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Comparative distribution of soil *Streptomyces* flora in different Jordanian habitats and their enzymatic and antibiotic activities.

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ABSTRACT

Seventeen soil samples were collected from different habitats from northern, eastern and southern Jordan, then characterized chemically and physically, and biologically for their *Streptomyces* content. pH and salinity values of the soil samples ranged between an average of 7.57 and 8.28, 9223 and 464.8 µs/cm, respectively. Organic matter content and moisture percentage was found with an average ranged between 2.9% and 9.25 %, 0.64% and 2.58 %, respectively. The average *Streptomyces* count was 1.98×10^5 cfu/g, 1.53×10^5 cfu/g, and 1.45×10^5 cfu/g in soil samples from northern, southern and eastern regions, respectively. A total of 148 different soil *Streptomyces* isolates were recovered with 42 %, 33 % and 25 % of them were from the northern, southern and eastern regions, respectively. Enzyme activity of these isolates showed that most of them (36.5 %) were able to produce protease and 35.8% were able to produce amylase. However, 27.7 % of the isolates were unable to produce any of the enzymes which were mostly recovered from the northern region. It was found that only 6% of the total isolates were active against one or more of the tested multi-resistant Gram negative pathogens with more active isolates being recovered from the south region.

Keywords: Antibiotic, Enzymatic, Soil, Streptomycetes

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INTRODUCTION

Streptomycetes are widely distributed in soils, especially in dry, slightly acidic and rich in organic matter. These microorganisms (in propagule numbers) are frequently exceed the combined counts of all other bacteria and counts of over 1 million per gram are commonly obtained [38]. However, these counts do not state any thing about the number and frequency of species. Ishizawa & coworkers [1, 2] reported that chromogenic species of streptomycetes occur preferentially in volcanic soils, while non-chromogenic species in non-volcanic soils. Their study confirmed previous observations by Craveri *et. al*. [3] that volcanic soils are rich in physiologically active Streptomycetes. Vegetation and season was found to be effective on the numerical occurrence of some *Streptomyces* species in soil [4, 5, 6].

Having especial environmental characteristics, and being rich in actinomycete population, the microbiology of the Jordanian soils has to be further explored for new active strains of actinomycetes. Pioneering attempt was made to isolate and study a preliminary screening of actinomycetes in Jordan. Such line of research was aimed on the preparation of biofertilizers, biofungicides, and isolation of the active metabolites. Therefore, intensive campaign of collection of soil samples was done [7] and the recovered streptomycetes were characterized [7, 8, 9].

The purpose of this study was to investigate the numerical occurrence of *Streptomyces* flora in different terrestrial habitats in Jordan and correlate their content and metabolic activities with chemical and physical properties of soil.

MATERIALS AND METHODS

Location, sampling and sample processing

A total of 17 soil samples were collected from northern, eastern and southern Jordan (Table 1). A single sample was collected from each location during September-December, 2004. Samples were collected by scraping off an approximately 3cm of the soil surface with a spatula and taking an approximately 500 g sample at 10 cm below the surface. Samples were placed in plastic bags, transferred to the laboratory and stored at 4 °C.

Sample processing

Each soil sample was crushed, thoroughly mixed and sieved through a 2 mm pore size mesh (Retsch, Haan, Germany) to get rid of large debris and the sieved soil was used for detection of the pH, soil moisture, organic matter, and salinity in addition for the isolation of *Streptomyces*.

Soil pH, moisture and salinity measurements

Soil suspension was made by diluting the soil to a known volume of distilled water (1:2 w/v). The soil–water suspensions were shaken for 24 hours in an orbital shaker (TEQ, Portugal). The suspensions were centrifuged, and then supernatant was filtered through Whatman filter papers 90 mm. The electrode of pH meter (Hanna, Italy) was immersed into the filtrated sample solution for pH measurement. For moisture measurements, 10 g of each soil sample was weighted in crucible dish then placed in an oven (WTB binder, Germany) for 24 hours at 110° C; then weighted again and the difference in weight between the initial and final readings represented the loss of water content [10]. Organic matter contents were determined by placing ten grams of each soil sample in crucible dish then burned in an oven (Nuve, Turkey) for 4 hours at 600° C, and then re-weighed again to calculate the difference.

Isolation of Streptomyces isolates

One g of each of the enriched soil sample was suspended in 99 ml sterile distilled water then incubated in orbital shaker incubator (TEQ, Portugal) at 28 °C with shaking at 140 rpm for 30 min. Mixtures were allowed to settle then serially diluted up to 10^{-6}. From each suitable dilution, 0.1 ml was taken and spread evenly with sterile L-shape glass rod over the surface of starch casein nitrate agar (SCNA) [11] to determine total *Streptomyces* count. SCNA plates were incubated under aerobic conditions in an ordinary

incubator (WTB Binder, Germany) at 28 °C for 10 days. Plated dilutions that gave 20-200 colonies on SCNA were chosen for further isolation. Repeated streaking on SCNA plates purified bacterial colonies that showed *Streptomyces*-like appearance [12].

Screening for amylase-producing Streptomyces

Pure isolates of *Streptomyces* were cultured on starch casein nitrate agar (SCNA) and incubated at 28° C for 4 days. Plates were then flooded with iodine solution and left for 30 minutes, then washed with distilled water. Bacterial colonies producing amylase will show clear zone against black color of stained starch [13].

Screening for protease-producing Streptomyces

Pure isolates of *Streptomyces* were cultured on a skimmed milk casein agar plates (Himedia, India). The appearance of clearing zones around colonies indicates the presence of proteolytic activity in these isolates by hydrolysis of casein [14].

Production of antimicrobial agents

This was tested by Bauer-Kirby method [15] against pathogenic *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Proteus* sp.. These pathogens were obtained and identified by the Clinical Laboratory of King Abdullah University Hospital, Irbid-Jordan. Commercial antibiotics were tested against all these isolates such as Ceftriaton (CRO), Cefuroxine (CXM), Tetracyclin (T), Augmetin (AUG) Linezoid (LZD), Rifampicin (RP), Gentamicin (GM), Streptomycin (S), Levofloxacin (LEV), Vancomycin (VA), Teicoplanin (TEC), Nitrofurantion (NI), and Erythromycin (E). Isolates were grown on oatmeal agar for 14 days; discs (9 mm in diameter) were cut and placed on nutrient agar seeded with the test organisms and incubated at 28 °C. Inhibition zones were observed after 24 hrs.

Characterization of Streptomyces isolates

Streptomyces isolates with positive enzymatic and antibiotic activities were characterized morphologically and physiologically following criteria approved by the International *Streptomyces* project (ISP) [16]. General morphology was determined on oatmeal agar plates, incubated in the dark at 27 ˚C for 21 days, by direct light microscopy examination of the surface of crosshatched cultures. Colors were determined according to the scale adopted by Prauser [17] and isolates were grouped into separate color series according to the system proposed by Nonomura [18]. Melanin reactions were detected by growing the isolates on at least one of the ISP media (No. 6 and No. 7) [16]. The spore chain pattern was determined by observing the 21-day old crosshatched cultures under a compound light microscope (Nikon, Japan) at 100X. The isolates were divided into 3 sections according (not clear) to Nonomura [18] to include: *Rectiflexibiles* (RF), *Retinaculiaperti* (RA) and *Spirales* (S). Carbon utilization test was performed according to the ISP [17].

RESULTS

Characterization of soil samples

Twenty seven soil samples were collected from different locations representing six regions in Jordan. Soil characters such as organic matter, soil moisture, pH and salinity were tested (Table 1). The highest organic matter and moisture percentage was in the soil samples collected from northern regions with an average of 9.25 % and 2.58 %, respectively. However, soil samples from the eastern and southern regions showed the lowest percentage with an average of 5.05/2.9 % and 0.7/0.64 %, respectively (Table 1). Salinity of soils from the eastern regions had the highest values with an average of 9223 µs/cm. However, the lowest salinity values were recorded in soil samples from northern and southern regions with an average of 464.8 and 970 µs/cm respectively (Table 1). pH values for all samples are slightly alkaline with average ranging between 7.57 and 8.28 (Table 1). The highest salinity values were recorded in dry regions; Mafraq, Um el Quttein, and Al Jafr, with 26300, 9420, and 4220 µs/cm, respectively. The highest organic matter content was recorded from soil samples collected from forested regions such as Ajlun and Ba'aun with 12.1 % and 17 %, respectively.

Streptomyces content

Results showed that the average *Streptomyces* count was maximally observed in soil samples taken from the north with 1.98 × 10^5 CFU/gm. However, lowest count was observed in soils from the east and the south with an average of 1.45 and 1.53 × 10^5 CFU/gm, respectively (Table 1).

Isolation and characterization of Streptomyces isolate

A total of 148 *Streptomyces* isolates were recovered from the different 17 soil samples (Table 1). All isolates were selected based on their colony morphology that resembles *Streptomyces* species. The colony morphology of *Streptomyces* isolates cultured on starch casein nitrate agar plates (SCNA) after 10 days of incubation at 27° C revealed small colonies (1-10 mm diameter), discrete and leathery, initially relatively with smooth surface but later developed a weft of aerial mycelium that appeared granular, powdery and velvety.
The overall distribution of the different color series of *Streptomyces* isolates showed high prevalence for the white (77 isolates) and grey (35 isolates) colors series. However, the lowest occurrence was for yellow (9 isolates) and green (6 isolates) colors series (Table 2). *Streptomyces* isolates were characterized morphologically and physiologically. Table 2 shows pigmentation production by all *Streptomyces* isolates with 40%, 95% and 24% of all isolates were able to produce soluble, reverse side, and melanin pigments, respectively, with more pigmentation for the isolates from the north (77.7%) than isolates from the east (35%) and south (46%). Data indicated that most isolates bear spiral sporophore morphology followed by flexibilis (25%), rectinaculum-apertum (20%) then rectus (18%).

Occurrence of Streptomyces isolates in soils of different regions in Jordan

The occurrence of *Streptomyces* color series through different soil samples are shown in Table 3. Data indicated that the white color series (52 %) was the most abundant through different regions with 25 % of the white isolates being recovered from the north region. Green series was the least abundant (4 %) through the different regions. All color series were found in north region, while the other regions lacked one of the color.

The sporophore morphology for the different *Streptomyces* isolates is summarized in Table 3. Simple spiral is the most abundant (41 %) sporophore arrangement among the different *Streptomyces* isolates with 17.5 %, 7 % and 16 % of the isolates being recovered from north, east and south regions, respectively. Rectus and retinculum-apertum sporophore have almost the same percentage among all regions with 20% and 18%, respectively. However, flexibilis sporophore arrangement constituted 25% of all arrangements.
series

Occurrence of Streptomyces isolates in soils of different regions and their enzymatic activity

Table 3 shows that most of the active enzyme producers were isolated from soils samples collected from the north (49%) followed by the eastern (42%) and the southern (27%) regions. Data showed that most of the isolates (36.5 %) were able to produce amylase enzyme, and (35.8 %) of the isolates were able to produce protease enzyme. In addition it was found that 46% of the isolates were able to produce at least one enzyme, 27.3 % were able to produce any of these enzymes (Fig. 2). The ability of these isolates to produce these enzymes ranged from weak to strong depending on the width of the diameter of the clear zone formed around the isolates in the screening process. As indicated in Table 4, the different active isolates were distributed into 5 groups according to the diameter of the clear zone on different agar plates. The isolate Um2 was able to produce both enzymes with a clear zone of 31-40 mm on SCNA, 21-30 on skimmed milk agar and peptone yeast extract agar plates. The active isolates were further characterized (Table 4).

Occurrence of *Streptomyces* isolates in soils of different regions and their antibiotic activity against Gram negative multi-resistant pathogens

Several Gram negative multi-resistant bacteria namely; *Escherichia coli*, *Klebsiella pneumonia*, *Proteus* sp. and *Pseudomonas aeruginosa* obtained from the laboratory of King Abdullah University Hospital were re-tested for their susceptibility to different antibiotics (Table 5). Some of these pathogens such as *P. aeruginosa* were resistant to 10 different antibiotics. When the recovered *Streptomyces* isolates were tested

for their activity on these different Gram negative multi-resistant pathogens, data indicated that only 9 isolates have an activity against one or more pathogens (Table 5). Data indicated that none of the active isolates were recovered from soils samples collected from the eastern region. These 9 active isolates were further characterized (Table 5).

Results indicated that active *Streptomyces* isolates inhibited the growth of different multi-resistant pathogens with an inhibition zone diameter ranged from 7 to 23 mm. *P. aeruginosa* was not susceptible to all of the tested active *Streptomyces* isolates except for isolate Pe3. This isolate was able to inhibit all the multi-resistant pathogens with an inhibition zone diameter of 11 mm. When Pe3 *Streptomyces* isolate was compared to the susceptibility of the different pathogens to antibiotics (Table 5), data indicated that the active compound produced by this active isolate is similar in activity to the antibiotic streptomycin. However, this compound may be different from Cefuroxine (CXM), Levoflaxin (Lev), Cefltriaton (CRO), Augmetin (AUG) and Nitofurantion (NI) antibiotics.

DISCUSSION

Streptomyces species are heterotrophic feeders and they can utilize both simple and complex molecules as nutrient. About three-fourth of the *Streptomyces* species may produce antibiotics. In addition to antibiotics, *Streptomyces* species liberate extracellular enzymes such as; proteases and amylases [19]. Nutrient availability is a major factor governing the distribution and activity of soil *Streptomyces*; temperature, pH, moisture content, organic matter content, and soil type also extent an influence, as do seasons and climate [20, 21].

Soil salinity is one of the characters that have been studied. The arid soil samples from Mafraq, Um el-Quttein and Al Jafr had the highest salinity content because of there climatic condition which include high temperature, low rainfall and highest evaporation rate. Microorganisms found in these regions are considered halotolerant; they grow in salt concentrations above 0.2 mol 1^{-1} NaCl. This finding is in agreement with Basilio *et al* [22] who recovered halotolerant and halophilic *Streptomyces* isolates in eight of 17 soil samples collected from different locations in Philippines, Spain, Switzerland, Costa Rica, Sri Lanka and Mexico**.**

pH of a soil is an indicator of the activity of the hydrogen ion in the soil. Due to pH differences between different soils in different regions, most *Streptomyces* behave as neutrophiles in culture, grow between pH 5.0 and 9.0 with an optimum close to neutrality. Only a few of the type strains studied by Williams *et al* [23] were able to grow at pH 4.3, but acidophilic strains have been isolated from acidic soils and other materials. Acidophilic isolates grow in the range from about pH 3.5 to 6.5 with optimum rates at pH 4.5 - 5.5. Alkanophilic strains, which grew between pH 8 and 11.5 with optimum at pH 9 - 9.5. *Streptomyces* show their optimum growth in neutral and slightly alkaline conditions with range of 6.5 – 8 [16]. Almost all of our soil samples are slightly alkaline with pH range 7.54 – 8.28.

The water content of soil varies over time and with precipitation events. Adequate water content is required to sustain microbial activity in soil. The variation in water content in soil samples is due to climatic variation which includes rainfall rate, temperature and evaporation rate. In dry soil, *Streptomyces* count decrease markedly at low moisture content, but their proportion to other bacteria may be higher because their spores are more resistant to desiccation than are the vegetative cells of bacteria. Optimum count from neutral soil and optimum growth of *Streptomyces* found in high moisture content. In his study in Oklahoma, Stan Lake [24] reported that the increase in soil moisture would increase microbial activity and microbial counts. Our study also shows that the soil samples that have the highest moisture content have the highest *Streptomyces* count, when compared to other samples from the same regions, for example soil samples from Ajlune had the highest moisture content (10.3 %), with highest *Streptomyces* count (4.7×10^5 /gm), while the count was the lowest in Raymon (5×10^4 /gm soil) when compared to other samples from the same region. The average *Streptomyces* count ranged from 2.5×10^4 to 4.7×10^5 CFU/g, which is lower than the count that was reported in Northern Jordan [25] and Jordan Valley [26]. Barakat *et al* [27] found that the *Streptomyces* count ranged from 0.5 to 8.78 % in Moroccan rhizosphers soil, and Saadoun *et al.* [28] reported that the *Streptomyces* accounted for about 1 – 20 % of total bacterial count in soil samples collected from northern Jordan.

Most soils contain a significant proportion of clay and humic colloids. Such colloidal material can markedly affect the microbial activity at the micorenvironmental level. In general actinomycetes increased in number in accordance with the increase in soil humus contents. Zaitlin *et al* [19] found that the soil organic matter content, pH, moisture failed to influence actinomycetes (streptomycetes) communities in western Australian soils as the case of this study. Streptomycetes have the potential to degrade naturally occurring polymers such as chitin, keratin, fungal cell wall material, and humic acid and are usually considered to be most active in the more advanced stages of decomposition of plant and other materials [21].

All recovered isolates were categorized culturally and morphologically with distribution into six color series (Table 2). Members of the white series were found to represent 52 % of the total number of isolates; however, the lowest occurrence was noted for the green series (4 %). This is in contrast with previous studies on the distribution of streptomycetes in Jordan [7, 29], Morracco [27] and Tanzania [10] which reported the dominance of the grey color series. A study on *Streptomyces* from Iraqi soils also reported the dominance of the grey (51%) and white (23%) series over other colors [30].

Several streptomycetes isolates produced soluble pigments of different colors. Similar pigments were also reported from Etruscan tombs [31], and from Necropolis of Carmona [32]. Pigments produced by the isolates of this study are higher than what was reported by Saadoun and his colleagues [7, 12, 25, 28] and by Barakat *et al* [27]. The variation in the percentage of distinctive color is due to differences in soil habitat and vegetation which may be responsible for species composition in different habitats, and cause differences in morphology and characteristics.

The morphology of spore indicate that 41% of the isolates showed spirales sporophore, 25% showed flexuous, rectus and retiniaculum-apertum with 18% and 20%, respectively. The results of this study are in accordance with other studies [7, 25, 28].

All of the 148 *Streptomyces* isolates were screened for protease and amylase enzyme production (Fig 2). The results showed that the amylase enzyme had the highest percentage 36.5 %, then protease (35.8 %). Amylases are well known to be the responsible enzyme group in hydrolyzation starch to dextrin and sugars [33]. 36.5% of the isolates were able to produce amylase enzyme. Our results are similar to those obtained by Franqui-Espiet [34] who screened two mangrove swamp samples with a high number of organisms and an ability to degrade starch compared with the total number of cultured cells. Protease represents one of the largest groups of industrial enzymes and accounts for about 60 % of the total world -wide enzyme. Of the 148 *Streptomyces* isolates, 35.8 % were protease producers which mean that these isolates have great ability to degrade keratin, hair, leather and other organic matter that may be present in the soil [21]. Similar were obtained by Gesheva *et al*. [35] that most *Streptomyces* isolates were protease producers. Data showed that the isolate UM2 was able to produce protease and amylase with a clear zone of 31-40 mm. Further studies are recommended on this isolate to determine its ability for production of other enzymes such as cellulose, pectinase and chitinase and study its activity to degrade keratin, feathers and wood.

Our results showed that only 6% of the recovered isolates have antimicrobial activity against at least one of the tested pathogens. This is by far much lower than results obtained in previous studies from different soils in Jordan [7, 12]. In the case presented here, the use of multi-resistant pathogens may explain these differences. Panady *et al* [36] in their study on actinomycetes isolated from the Khumu region of Nepal reported that only two isolates with activity against Gram negative bacteria. Data indicated that no active isolates were recovered from the eastern region. Climate, vegetation, and soil type probably play an important role in this difference [19, 25, 28]. Some of the isolates exhibited a wide zone of inhibition against antibiotic-resistant pathogens which signifies their strong activity. Among these isolates, Pe3 was able to inhibit the growth of all tested bacterial pathogens. The fact that this active isolate was recovered from Petra soil sample may be explained that this isolate may be thermotolerant and their production of secondary metabolites may serve a survival function in the soil with elevated temperatures. The low nutrient habitat of this location may explain the observation that the deficiency of growth-limiting component(s) triggers secondary metabolite biosynthesis [37] along with the competitive function of antibiotics [38]. In addition to low nutrient levels in Jordanian desert, the dry and harsh environmental conditions could trigger or favors streptomycetes to synthesize unusual metabolites. The unusual antibiotic profile of this active strain underlined its potential as a source of novel antibiotics. Therefore, further studies are encouraged to see if this active *Streptomyces* strain can produce novel compounds other than streptomycin which is inhibits all tested Gram negative pathogens

and identify these compounds. Furthermore, it is conceivable that through changing their growth conditions, the activity of this isolate may increase several fold.

Table 1. Soil samples collected from different regions of Jordan and their properties and their *Streptomyces* content.

Location	Abbreviation	Soil Properties				*Streptomyces* Count CFU x 10^5/g soil	No. of *Streptomyces* Isolates
		Salinity µs/cm	pH	Moisture %	Organic Matter %		
North Region							
Dibbin	Di	672	7.9	1.9	9.7	1.1	1
Kufr Khall	Kf	227	8.16	0.58	4.7	1.95	13
Raymon	Ra	500	7.93	0.5	5.7	0.5	12
Ajlun	Aj	300	7.93	10.3	12.1	4.7	9
Ba'aun	Ba	560	8.18	0.88	17	2.4	22
Kufrunjeh	Ku	530	8.03	1.3	6.3	1.2	5
Average		464.6	8.02	2.58	9.25	1.98	**Total: 62**
East Region							
Mafraq	Mf	26300	7.54	0.73	6.3	1.4	13
En-Nabi	En	758	8.19	0.43	4.1	1.6	8
Hamamat el-e'lemat	Hm	413	8.17	1.4	4.4	1.9	13
Um el Quttein	Um	9420	8	0.94	5.4	0.9	3
Average		9223	7.98	0.70	5.05	1.45	**Total: 37**
South Region							
Aljafr	Ja	4220	7.57	0.51	4	4.5	21
Aqaba	Aq	754	7.93	0.18	0.41	0.7	3
Ras An Naqab	Ra	360	8.13	0.48	3.2	0.25	1
Petra 1	Pe1	279	8.15	0.11	1.1	1.9	9
Petra 2	Pe2	282	8.15	0.62	3.2	1.5	7
Hwalah	Hw	278	8.18	1.9	4.9	0.5	6
Shawbak Desert HW	Sh	339	8.28	0.72	3.5	1.35	2
Average		970	8.05	0.64	2.9	1.53	**Total: 49**
Total							**148**

Di: Dibbin; Kf: Kufr Kall; Ra: Raymon; Ba: Ba'aun; Ku: Kufrinjah; Ja: Aljafr; Aq: Aqaba; Rs: Ra's an Naqab; Pe: Petra; Hw; Hwalah; Mf: Mafraq; En: En-Nabi; Hm: Hamamat el-e'lemat; Um: Um el Quttein

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Table 2. Overall distribution of the different *Streptomyces* isolates in soil of different regions of Jordan.

Color series							
	Grey	White	Yellow	Blue	Green	Variable[a]	Total
Number of isolates	35 (23.5)	77 (52)	9 (6)	10 (7)	6 (4)	11 (7.4)	148
Pigmentation							
Soluble Pigment	15 (25.4)	27 (46)	3 (5)	7 (8.6)	2 (3.4)	5 (8.4)	59 (40)
Reverse Side Pigment	35 (25.5)	69 (50.4)	9 (6.6)	9 (6.6)	4 (2.9)	11 (8)	137 (93)
Melanin Pigment	5 (18.5)	14 (51.9)	2 (7.4)	5 (18.5)	1 (3.7)	0	27 (18)
Sporophore Morphology							
Spiral	15 (26)	29 (50)	5 (8.6)	4 (7)	2 (3.4)	3 (5)	58 (39.2)
Flexibilis	9 (23.6)	19 (50)	2 (5.2)	2 (5.2)	3 (8)	3 (8)	38 (26)
Rectus	4 (13.8)	18 (62)	1 (3.4)	2 (7)	0	4 (13.8)	29(19.6)
Retinaculum-Apartum	7 (30.4)	11 (47.8)	1 (4.3)	2 (8.9)	1 (4.3)	1 (4.3)	23 (15.5)

[a] Variable: Violet, Orange and Pink.

Numbers in parentheses represent % out of total.

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Table 3. Number of *Streptomyces* isolates recovered from north (N), east (E) and south (S) regions of Jordan and their different morphological properties, enzymatic and antibiotic activities.

Property		Color Series						Total
		Grey	White	Yellow	Blue	Green	Variable a	
Number of Isolates	N	9 (6)	37(25)	5 (3)	4 (3)	3 (2)	4 (3)	62 (42)
Number of Isolates	E	12 (8)	20 (13.5)	1 (1)	0	3 (2)	1 (1)	37 (25)
Number of Isolates	S	14 (9.5)	20 (13.5)	3 (2)	6 (4)	0	6 (4)	49 (33)
Pigment Production								
Soluble	N	7 (21.2)	17 (51.5)	2 (6.1)	3 (9.1)	1 (3)	3 (9.1)	33 (22.2)
Soluble	E	2 (22.2)	5 (56)	1 (11)	0	1 (11)	0	9 (6)
Soluble	S	6 (37.5)	5 (31.3)	0	4 (50)	0	1 (6.3)	16 (10.8)
Reverse Side	N	9 (15)	34 (57)	7 (12)	4 (7)	2 (3.3)	4 (7)	60 (40.5)
Reverse Side	E	12 (33.3)	19 (53)	1 (3)	0	2 (6)	2 (3.3)	36 (24)
Reverse Side	S	14 (33)	16 (37.2)	3 (7)	5 (11.6)	0	5 (11.6)	43 (29)
Melanin	N	2 (33.)	3 (50)	0	0	1 (17)	0	6 (4)
Melanin	E	1 (14.3)	4 (57.1)	0	2 (28.6)	0	0	7 (4.7)
Melanin	S	0	4 (50)	1 (12.5)	3 (37.5)	0	0	8 (5.4)
Sporophore Morphology								
Spiral	N	2 (8.3)	15 (62.5)	3 (12.5)	1 (4.2)	2 (8.3)	1 (4.2)	24 (16.2)
Spiral	E	6 (43)	7 (50)	1 (7)	0	0	0	14 (9.5)
Spiral	S	7 (35)	7 (35)	1 (5)	3 (15)	0	2 (10)	20 (13.5)
Flexibilis	N	3 (18.7)	8 (50)	1 (6.3)	1 (6.3)	1 (6.3)	2 (12.5)	16 (10.8)
Flexibilis	E	2 (20)	6 (60)	0	0	2 (20)	0	10 (7)
Flexibilis	S	4 (33.3)	5 (41.8)	1 (8.3)	1 (8.3)	0	1 (8.3)	12 (8.1)
Rectus	N	1 (11)	7 (78)	0	0	0	1 (11)	9 (6)
Rectus	E	1 (17)	5 (83)	0	0	0	0	6 (4)
Rectus	S	2 (14.3)	6 (43)	1 (7)	2 (14.3)	0	3 (21.4)	14 (9.5)
Retinaculum-Aperturae	N	3 (23.1)	7 (53.8)	1 (8)	2 (15.4)	0	0	13 (9)
Retinaculum-Aperturae	E	3 (43)	2 (29)	0	0	1 (14)	1 (14)	7 (4.7)
Retinaculum-Aperturae	S	1 (33)	2 (67)	0	0	0	0	3 (2)
Enzymatic Activity								
No. of active Isolates	N	8 (5.4)	28 (18.9)	2 (1.35)	8 (5)	0	2 (1.35)	48 (32)
No. of active Isolates	E	21 (14.2)	12 (8)	1 (0.7)	0	1 (0.7)	1 (0.7)	36 (24.3)
No. of active Isolates	S	3 (2)	9 (6)	0	6 (4)	0	3 (2)	21 (14)
Amylase	N	4 (18.2)	11 (50)	2 (9.1)	4 (18.2)	0	1 (4.5)	22 (15)
Amylase	E	11 (55)	8 (40)	0	0	1 (5)	0	20 (13.5)
Amylase	S	1 (8)	6 (46)	0	4 (31)	0	2 (15)	13 (0.9)
Protease	N	4 (15.3)	17 (65.4)	0	4 (15.3)	0	1 (4)	26 (18)
Protease	E	10 (62.5)	4 (25)	1 (6)	0	1 (6)	0	16 (10.8)
Protease	S	2 (25)	3 (37.5)	0	2 (25)	0	1 (12.5)	8 (5.4)
Antibiotic Activity								
No. of active Isolates	N	0	3 (2)	0	0	0	0	3 (2)
No. of active Isolates	E	0	0	0	0	0	0	0
No. of active Isolates	S	0	5 (3.4)	0	0	0	0	5 (3.4)
Escherichia coli	N	0	3 (100)	0	0	0	0	3 (100)
E.coli	E	0	0	0	0	0	0	0
E.coli	S	0	5 (100)	0	0	0	0	5 (100)
K.pneumonia	N	0	3 (100)	0	0	0	0	3 (100)
K.pneumonia	E	0	0	0	0	0	0	0
K.pneumonia	S	0	5 (100)	0	0	0	0	5 (100)
Proteus	N	0	1 (33.3)	0	0	0	0	3 (100)
Proteus	E	0	0	0	0	0	0	0
Proteus	S	0	2 (40)	0	0	0	0	5 (100)
P.aeruginosa	N	0	0	0	0	0	0	3 (2)
P.aeruginosa	E	0	0	0	0	0	0	0
P.aeruginosa	S	0	1 (20)	0	0	0	0	5 (3.4)

[a] Variable: Violet, Orange and Pink.

Numbers in parentheses represent % out of total.

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ISSN: 0975-8585

Table 4. Characterization of antibiotic active *Streptomyces* producer isolates

Isolates	Cultural characters				Sporophore	Enzymatic Activity		Sugar utilization								
Isolates	AM	ME	RS	SP	Sporophore M	Amylase	Protease	G	Rf	M	X	I	R	F	S	A
J2	Grey	-	-	+	S	-	++	+	-	+	+	+	+	-	-	+
P1	White	-	-	+	F	++++	+++	+	-	+	-	-	+	-	-	+
Um2	White	+	+	-	S	++++	++++	+	+	-	-	-	+	+	-	+
Mf3	Grey	-	+	-	S	+++	++	+	+	-	+	-	+	+	-	+
Mf5	Grey	-	+	-	RA	++	++	ND	ND	ND	ND	ND	ND	ND	ND	ND
Hm1	Green	-	+	-	F	+++	+++	ND	ND	ND	ND	ND	ND	ND	ND	ND
Hm4	White	-	+	-	R	+++	+++	+	+	+	+	-	-	+	-	+
Kf2	White	+	+	-	R	+++	++++	ND	ND	ND	ND	ND	ND	ND	ND	ND
Ba4	Grey	-	+	+	RA	+++	++++	+	+	-	+	+	+	-	+	+

Cultural characters: AM: aerial mycelium; ME: melanin pigments; RS: reverse side pigments; SP: soluble pigments; Spore morphology: S: spirales; F: Flexibilis; R: Rectus; RA: Retiniaculum Apertum; Sugar utilization: G: glucose; Rf: raffinose; M: manitol; X: xylose; I: inistol; R: rhaminose; F: fructose; S: sucrose; A: arabinose. Diameter of clear zone :(+): <10 mm; (++): 11-20 mm; (+++): 21-30; (++++): 31-40 mm; (+++++): 41-50 mm

Table 5. Characterization of antibiotic active *Streptomyces* producer isolates

Isolates	Cultural characters				Spore	Antibiosis								Sugar utilization							
Isolates	AM	ME	RS	SP	Spore chain	*E. coli*		*P. aeruginosa*		*K. pneumonia*		*Proteus sp.*		G	Rf	M	X	I	R	F	S	A
						S	R	S	R	S	R	S	R									
						CRO S Lev NI	CMX T AUG LZD RP GM VA TEC E	GM S Lev	CRO CMX T AUG LZD RP VA TEC NI E	CRO AUG GM S	CMX T LZD RP Lev VA TEC NI E	CRO T RP GM S NI E	CMX AUG LZD RP Lev VA TEC									
Pe 2	white	-	+	-	S	+		-		++		-		+	-	+	+	+	+	-	-	+
Pe 3	White	-	+	+	F	++		++		++		++		+	-	+	-	-	+	-	-	+
Pe 4	White	-	+	-	F	+		-		+		-		ND	ND	ND	ND	ND	ND	ND	ND	ND
Aq 1	White	-	+	+	RA	+		-		++++		-		+	+	-	+	+	+	-	+	+
Di 2	White	-	+	-	S	+		-		++		-		+	-	+	-	-	-	-	+	+
Di 3	white	-	-	-	RA	+		-		++		-		+	-	+	+	+	-	-	+	+
Di 4	White	-	+	+	S	+		-		++		-		+	-	-	-	+	+	-	-	+
Kf 7	White	-	+	+	F	-		-		++		-		ND	ND	ND	ND	ND	ND	ND	ND	ND
H 1	Grey	-	+	+	S	+++		-		+++		-		+	-	+	+	-	+	-	-	-

Cultural characters: AM: aerial mycelium; ME: melanin pigments; RS: reverse side pigments; SP: soluble pigments; Spore morphology: S: spirales; F: Flexibilis; R: Rectus; RA: Retiniaculum Apertum; Sugar utilization: G: glucose; Rf: raffinose; M: manitol; X: xylose; I: inistol; R: rhaminose; F: fructose; S: sucrose; A: arabinose. Diameter of clear zone :(+): 5-10 mm; (++): 11-16 mm; (+++): 17-22 mm; (++++): 23-28 mm.
S: Sensitive to; R: Resistant to
CRO: Cefltriaton; CMX: Cefuroxine; T: Tetracycline; AUG: Augmetin; LZD: Linezoid; RP: Rifompicin; GM: Gentamycin; S: Streptomycin; Lev: Levoflaxin; VA: Vancomycin; TEC: Teicoplanin; NI: Nitofurantion; E: Erythromycin.

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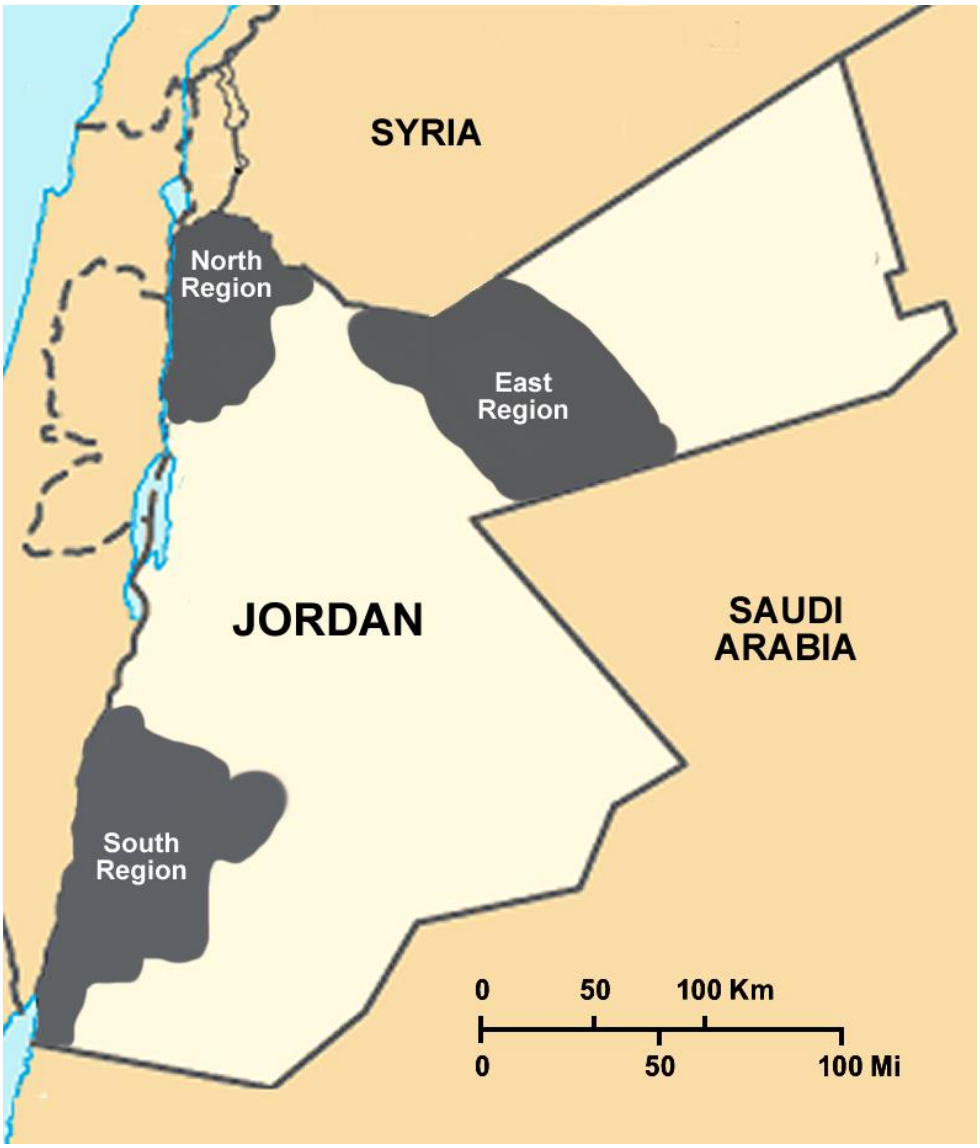
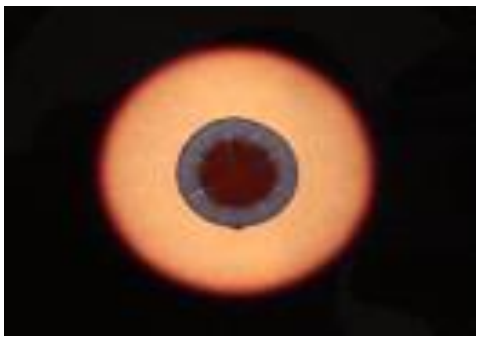


Figure 1. Map of Jordan showing the sites from where soil samples were collected (for locations of collection, see Table 1).



A

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B

Figure 2. Plate diffusion method to detect the enzymatic activity of *Streptomyces* isolates. (A) Amylase enzyme production on SCNA plate. (B) Protease enzyme production on skimmed milk casein agar plate.

CONCLUSION

Streptomycetes are abundant in the tested soil samples with the highest count found of soil samples with high moisture and organic matter content. The average count of *Streptomyces* per gram dry weight of soil is located within the range that is found in the temperate regions. Soils from northern and southern regions had the highest and lowest average *Streptomyces* count, respectively. Most of the active enzyme *Streptomyces* producers occurred in soil samples of north region and with highest percentage of amylase and protease enzyme activity over other regions. The antibiotic active *Streptomyces* producers occurred mainly in soil samples of south region with the highest active isolates against all tested multi-resistant

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