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Full Length Research Paper

Isolation, characterization and antimicrobial activity of *Streptomyces* strains from hot spring areas in the northern part of Jordan

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A total of 30 *Streptomyces* isolates (28 from soil and 2 from water) were isolated and purified from hot-springs areas in the northern part of Jordan. Four strains were thermopile. They grew at 45 and 55°C but not at 28°C. Strains were described morphologically on four different media: on glycerol yeast extract, oatmeal, yeast malt-extract and starch casein agar. White and grey color series were the most frequent series on all media. The results showed that glycerol yeast extract and starch casein were the best media for sporulation. And yeast malt-extract was the best medium for the production of soluble pigment. Physiological and biochemical tests showed that the highest number of *Streptomyces* isolates were able to hydrolyze tyrosine was 26 (87%). This was followed by 25 (83%) for starch, 24 (80%) for urea, 21 (70%) for casein and 10 (33%) for gelatin. Twenty two (73%) strains showed the ability to reduce nitrate and 8 (27%) strains produced melanin. Carbon source utilization showed that 26 (87%) strains were able to utilize L- arabinose, 25 (83%) strains were able to utilize meso-inositol, 8 (27%) strains were able to utilize D-sorbitol, 18 (60%) strains were able to utilize D-mannitol, 28 (93%) strains were able to utilize L-rhamnose and all isolates exhibited the ability to utilize D-fructose and D- glucose. The ability to exhibit antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* was detected among 20 and 26% of the isolates, respectively, while the ability to exhibit antifungal activity against *Candida albicans* was detected among 23% of the isolates. Molecular identification of the 8 antibiotics producers was carried out by PCR technique using two sets of primers specific to *Streptomyces* 16S rDNA gene sequences; strepB/strepE and strepB/strepF which amplified 520 and 1070 bp, respectively. All these antibiotic producer isolates showed positive results for the genus *Streptomyces* specific primers.

Key words: Characterization, streptomyces, antimicrobial activity, hot springs, thermophile, PCR.

INTRODUCTION

Since the discovery of penicillin from the filamentous fungus, *Penicillium notatum,* by Fleming in 1929 and the observation of the broad therapeutic use of this agent in the 1940s, the so-called “Golden Age of Antibiotics”, many countries around the world have developed intensive programs to increase the number of described antibiotics or to find new one's (Abussaud, 2000; Cragg and Newman, 2005).

In spite of the large number of antibiotics that have been discovered since that time, a large number of pathogenic bacteria have became resistant to antibiotics in common use (Mellouli et al., 2003; Cirz et al., 2005). As a result of the increasing prevalence of these antibiotic-resistant pathogens and the pharmacological limitations of the present antibiotics, searching for new antibiotics or modification of the present types has become an urgent focus for many researches (Rintala, 2001; Sahin and Ugur, 2003).

Filamentous bacteria belonging to the genus *Streptomyces* are well-known as the largest antibiotic-producing genus in the microbial world discovered so far (Taddei et al., 2006; Jayapal et al., 2007). Most *Streptomyces* and other Actinomycetes produce a diverse array of antibiotics including aminoglycosides, anthracyclins, glycolpeptides, β-lactams, macrolides, nucleosides, peptides,

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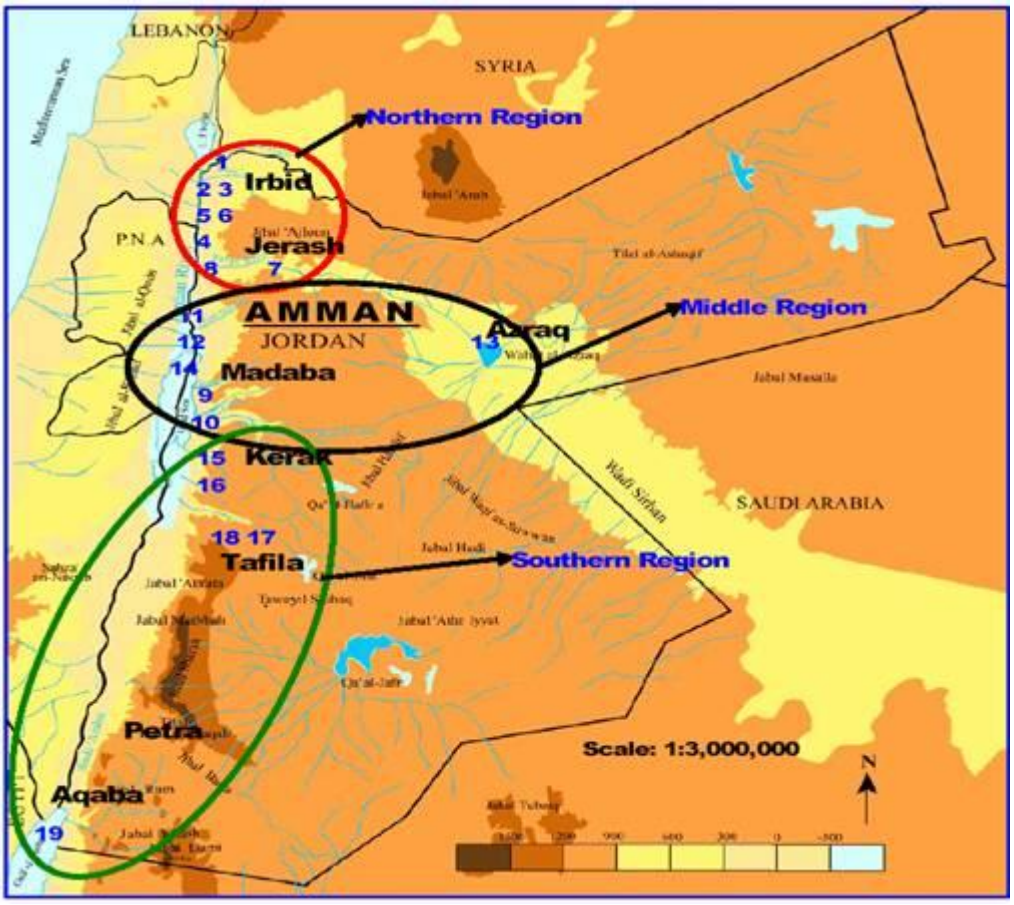

Figure 1. Distribution of hot-springs in in northern region of Jordan. 1: Al Hammah; 2: Ashouneh; 3: Abu Dablah; 4: Waggas; 5: Al Mansheyyah; 6: Abu Ziad; 7: Jerash; 8: Deir Alla.

polyenes, polyethers and tetra-cyclines. They produce about 75% of commercially and medically useful antibiotics (Mellouli et al., 2003; Sahin and Ugur, 2003).

The genus *Streptomyces* proposed by Waksman and Henrici in 1943, are a Gram-positive, aerobic, filamentous soil bacteria, produce an extensive branching substrate and aerial mycelium bearing chains of arthrospores. The substrate mycelium and spores could be pigmented, but also diffusible pigments could be produced. *Streptomyces* have high G+C (69 - 78%) content in their DNA and their cell wall is characterized as Type I (Lechevalier and Lechevalier, 1970; Williams et al., 1989; Rintala, 2001).

In the course of screening for new antibiotics, attention has primarily been concentrated to isolate *Streptomyces* from soil. Most recently, attention has been focused on greater diversity of organisms, those which are considered "rare", those which are difficult to isolate and/or culture and those which grow under extreme conditions such as thermophiles, acidophiles, halophiles etc (Yallop et al., 1997; Thakur et al., 2007).

Mesophilic *Streptomyces* are usually cultivated at temperature from 22-37°C while thermophilic *Streptomyces* grow between 25 and 55°C, they grow quite well at 50°C (Kim et al., 1999; Rintala, 2001). These organisms are useful as producer of antibiotics, enzymes and other bioactive metabolites because of their rapid autolysis of mycelium (Xu et al., 1998).

In continuing our screening program for *Streptomyces* flora in Jordan (Abussaud, 1996; Abussaud, 2000), we tend our attention to isolate *Streptomyces* strains from new locations and conditions such as hot-springs areas and test their capability to produce antimicrobial substances in order to look for the possibility of finding novel antibiotics. We started with 4 locations in the northern part of Jordan: Alshouneh, Waggas, Almansheyyah and Deir Alla springs (Figure 1).

MATERIALS AND METHODS

Collection of sample

A total of 12 soil samples and 12 water samples were collected from four different hot spring areas (Figure 1) Alshouneh, Waggas, Al-Mansheyah and Deir Alla. Six samples (3 soil samples and 3

water samples) from each location were collected as follows.

Water samples from the spring water column

About 2 l of water have been collected in sterile container, closed immediately and stored in ice box, in the presence of ice pads until shipped to the laboratory for analysis.

Soil samples

Soil samples were taken from sites along water streams at a depth of 10 cm, after removing approximately 3 cm of the soil surface. Samples were placed in polyethylene bags, closed tightly and stored in ice box as previously described. Physical factors such as temperature of water were directly measured at the sampling site by using a thermometer (Brannan co. England). The pH was also measured by using pH indicator paper (Whattman co. England).

Isolation of *Streptomyces* strains

Soil samples

Soil samples were analyzed following a modification of the procedure of Abussaud and Saadoun (1991): one gram of soil was suspended in 100 ml sterile distilled water, shaked in a reciprocal shaker at 190 rpm for 30 min, and then allowed settling. Serial dilutions (10^{-1} to 10^{-6}) were made. A 0.1 ml of each dilution was pipetted and spread evenly over the surface of Starch Casein (SCM) agar plates (g/l): (starch 10, casein 0.3, $NaNO_3$ 2, K_2HPO_4 2, NaCl 2, $MgSO_4.7H_2O$ 0.05, $CaCO_3$ 0.02, $FeSO_4.7H_2O$ 0.01 and agar powder 20, pH = 7.2) supplemented with cyclohexamide (50 ug/ml) and filter-sterilized rifampicin (0.5 ug/ml) using a sterile L-shaped glass rod. These plates were incubated at 28 and 55°C until good growth occurred. Dilutions that gave about 100 colonies per plate were chosen for the isolation of *Streptomyces* isolates.

Water samples

(a) 100 ml of each water sample was filtered through a Millipore membrane (0.22 - 0.45 µm pores, Sartorious. Germany), after that, the membranes were transferred to the surface of Starch Casein (SCM) plates and incubated at 55 and 28°C for 7 - 14 days.
(b) 10 ml of each water sample was inoculated into 90 ml starch casein (SCM) broth and Tryptone-yeast extracts broth (TYE): (g/l) Tryptone 5, yeast extract 3, pH = 7.2) in 250 ml flask, then incubated for 24 h with shaking; (225 rpm, HT shaker. Germany), at 55 and 28°C, after that about 200 µl were transferred to starch casein (SCM) plates. These plates were incubated following the previous procedure of incubation.
(c) A combination between the previous two steps was done to ensure our results, in details: 100 ml of each water sample was filtered through a Millipore membrane; the membranes were transferred to 100 ml starch casein (SCM) broth in 250 ml flask, incubated at 55 and 28°C for 24 h with shaking at 225 rpm. Then aliquots (0.2 ml) of 10^{-2} to 10^{-5} ten-fold serial dilutions were spread over the surface of dried SCM agar plates.

The plates were incubated as described previously at 55 and 28°C for 7 - 14 days.

Streptomyces colonies were then picked up and transferred to yeast malt-extract agar (g/l) (yeast-extract): (3, malt-extract 3, peptone 5, glycerol 10 ml/l, agar 20, pH = 7.0 ± 0.2), starch casein agar plates, glycerol yeast-extract agar plates (g/l): yeast-extract 2, K_2HPO_4 1, glycerol 5 ml/l, agar 20, pH = 7.2 and oatmeal agar for further purification.

Maintenance media

After purification, *Streptomyces* isolates were maintained as suspend-sions of spores and mycelia fragments in 20% glycerol (v/v) at -20°C.

Morphological characterization

Morphological characterization of *Streptomyces* isolates were done according to the ISP recommendations (Shirling and Gottlieb, 1966). A pure culture of each isolate was picked up and transferred to grow on four different media: Yeast Malt-Extract (YME) agar (ISP2), Starch Casein (SCM) agar, Glycerol Yeast-Extract (GYE) agar and Oatmeal agar (ISP3) for 5 - 10 days at 55°C for themophilic *Streptomyces* and at 28°C for mesophilic *Streptomyces*. Then, colors of the aerial and substrate mycelium and those of the soluble pigments were examined.

Cultural, physiological and biochemical tests

Determination of the cultural, biochemical and physiological characteristics was carried out according to Williams et al. (1983), Brown et al. (1999) and Babcock (1979).

Growth temperature

Also the ability of the isolates to grow at different temperatures (28, 37, 45 and 55°C) was studied.

Carbon source

The ability of the strains to use different carbon sources was determined according to the ISP recommendation (Shirling and Gottlieb, 1966); seven sugars were used as a carbon sources: L-arabinose, Meso-inositol, D-sorbitol, D-mannitol, L-rhamnose, D-fructose and D- glucose, the results were determined after 14 days incubation at optimum temperatures.

Antimicrobial activity

Antimicrobial activity on agar media

Antimicrobial activity was tested by growing *Streptomyces* strains on agar plates until good and thick growth occurred. Then agar block from these plates were transferred to plates previously seeded with the indicator organisms (*Escherichia coli, Staphylococcus aureus* and *Candida albicans)*. The plates were incubated at appropriate temperature for each indicator. Activity was measured as inhibition zone in millimeters around the agar block (Abussaud and Saadoun, 1991).

Antimicrobial activity in broth media (antibiotics fermentation)

Spores from each 10 days-old culture of isolates grown on glycerol yeast-extract agar plates were used to inoculate 100 ml of glycerol yeast-extract broth into 250-ml Erlenmeyer flasks. These cultures were grown in a rotary shaker at 150 rpm, 28°C, for eight days. Glycerol Yeast-Extract medium was preliminarily tested and was found to be suitable for antibiotic production by our isolates.

During fermentation, 2 ml sample of each culture were collected in eppendorf tube every 2 days and tested for antibiotic activity against *E. coli, S. aureus* and C. *albicans*. Activity was tested by

Table 1. Primers used for detection of *Streptomyces* in PCR reactions.

Primer	Sequence (5′ 3′)	Primer target sequence	Amplicon length (bp)	Reference
strepB(F) /strepE(R)	ACAAGCCCTGGAAACGGGGT CACCAGGAATTCCGATCT	16S rDNA	520	Rintala et al., 2002
strepB(F) /strepF(R)	ACAAGCCCTGGAAACGGGGT ACGTGTGCAGCCCAAGACA	16S rDNA	1070	Rintala et al., 2002

Table 2. Physical properties of hot springs.

Location	Temperature (°C)	pH
Alshouneh (Sh)	54	6.5
Waggas (Wg)	47	8
Almansheyyah (Mn)	48	8
Deir Alla (DA)	36	7

preparing wells into agar plates previously seeded with the indicator organism and transferring 100 microliters from the centrifuged fermentation broth into these wells.

Molecular identification of antibiotics producer isolates

Extraction of genomic DNA from pure culture

Genomic DNA was isolated from the isolates using a bacterial genomic DNA isolation kit (Biobasic Inc. Canada). One separate colony from each bacterial isolate was inoculated into 10 ml nutrient broth and incubated overnight at 28°C. Then 1 ml was taken and centrifuged at 14000 rpm for 15 min at room temperature, the pellets were suspended in 200 µl cold TE (10 mM tris base, 1 mM EDTA, pH 8.0) buffer and 400 µl digestion solution and mixed well, then a 3 µl of Proteinase K were added and incubated at 55°C for 2 h.

After incubation, 260 µl of 100% ethanol were added to the solution, and then the whole mixture was applied into 2 ml EZ-10 column provided with the Kit and centrifuged at 8,000 rpm for 1 min. The pellets were resuspended again with 500 µl of wash solution and centrifuged at 8,000 rpm for 1 min, this step was repeated again.

After that, the column was placed in a clean microfuge tube and a 50 µl of elution buffer were added to the center of the column, incubated at 37°C for 2 min and finally centrifuged at 10,000 rpm for 1 min to elute the DNA.

Polymerase chain reaction (PCR)

PCR amplification of 16S rDNA was carried out in 50 µl volumes containing: 25 µl of Econo Taq PLUS GREEN 2X Master Mix, 0.25 µl (100 pmol) of each primer, 2 µl (10 ng) of DNA template and 22.5 µl DNAse free water, each primer pair has its program that will be mentioned later.

For each PCR reaction a negative PCR reaction tube was performed where no DNA template was added (not shown in figures) and all PCR reactions were performed in a Perkin Elmer DNA thermal cycler (Perkin Elmer 480).

Identification of bacterial isolates using StrepB/StrepE primer pair specific to genus Streptomyces 16S rDNA gene sequences

The primer pairs StrepB/StrepE (sequences listed in Table 1) amplified 520 bp fragments, nucleotides 139 - 657. The PCR was programmed as follows: after the initial denaturation for 5 min at 98°C, 30 cycles of denaturation (1 min at 95°C), primer annealing 40 s at 54°C and primer extension (2 min at 72°C) were performed. A final extension at 72°C for 10 min had followed.

Identification of bacteria isolates using StrepB/StrepF primer pair specific to genus Streptomyces 16S rDNA gene sequences

The primer pairs StrepB/StrepF and StrepB/StrepE amplified 1070 and 520 bp fragments, nucleotides 139 -1212. The PCR were programmed as follows: after the initial denaturation for 5 min at 98°C, 30 cycles of denaturation (1 min at 95°C), primer annealing 40 s at 58°C and primer extension (2 min at 72°C) were performed. A final extension at 72°C for 10 min followed.

Gel electrophoresis and photography

The PCR amplified products were separated on 1% w/v ultra-pure agarose powder in 1X TBE buffer (pH 8.3) at 100 V for 60 - 70 min using mini-gel set (Bio Rad,). Gels were stained with ethidium bromide (0.5 µg/ml) and analyzed using BioDocAnalyze (Biometra, Germany). A 250 base pair (bp) molecular weight marker was included on every gel.

RESULTS

Physical properties of hot springs

Physical properties such as temperature and acidity degree (pH) for each sampling location (water sample) are indicated in Table 2.

Isolation of *Streptomyces*

Thirty different bacterial isolates were isolated during this study. Six isolates were isolated from Deir Alla, only one from water (DA1-DA6); 8 isolates were isolated from Almansheyyah (Mn1-Mn8); 9 isolates were isolated from Alshouneh, only one from water (Sh1-Sh9) (two of these isolates were thermophilic they grow at_55°C) and 7 isolates were isolated from Waggas (Wg1 - Wg7); also two of these isolates were thermophilic; (Wg6 and Wg7).

Morphological characterization

The aerial mycelium color of the isolates ranged from red,

Table 3. Physiological and biochemical characteristics of *Streptomyces* isolate (number of positive/total in each site).

Location	Starch hydrolysis	Urea hydrolysis	Casein hydrolysis	L-tyrosine hydrolysis	Gelatin lequification	Nitrate reduction	Melanin formation
DA	4/6	6/6	6/6	6/6	1/6	4/6	1/6
Mn	7/8	6/8	7/8	7/8	3/8	6/8	3/8
Sh	9/9	7/9	6/9	7/9	4/9	6/0	3/9
Wg	5/7	5/7	2/7	6/7	2/7	6/7	1/7

Table 4. Ability of *Streptomyces* isolates to grow at different temperatures.

Location	28°C	37°C	45°C	55°C
DA	6/6	6/6	3/6	0
Mn	8/8	8/8	6/8	0
Sh	7/9	7/9	5/9	2/9
Wg	5/7	6/7	4/7	2/7

white, blue and green, grey to purple. While the substrate mycelium color shows narrower diversity. On the other hand, the isolates produced different pigments: yellow, pink and grey. These differences might reflect the diversity among the isolates.

On GYE medium, out of 30 isolates, 14 were white, 10 grey, 2 red, 2 green, 1 blue and 1 purple. On the other hand, 9 isolates were found to be producers of diffusible pigment, 7 of them produced brown color while the other produced a yellow pigment.

On Oatmeal medium, out of 30 isolates, 12 isolates were white, 9 grey, 2 pink, 1 brown, 1 purple and 1 red and 4 isolates failed to sporulate on this medium. 10 isolates produced diffusible pigment, 5 of them brown, 4 yellow and one isolates produced pink pigment.

On YME media, out of 30 isolates, 10 isolates were white, 7 grey, 1 green, 1 brown, 1 yellow and 10 of them were not able to sporulate. 12 isolates were able to produce diffusible pigment, 7 produced brown pigment and the rest produced yellow pigment.

On STC media, out of 30 isolates, 13 isolates were white, 10 grey, 3 pink, 2 purple, 1 green and 1 brown, only 3 isolates were able to produce diffusible pigment, 2 produced brown color and 1 produced red color.

Physiological and biochemical characteristics of *Streptomyces* isolates

As indicated in Table 3, 25 (83%), 24 (80%), 21 (70%), 26 (87%), 10 (33%), 22 (73%) and 8 strains (27%) showed the ability to degrade starch, hydrolyze urea, degrade casein, hydrolyze tyrosine, liquefy gelatin, reduce nitrate and produce melanin, respectively.

Also the ability of the isolates to grow at different temperatures (27, 37, 45 and 55°C) was examined. The results depicted in Table 4 showed that, out of 30 isolates only 4 (Thermophilic) isolates were able to grow at 55°C, 18 isolates included thermophilic isolates were able to grow at 45°C, 27 at 37°C and 26 at 27°C.

Carbon source utilization

Table 5 represents the ability of *Streptomyces* to utilize different carbon sources (7 different sugars were used). Out of 30 isolates, 26 (87%) isolates were able to utilize L- arabinose, 25 (83.%) isolates were able to utilize Meso-inositol, 8 (27%) isolates were able to utilize D-sorbitol, 18 (60%) isolates were able to utilize D-mannitol, 28 (93%) isolates were able to utilize L-rhamnose and all isolates exhibited the ability to utilize D-fructose and D-glucose.

Antimicrobial activity on agar media

Antimicrobial activity was tested against *E. coli, S. aureus* and *C. albicans.* Out of 30 isolates 8 were antibiotics producers. These isolates were assigned: Mn1, Mn3, Mn8, Sh1, Sh3, Sh6, Wg4 and Wg5. Six of them showed antibacterial activity against G-ve (represented by *E. coli*), all of them were active against G+ve bacteria (represented by *S. aureus*) and 7 of them were able to produce antifungal activity against *C. albicans*.

Table 6 represents the antimicrobial activity during a period of 8 incubation days. After 2 days, only 1 (12.5%) isolate was active against all tested microorganisms. After 4 days, 3 (37.5%) of the isolates were active against all tested microorganisms. After 6 days, 5 (62.5%) isolates showed activity, 40% of them were active against *E. coli*, all of them exhibited activity against *S. aureus* and 80% of them exhibited activity against *C. albicans.* After 8 days, 4 (50%) of the isolates exhibited the activity, 75% of them were active against *E. coli* and all of the rest were active against *S. aureus* and *C. albicans*.

Molecular identification of the bacterial isolates

Genomic DNA was isolated from the 8 antibiotic producers (Mn1, Mn3, Mn8, Sh1, Sh3, Sh6, Wg4 and Wg5) and from the positive control (*Streptomyces halstedii* ATCC

Table 5. Utilization of carbon sources (positive/total).

Location	Arabinose	Inositol	Sorbitol	Mannitol	Rhamnose	Fructose	Glucose
DA	5/6	5/6	3/6	4/6	5/6	6/6	6/6
Mn	8/8	5/8	1/8	5/8	8/8	8/8	8/8
Sh	7/9	8/9	2/9	6/9	9/9	9/9	9/9
Wg	6/7	7/7	2/7	3/7	6/7	6/7	7/7

Table 6. Antimicrobial activity *of *Streptomyces* isolates (on agar media).

Location	*E. coli*	*S. aureus*	*C. albicans*
DA	0	0	0
Mn	3/8	3/8	2/8
Sh	3/9	3/9	3/9
Wg	0	2/7	2/7

*Number of active isolates/total number.

10897). A large amount and good quality of genomic DNA was obtained from each bacterial isolates.

Two primer pairs were used in the PCR reactions to identify the bacterial isolates as a *Streptomyces* isolates (StrepB/StrepE and StrepB/StrepF). Using 16S rDNA StrepB/StrepE primer pair, all antibiotic producers showed positive results with 520 bp PCR amplification products (Figure 2)

Using 16S rDNA StrepB/StrepF primer pair all antibiotic producers and the control *S. halstedii* showed positive results with 1070 bp PCR amplification product (Figure 3).

DISCUSSION

Streptomyces represent an important source of biologically active compounds. They are used extensively in industry as producers of antibiotics, enzymes, enzyme inhibitors and antitumour agents. However, it is important to continue the screening for novel bioactive compounds as the number of microorganisms resistant to the existing antibiotics is growing every year. However, it is becoming increasingly difficult to discover new commercially useful secondary metabolites from common streptomycetes, thereby emphasizing the need to isolate, characterize and screen novel members of the genus *Streptomyces*. *Streptomyces* from under explored habitats are proving to be a rich source of new bioactive compounds, including antibiotics (Berdy, 2005; Okoro et al., 2009). Therefore, we decided to isolate *Streptomyces* strains from Jordanian hot springs and study their capability to produce antibiotic activity.

A total of 30 different bacterial isolates were recovered during this study from non-cultivated hot spring areas in Jordan. All bacterial isolates were typically *Streptomyces*; they grew on a range of agar media showing morphology typical of *Streptomyces*.

The majority of the isolates showed good growth on SCM agar medium. This medium seems to be specific and sensitive for *Streptomyces*, since it contains starch that most *Streptomyces* use as a carbon source and the basic minerals that are needed for good growth. In addition, its transparency facilitates colony observation. Earlier studies have shown the importance of the constituents of the screening media under which the producing microorganisms were cultivated (Williams et al., 1989; Rintala, 2001).

The number of *Streptomyces* isolates that were isolated from soil samples (28 isolates) was highly greater than that isolated from water samples (2 isolates), this may by due to the presence of organic matter that make *Streptomyces* abundant in soil, it is the dominant genus in the soil that gives it it's odor (Kutzner, 1986 ; Rintala, 2001).

Streptomyces have been isolated from fresh water as well as marine environments (Delabre et al., 1998), although, it has been a subject of debate. Many scientists considered *Streptomyces* to be part of the marine ecosystem, while many others failed to isolate *Streptomyces* from water samples and did not consider *Streptomyces* to be indigenous to the marine environments (Okazaki, 2006) and the debate point was whether they are indigenous, or have been washed off from the surrounding soils, so such studies could explain the presence of few *Streptomyces* strains in hot-springs water samples, since its water coming from under ground, surrounded by rocks not soils (Goodfellow and Simpson, 1987; Rintala, 2001).

All of these isolates fitted the genus description as reported by several studies (Shirling and Gottlieb, 1966; Kutzner, 1986; Williams et al., 1989). The color of the substrate mycelium and aerial spore mass was varied which reflect the diversity of *Streptomyces* isolates.

Msameh (1992) in his study on distribution and antibiotic activity of *Streptomyces* flora in Jordan reported that the white and grey color series showed the highest percentage of occurrence (43.6 and 28.3%, respectively). In the present study, 50% of the isolated *Streptomyces* isolates were from Alshoneh and Deir Alla (30 and 20%, respectively). White and grey color series had also the percentage (46 and 33.3%, respectively).

The comparison of the physiological and biochemical characteristics of the presented isolates with the actinomycetes as described in Bergey's Manual of Determinative

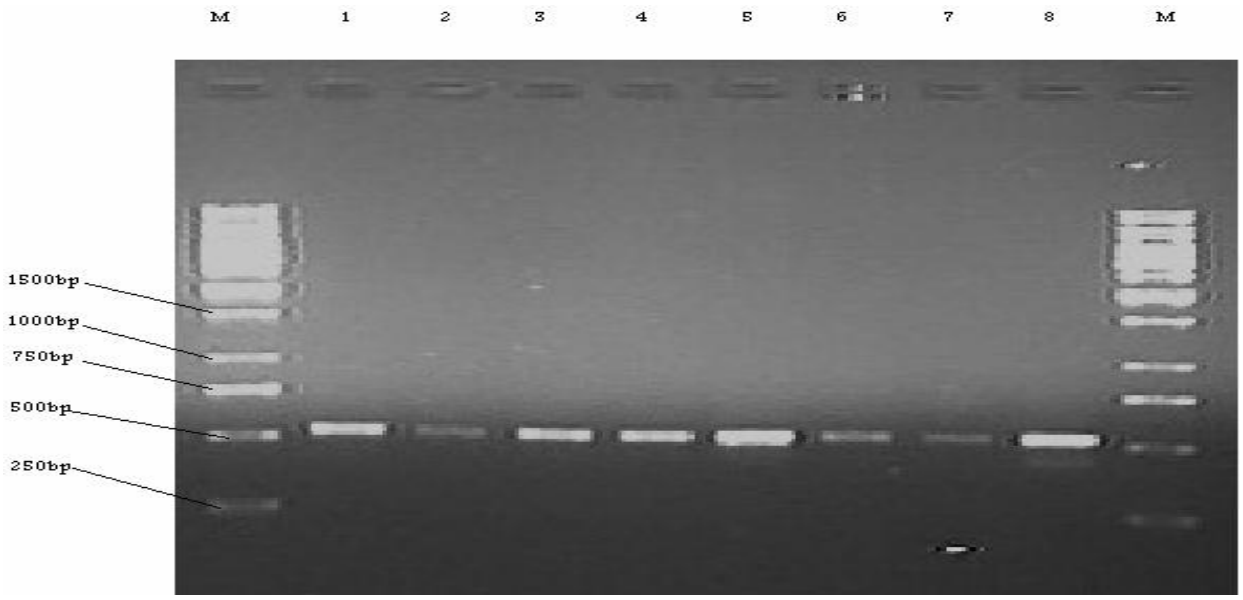

Figure 2. Agarose gel electrophoresis of PCR amplification products of genomic DNA isolated from *Streptomyces* pure culture using strepB(F)/strepE(R) of 16S rDNA gene. Lane M, 1 kb DNA ladder; lane 1, Mn8; lane 2, Sh3; lane 3, Mn1; lane 4, Sh6; lane 5, Wg5; lane 6, Wg4; lane 7, Sh1; lane 8, Mn3.

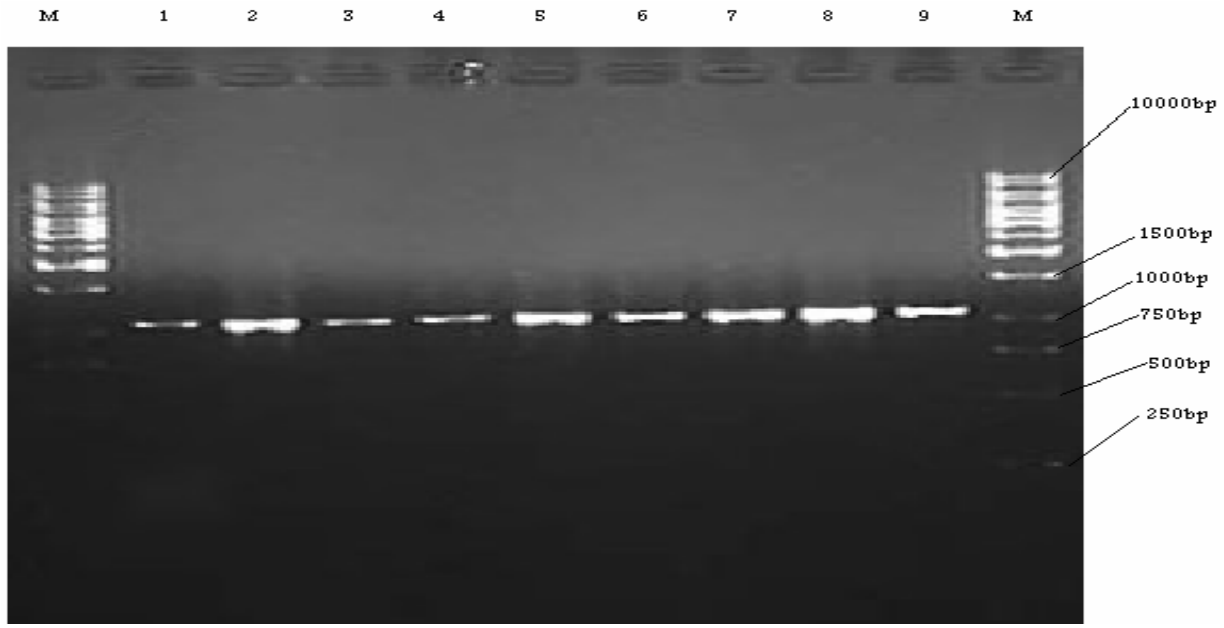

Figure 3. Agarose gel electrophoresis of PCR amplification products of genomic DNA isolated from *Streptomyces* pure culture using strepB(F)/strepF(R) of 16S rDNA gene. Lane M, 1 kb DNA ladder; lane 1, Mn8; lane 2, Sh3; lane 3, Mn1; lane 4, Sh6; lane 5, Wg5; lane 6, Wg4; lane 7, Sh1; lane 8, Mn3) and lane 9, *S. halstedii*.

Bacteriology determined that these isolates belongs to the genus *Streptomyces*.

Antibacterial activity and antifungal activity was observed in 8 (27%) and 7 isolates (23%), respectively. In former studies, it was shown that the isolation rate of *Streptomyces* with antimicrobial activity was higher than 40% (Lemriss et al., 2003) and in others less than 10% (Jiang and Xu 1996). This variation may be due to many factors example, soil type, climate, strain type and isolation methods. We found the best percentage (37.5%) of antibacterial and antifungal activity among Almansheya strains, followed by those from Alshouneh and Waggas

Table 7. Antimicrobial activity of antibiotics producers (in broth media).

Strain	2 days against			4 days Against			6 days against			7 days against		
	E. coli	*S. a*	*C. alb*	*E. coli*	*S. a*	*C. alb*	*E. coli*	*S. a*	*C. alb*	*E. coli*	*S. a*	*C. alb*
Mn1	-	-	-	-	-	-	-	-	-	-	-	-
Mn3	+	+	+	+	+	+	+	+	+	+	+	+
Mn8	-	-	-	-	-	-	-	+	-	-	-	-
Sh1	-	-	-	-	-	-	ND	+	+	+	+	+
Sh3	-	-	-	+	+	+	+	+	+	-	-	ND
Sh6	-	-	-	+	+	+	ND	+	+	+	+	+
Wg4	-	-	-	-	-	-	-	-	-	-	+	+
Wg5	-	-	-	-	-	-	-	-	-	-	-	-

E. coli: *Escherichia coli, S. a: Staphylococcus aureus*, *C. alb*: *Candida albicans.* + = Active, - = not active, ND = not determined.

areas 33.3 and 28.5%, respectively. No antibiotics producers were isolated from Deir Alla area.

The highest percentage of activity was recorded against Gram-positive bacteria followed by Yeast and Gram-negative bacteria. Some isolates did not show activity in liquid media. Out of the 8 active isolates on agar medium, only 5 (62.5%) isolates were found to exhibit antibacterial activity in liquid media (Table 7).

During the screening of the secondary metabolite, *Streptomyces* isolates were often encountered which show antimicrobial activity on agar but not in liquid culture (Thakur et al., 2007).

Molecular identification

Molecular identification was performed using polymerase chain reaction (PCR) which is currently used as a sensitive and specific detection method for micro-organisms (Rintala et al., 2002). The 16S rDNA gene was chosen as the target gene for the PCR primers in the PCR assay, aiming at the detection of the 8 antibiotic producers of the *Streptomyces* isolates. In this study two sets of primers strepB/strepE and strepB/strepF specific to 16S rDNA gene fragment were used to identify bacterial isolates; positive results were recorded for all bacterial isolates with amplification and corresponding to 520 and 1070 bp and thus, confirm that all antibiotics produce bacterial isolates belong to *Streptomyces* species.

In order to detect the presence of *Streptomyces* isolates in the water samples, the same two sets of primers were used to amplify the 16S rDNA gene collected from water samples; all samples exhibited negative results which indicated the inexistence of *Streptomyces* in these water samples.

In comparison between cultural and molecular methods for identification of *Streptomyces* isolates, we could say that molecular methods are more sensitive, rapid and not laborious in opposite to cultural methods that are laborious and time consuming.

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