomyces* isolates to produce fiber hydrolytic enzymes		
Isolate	CMCase	Pectinase	Xylanase
Ib 27	(+3)	(+3)	(+2)
Ib 24D	(+3)	(+3)	(+3)
Ib 113	(+3)	(+3)	(+2)
Ib 54	(+3)	(+2)	(+3)
Ib 217	(+3)	(+2)	(+3)
Ib 43	(+3)	(+3)	(+2)
Ib 16	(+3)	(+3)	(+1)
Ib 24C	(+3)	(+1)	(+3)
Ib 128	(+3)	(+2)	(+2)
Ib 28	(+2)	(+2)	(+3)
Ib 34	(+3)	(+1)	(+2)
Ib 23A	(+2)	(+2)	(+2)
Ib 24	(+2)	(+2)	(+2)
Ib 62	(+1)	(+2)	(+2)
Ib 114	(+1)	(+1)	(+3)
Ib 41	(+3)	(+1)	(+1)
Ib 11	(+1)	(+2)	(+1)
Ra 8	(+2)	(+1)	(+1)
Ra 12	(+3)	(+1)	(+2)
Ra 10	(+3)	(+1)	(+3)
Ra 5	(+3)	(+1)	(+2)
Es 10	(+3)	(+2)	(+3)
Es 1	(+3)	(+1)	(+3)
Es 7	(+2)	(+1)	(+1)

*Diameter of clear zone, (+1); < 5 mm; (+2): 5-10 mm; (+3): > 10 mm

Morphological and physiological characterization of the active isolates: After applying the characterization methods for *Streptomyces* species, results showed that 24 isolates out of 35 fitted the genus description given by Nonomura (1974) and Shirling and Gottlieb (1966). The other active isolates were characterized as *Streptoverticilium* and/or other actinomycetes.

The 24 active fiber-hydrolytic enzymes producing-*Streptomyces* isolates were categorized into 4 colour series depending on the colour of mature sporulated aerial mycelia, white (54.2%) and grey (29.2%) were the most common colour series; however, the lowest occurrence was for the yellow and the variable (pink and violet) colour series and was represented by 8.3% for each (Table 2).

Pigmentation characterization: Regarding the colour of the substrate mycelium (reverse colour), ten of the active isolates exhibited distinctive reverse side pigmentation and 11 of them were able to produce soluble pigments. However, only two isolates (Ra.8) and (Ib.14) were able to give a positive reaction on tyrosine agar slants (ISP medium No. 7) but not on yeast-extract iron agar slants (ISP medium No. 6) (Table 2).

Sporophore morphology: The most occurring sporophore arrangement was the rectiflexibile (RF) and represented by 14 of the active isolates, followed by the spiral arrangement (S) and represented by 6, while the retinaculiaperti (RA) was the least observed arrangement (4) (Table 2).

Identification of the most active isolate: The isolate Ib 24D was completely characterized as follows: grey aerial mycelium with distinctive reverse side color, unable to produce both soluble and melanin pigments; able to utilize L-arabinose, D-xylose, D-mannitol, D-fructose, rhamnose, mannose and sucrose; however, unable to utilize I-inositol and raffinose. This isolate bears rectiflexible (RF) sporophore arrangement and a smooth spore surface (Fig. 2). Comparing the results of characterization for this active isolate with those in Nonomura key for classification and identification of *Streptomyces* (Nonomura, 1974) data revealed that the isolate Ib 24D might be physiologically similar to *Streptomyces fulvoviridis*.

Extraction of genomic DNA from *Streptomyces* pure cultures: Genomic DNA was extracted using commercial DNA isolation kit. The extracted DNA was of a good quality as being checked by agarose gel electrophoresis. The extracted DNA was intact, clean and free of RNA (data not shown).

RAPD study: A total of 11 active soil *Streptomyces* isolates being able to produce the three enzymes were

Table 2: Morphological and cultural characteristics of the 24 *Streptomyces* isolates with their ability to produce the three fiber hydrolytic enzymes

	Colour series				
	White	Grey	Yellow	Variable**	Total***
No. of Isolates*	13 (54.2%)	7 (29.2%)	2 (8.3%)	2 (8.3%)	24 (100%)
Pigment Production					
Melanin	1	0	1	0	2
Reverse Side	3	5	1	1	10
Soluble	5	3	2	1	11
Sporophore Morphology					
Rectiflexible (RF)	8	4	1	1	14
Spirales (S)	3	2	0	1	6
Retinaculiperti (RA)	2	1	1	0	4

*These 24 active isolates were able to produce cellulase, xylanase and pectinase, **Variable: pink, orange and violet, ***Numbers in parentheses represent the percentage out of the total

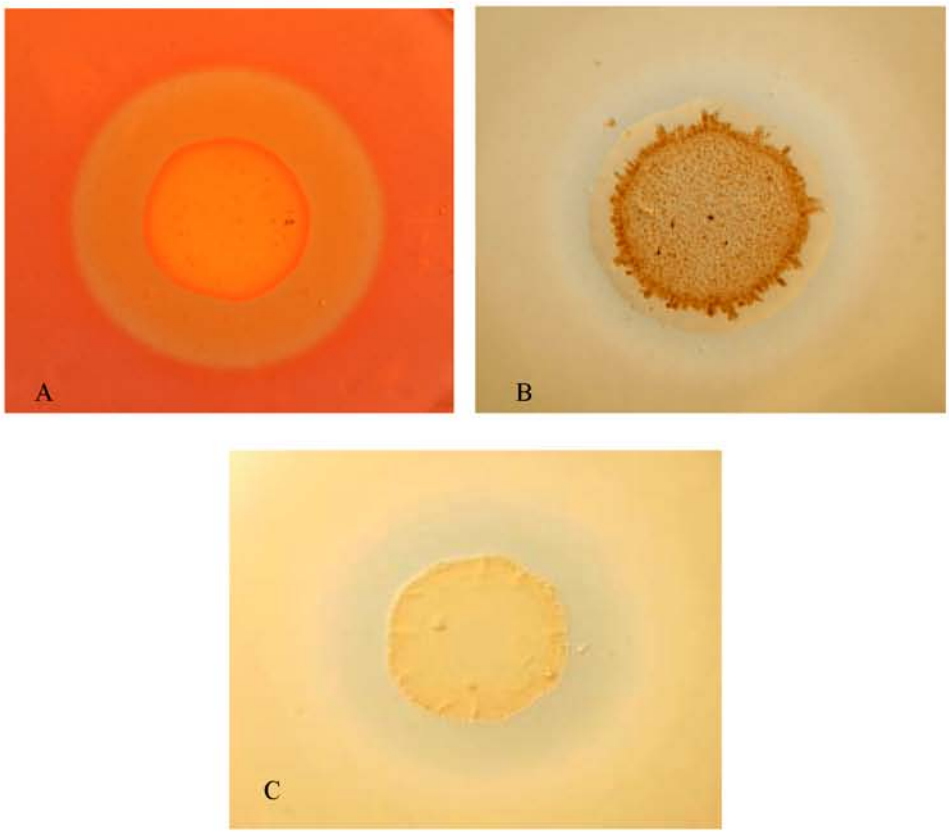

Fig. 1: Plate diffusion method showing the fiber hydrolytic enzymes activity (A) detection of CMCase production (B) detection of pectinase production (C) detection of xylanase production

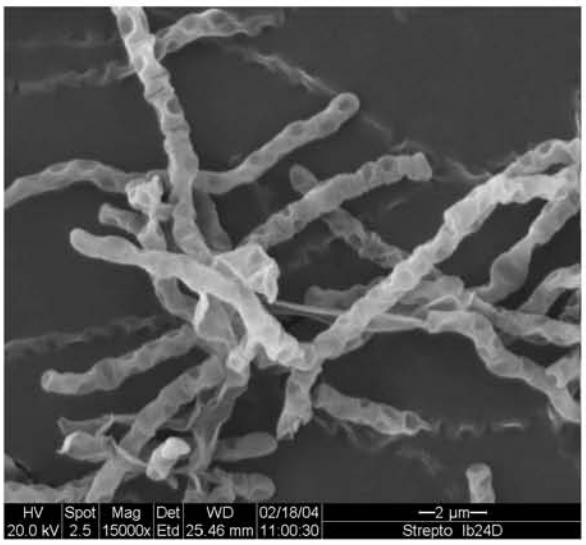

Fig. 2: Scanning electron micrograph of spore chains of strain Ib 24D

Table 3: Characteristics of 11 hydrolytic enzyme-producing *Streptomyces* strains with their fitness to RAPD clusters

	Cultural characters[a]						Production of Enzymes[c]		
Strain No.	AM	ME	RP	SP	Spore Chain[b]	RAPD Cluster	I[d]	II	III
Ib 54	White	-	+	-	S	Out	(+3)	(+2)	(+3)
Ib 43	White	-	-	-	RF	2	(+3)	(+3)	(+2)
Ib 128	White	-	-	-	S	1	(+3)	(+2)	(+2)
Ib 34	White	-	-	+	RF	2	(+3)	(+1)	(+2)
Ib 114	Gray	-	+	+	RF	1	(+1)	(+1)	(+3)
Ib 24D	Gray	-	+	-	RF	1	(+3)	(+3)	(+3)
Ib 24C	Gray	-	-	+	RF	Out	(+3)	(+1)	(+3)
Ib 23A	Gray	-	+	+	RA	Out	(+2)	(+2)	(+2)
Ib 27	Pink	-	-	-	S	2	(+3)	(+3)	(+2)
Ib 16	Violet	-	+	+	RF	1	(+3)	(+3)	(+1)
Ib 24	Yellow	-	+	+	RA	1	(+2)	(+2)	(+2)

[a]AM: aerial mycelium color; ME: melanin pigment; RP: Reverse pigment; SP: Soluble pigment, [b]RF: Rectiflexible; S: Spirales; RA: Retinaculiperti, [c]I: CMCase; II: Pectinase; III: Xylanase, [d]Numbers in parenthesis represent the diameter of clear zone, (+1); < 5 mm; (+2): 5-10 mm; (+3): > 10 mm

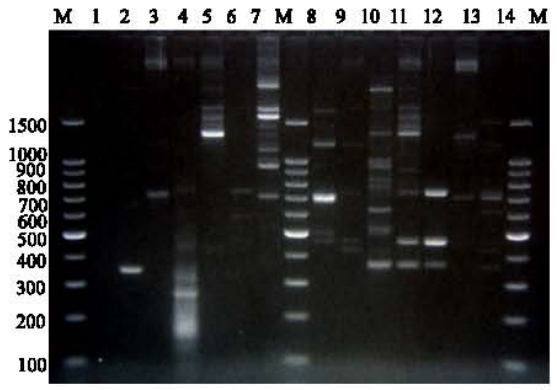

Fig. 3: RAPD fingerprints profile of *Streptomyces* isolates, on 2% agarose gel electrophoresis, amplified using primer OPA 09. Lane M: 100 bp DNA ladder molecular weight marker; lane 1:PCR negative control; lanes 2-14 corresponds to the *Streptomyces* isolates: lane 2: Ib 27, lane 3: Ib 24D, lane4: Ib 54, lane 5: Ib 43, lane 6: Ib 16, lane 7: Ib 24C, lane 8: Ib 128, lane 9: *S. halstedii* (ATCC 10897), lane 10: Ib 34, lane 11: Ib 23A, lane 12: Ib 24, lane 13: *S. violaceusniger*, lane 14: Ib 114

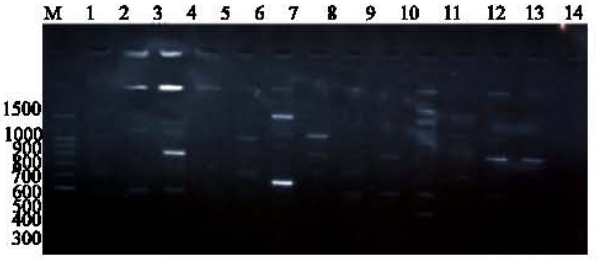

Fig. 4: RAPD fingerprints profile of *Streptomyces* isolates, on 2% agarose gel electrophoresis, amplified using primer OPO 20. Lane M: 100 bp DNA ladder molecular weight marker; lane 14: PCR negative control; lanes 1-13 corresponds to the *Streptomyces* isolates: lane 1: Ib 27, lane 2: Ib 24D, lane 3: Ib 54, lane 4: Ib 43, lane 5: Ib 16, lane 6: Ib 24C, lane 7: Ib 128, lane 8: *S. halstedii* (ATCC 10897), lane 9: Ib 34, lane 10:Ib 23A, lane 11: Ib 24, lane 12: *S. violaceusniger*, lane 13: Ib 114

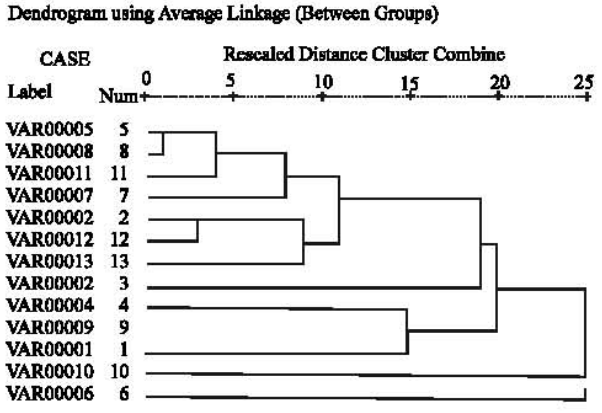

Fig. 5: Dendrogram using average linkage between groups, numbers 1-13 represents the soil isolates (1: Ib 27; 2: Ib 24D; 3: Ib 54; 4: Ib 43; 5: Ib 16; 6: Ib 24C; 7: Ib 128; 8: *S. halstedii* (ATCC 10897); 9: Ib 34; 10: Ib 23A; 11: Ib 24; 12: *S. violaceusniger*; 13: Ib 114)

further characterized culturally and morphologically (Table 3), then identified genetically by the analysis of their RAPD fingerprints using two arbitrary primers. The relatedness between *Streptomyces* strains was evaluated by comparison of their RAPD patterns generated by each primer. The RAPD-fingerprints of the various strains differed in fragments number, size and intensity depending on which primer being used in each run. UPGMA dendrograms were derived from the fingerprints generated by each primer. In order to increase the accuracy, the dendrogram presented in Fig. 5 was generated by combining the proximity matrices of the two primers in one matrix, by taking the average of three proximity readings for each isolate obtained from each primer (Mangin *et al*., 1999). Each RAPD experiment was

repeated three times with each primer and similar results were obtained. Based on that, typing scheme as reported

The number of polymorphic bands generated for each isolate was between 1 and 12 with a size ranged from 150 to 2538 bp. RAPD analysis revealed that two common bands were shared by approximately (90%) of the isolates and the reference strains with a size of 800 and 500 bp for the first and second, respectively (Fig. 3 and 4). The dendogram presented in Fig. 5 shows that the tested isolates in addition to the reference strains (*S. halstidii* ATCC 10897 and *S. violaceusniger*) fell into one super cluster. This super cluster consists of 2 clusters and one out group. Cluster 1 includes 5 isolates (Ib 16, Ib 24, Ib 24D, Ib 114 and Ib 128) in addition to *Streptomyces halstedii* ATCC 10897 and *S. violaceusniger* reference strain. Cluster 2 includes 3 isolates (Ib 43, Ib 34 and Ib 27). Isolate Ib 24D was found to be most related to the reference strain *S. violaceusniger*. Isolate Ib 54 formed an out group to cluster 1, isolates Ib 23A and Ib 24C were considered as out groups to both clusters. These out group isolates are less related to other isolates.

Data indicated that when RAPD clustering was compared to cultural and microscopic properties; most of the isolates within cluster 1 were able to produce diffusible pigments (3 out of 5) and were able to exhibit distinctive reverse colour (4 out of 5). Moreover, 3 out of 5 isolates beard rectiflexibile sporophores (Table 3). In the case of cluster 2, two isolates out of three were unable to produce diffusible pigments. However, two isolates (Ib 43 and Ib 27) produce both soluble and reverse side pigments.

DISCUSSION

Isolation and screening for streptomycetes producing fiber hydrolytic enzymes: All of the 110 *Streptomyces* like isolates were isolated from soils of Ajlun forests, which are rich in lignocellulosic materials from the thick vegetation cover or plant debris. Therefore it is expected that these soils would have rich bacterial populations and particularly actinomycetes inhabiting the forest soils. In these soils actinomycetes are adapted to utilize different plant polymers as carbon sources by being capable of hydrolyzing cellulose, xylan and pectin (Semedo *et al*., 2000).

The highest percentage among the other fiber hydrolytic enzymes was the CMCase. Such high percentage was also reported by Wachinger *et al*. (1989) who screened 180 *Streptomyces* isolates and found that nearly all of the isolates were able to hydrolyze Carboxy Methyl Cellulose (CMC).

Selection of those isolates that are able to produce the three fiber hydrolytic enzymes (CMCase, xylanase and pectin) makes the use of these isolates more efficient, in such a way that the production of these enzymes from a single microorganism makes its industrial and biotechnological application more feasible and economical (Beg *et al*., 2000).

Morphological and physiological characterization of the active isolates: Results indicate that the grey and the white colour series were the most abundant. Similar results were reported for *Streptomyces* isolates that have been recovered from other locations in Jordan (Saadoun and Al-Momani, 1997; Saadoun *et al*., 1999; Saadoun and Gharaibeh, 2002; Saadoun and Gharaibeh, 2003). However, variability in the results of the reverse side colour production from the other studies (Saadoun and Al-Momani, 1997; Saadoun *et al*., 1999) could be due to the variation in soil habitat and vegetation that could affect the species composition and their physiological and morphological characterizations. Also, production of water-soluble pigment by the recovered isolates of this study is relatively higher than what obtained by Saadoun and Gharaibeh (2003) and by Saadoun *et al*. (1999), which could be again due to the soil habitat variation.

RAPD study: RAPD profiles for the 11 fiber hydrolytic enzymes-producing isolates along with 2 reference strains were analyzed by UPGMA dendrograms. This analysis enabled a simultaneous comparison between these isolates. When the band patterns were analyzed, data revealed the presence of common bands between all the isolates and the reference strains, which may represent prospective *Streptomyces*-probes. On the other hand, fragments specific to some species were also observed. The size of the bands and even the bands number and profile didn't match with what was reported by Mehling *et al*. (1999) or Malkawi *et al*. (1999). However, at least the size of one common band of 800 bp was similar to the finding of Gharaibeh *et al* (2003) who determined three common bands in the size of 2777, 800 and 250 bp. This could be due to the use of different thermostable DNA polymerase and different thermocycler machine (Ceatano-Annolles and Gresshoff, 1994; Saadoun *et al*., 1999). This supports the previous findings about the effect of thermostable DNA polymerase, the thermocycler and other reagents on the RAPD profile (Caetano-Annolles and Gresshoff 1994, Power 1996). The bands size and pattern revealed in this study did not match with what was reported by Malkawi *et al*. (1999). This variability in results could be due to the use of different PCR reaction conditions such as different thermostable DNA polymerase and different thermocycler machine (Caetano-Annolles, 1993).

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REFERENCES

Al-Eisawi, D., A. El-Oqlah, S. Oran and J. Lahham, 2000. Jordan Country Study on Biological Diversity: Plant Biodiversity and Taxonomy. UNDP Publication, Amman, pp: 70.

Arbeit, T.L., 1994. Laboratory Procedures for Epidemiological Analysis. In Manual of Clinical Microbiology. Ed., Murray, P.R., E.J. Barn, M.A. Pfaller, F.C. Tenover and R.H. Yolher, 6th Edn. Washington DC: ASM Press.

Beg, Q.K., B. Bhushan, M. Kapoor and G.S. Hoondal, 2000. Effect of amino acids on production of xylanase and pectinase from *Streptomyces* sp. QG-11-3. World J. Microbiol. Biotechnol., 16: 211-213.

Blanchette, R.A., J.B. Sutherland and D.L. Crawford, 1981. Actinomycetes in discolored wood of living silver maple. Can. J. Bot., 59: 1-7.

Blanco, A., T. Vidal, J.F. Colom and F.I.J. Pastor, 1995. Purification and properties of xylanase A from alkali-tolerant Bacillus sp. strain Bp-23. Applied Enviorn. Microbiol., 61: 4468-4470.

Caetano-Annolles, G., 1993. Amplifying DNA with arbitrary oligonucleotide primers. PCR Methods Applications, 3: 85-94.

Caetano-Annolles, G. and P.M. Gresshoff, 1994. DNA amplification using arbitrary minihairpin oligonucleotide primers. Biotechnology, 12: 619-623.

Carder, J.H., 1986. Detection and quantitation of cellulase by congo red staining of substrates in a cup-plate diffusion assay. Anal. Biochem., 153: 75-79.

Coughlan, M.P. and G.P. Hazlewood, 1993. B-1,4-D-xylan degrading enzyme systems: Biochemistry, molecular biology and applications. Biotechnol. Applied Biochem., 17: 259-289.

Crawford, D.L. and J.B. Sutherland, 1979. The role of actinomycetes in the decomposition of lignocellulose. Dev. Ind. Microbiol., 20: 143-151.

Daffonchio, D., G. Baggi, G.P. Molinari, G. Ranalli and C. Sorlini, 1996. Effect of the herbicide molinate on the metabolic activities of degradative *Streptomyces* griseus strain. J. Enviorn. Sci. Health, 31: 257-268.

Demeke, T. and R.P. Adams, 1994. The Use of PCR-RAPD Analysis in Plant Taxonomy and Evolution. In PCR Technology: Current Innovations. Griffin, H. and A. Griffin (Eds.). London: CRC Press, pp: 179-191.

Elegir, G., G. Szakaacs and T.W. Jeffries, 1994. Purification, characterization and substrate specificities of multiple xylanases from *Streptomyces* sp. strain B-12-2. Applied Enviorn Microbiol., 60: 2609-2615.

El-nakeeb, M.A. and H.A. Lechevalier, 1963. Selective isolation of aerobic actinomycetes. Applied Microbiol., 11: 75-77.

Gilbert, M., R. Morosoli, F. Shareck and D. Kluepfel, 1995. Production and secretion of proteins by streptomycetes. Crit. Rev. Biotech., 15: 13-39.

Gharaibeh, R., I. Saadoun and A. Mahasneh, 2003. Genotypic and phenotypic characteristics of antibiotic-producing soil *Streptomyces* investigated by RAPD-PCR. J. Basic Microbiol., 43: 18-27.

Hopwood, D.A., M.J. Bibb, K.F. Chater, T. Kieser, C.J. Bruton, H.M. Kieser, D.J. Lydiate, C.P. Smith and H. Schrempf, 1985. Genetic Manipulation of *Streptomyces*: A Laboratory Manual. John Innes Foundation. Norwich. United Kingdom, pp: 79-80.

Katsifas, E.A., E.P. Giannoutsou and A.D. Karagouni, 1999. Diversity of streptomycetes among specific Greek terrestrial ecosystems. Lett. Applied Microbiol., 29: 48-51.

King, B. and H.O.W. Eggins, 1977. Micromorphology of streptomycete colonization of wood. J. Instrum. Wood Sci., 7: 24-29.

Kuster, E. and S.T. Williams, 1964. Selection media for the isolation of streptomycetes. Nature, 202: 928-929.

Malkawi, H.I., I. Saadoun, F. Moumani and M.M.M. Meqdam, 1999. Use of RAPD-PCR to detect genetic diversity of soil *Streptomyces* isolates. New Microbiol., 22: 53-58.

Mangin, I., D. Corroler, A. Reinhardt and M. Gueguen, 1999. Genetic diversity among diary lactococcal strains investigated by polymerase chain reaction with three arbitrary primers. J. Applied Microbiol., 86: 514-520.

Mehling, A., U.F. Wehmeier and W. Piepersberg, 1995. Nucleotide sequences of streptomycete 16S ribosomal DNA: Towards a specific identification system for streptomycetes using PCR. Microbiology, 141: 2139-2147.

Nanmori, T., T. Watanabe, R. Shinke, A. Kohno and Y. Kawamura, 1990. Purification and properties of thermostable xylanase and β-xylosidase produced by a newly isolated *Bacillus stearothermophilus* strain. J. Bacteriol., 172: 6669-6672.

Niehaus, F., C. Bertoldo, M. Kahler and G. Antranikiar, 1999. Extremophiles as a source of novel enzymes for industrial application. Applied Microbiol. Biotechnol., 5: 711-729.

Nonomura, H., 1974. Key for classification and identification of 458 species of the streptomycetes included in ISP. J. Ferm. Technol., 52: 78-92.

Power, E.G.M., 1996. RAPD typing in microbiology-a technical review. J. Hosp. Infec., 34: 247-265.

Prauser, H., 1964. Aptness and application of colour for exact description of colours of *Streptomyces*. Zeitschrift fur Allgemeine Mikrobiologie ,4: 95-98.

Saadoun, I. and F. Al-Momani, 1997. Streptomycetes from Jordan soils active against *Agrobacterium tumefaciens*. Actinomycetes, 8: 29-36.

Saadoun, I., F. AL-Momani, H. Malkawi and M.J. Mohammad, 1999. Isolation, identification and analysis of antibacterial activity of soil streptomycetes isolated from north Jordan. Microbios., 100: 41-46.

Saadoun, I. and R. Gharaibeh, 2002. The *Streptomyces* flora of Jordan and its potential as a source of antibiotics active against antibiotic-resistant gram-negative bacteria. World J. Microbiol. Biotechnol., 18: 465-470.

Saadoun, I. and R. Gharaibeh, 2003. The *Streptomyces* flora of Badia region of Jordan and its potenttial as a source of antibiotics active against antibiotic-resistant bacteria. J. Arid Environ,. 53: 365-371.

Sambrook, J., E.F. Fritsch and T. Maniatis, 1989. Molecular Cloning: A Llaboratory Manual. New York: Cold Spring Harbor Laboratory, pp: 150-478.

Semedo, L.T., R.C. Gomes, E.P. Bon and R.R. Coelho, 2000. Endo cellulase and exocellulase activities of two streptomycetes strains isolated from a forest soil. Applied Biochem. Biotechnol., 84: 267-276.

Shirling, E.B. and D. Gottlieb, 1966. Methods for characterization of *Streptomyces* species. Int. J. Syst. Bacteriol., 16: 313-340.

Sneath, P.H.A. and R.R. Sokal, 1973. Numerical Taxonomy. San Francisco: W.H. Freeman, San Francisco, USA, pp: 100-150.

Squires, M.W., E.C. Naber and V.D. Toelle, 1991. The effects of heat, water, acid and alkali treatment of tomato cannery wastes on growth, metabolizable energy value and nitrogen utilization of broiler chicks. Poult. Sci., 71: 522-529.

Sutherland, J.B., R.A. Blanchette, D.L. Crawford and A.L. Prometto, 1979. Break down of Douglas-fir phloem by a lignocellulose-degrading *Streptomyces*. Curr. Microbiol., 2: 123-126.

Tsao, P.H., C. Leben and G.W. Keitt, 1960. An enrichment method for isolating actinomycetes that produce diffusable antifungal antibiotics. Phytopathology, 50: 88-89.

Tsujibo, H., T. Sakamoto, N. Nishino, T. Hasegawa and Y. Inamori, 1990. Purification and properties of three types of xylanases produced by an alkalophilic actinomycete. J. Appl. Bacteriol., 69: 398-405.

Wachinger, G., K. Bronnenmeier, W.L. Staudenbauer and H. Schrempf, 1989. Identification of mycelium-associated cellulase from *Streptomyces* reticuli. Applied Environ. Microbiol., 55: 2653-2657.

Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski, and S.V. Tingey, 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl. Acids Res., 18: 6531-6535.

Williams, S.T., M. Shameemullah, E.T. Watson and C.I. Mayfield, 1972. Studies on the ecology of actinomycetes in soil VI. The influence of moisture tension on growth and survival. Soil Biol. Biochem., 4: 215-225.