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Characterization and Identification of A ctinomycetes Isolated From Contaminated Soil in Riyadh

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Abstract— In this study, twenty soil samples were collected to isolate heavy metals resistant actinomycetes. The collection sites were chosen toward the four geographical directions, east, west, south and north of the gold factory in the second industrial city in Riyadh region at Saudi Arabia. Results has been shown that the best growth medium for actinomycetes isolation was the Agar-Albumin medium. From soil samples collected six isolates were able to grew in media containing 1 mM/100 ml of either aluminum, silver or cobalt ions .All isolates were found to be belonging to the genus Streptomyces. The isolates were classified on the basis of color into four strains. The first isolate was characterized by white color chain and was identified as *S. albus*, two isolates has gray color chain and were scored as *S. diastaticus and S. atroolivaceous*, those with red chain and comprised two isolates ,they were identified as *S. violaceus* and *S. exfoliate* and one isolate with yellow color chain identified as *S. niveus*. The recorded results showed that *S. diastaticus* and *S. albus* were the most tolerant actinomycetes to the concentration 1 mM/25 ml medium of aluminum, silver and cobalt.

Index Terms— Aluminum, silver and cobalt resistant actinomycetes, Characterization and Identification of actinomycetes , Riyadh, Saudi Arabia.

1 INTRODUCTION

MOST of the point sources of heavy metal pollutants are industrial wastewater from mining and metal processing. The heavy metals are transported by runoff water and contaminate water sources downstream from the industrial site[1]. As a consequence, the environment becomes acid and rich in heavy metals. Selecting microorganisms able to survive in this condition , which are of great interest as bioremediation agent [2].Heavy metals resistance actinomycetes have shown in some previous studies, such as , Albarracı´n *et al.*(2005) isolated fifty actinomycetes from copper contaminated and non-contaminated area. Primary qualitative screening assays showed that 100% of the isolated microorganisms of the contaminated area were resistant up to 80mg L^{-1} of $CuSO_4$. Polti(2006) used forty-one isolated actinomycetes to study qualitative and semi-quantitative screening of chromium(VI) resistance. Eleven Cr(VI) resistant strains were characterized and identified as species of the genera Streptomyces (10) and Amycolatopsis (1). Schmidt, A. *et al.*,(2007) showed that strain, Streptomyces acidiscabies E13 toleratant high concentrations of heavy metals (Ni, Cu, Cd, Cr, Mn, Zn, Fe). Al-Kadeeb *et al.* (2009) isolated *S. albus* ,*S. griseoflavus S. omiyaensis, S. lydicus, S. griseoflavus* ,*S. pactum, S. griseoviridis, S. cellulosae* and *S. sulphureus* from Riyadh soil. They reported that *S. griseoflavus* and *S. pactum* were the most tolerate lead, mercury and copper at (1 mM/ 25 ml) followed by *S. albus* and *S. griseoflavus*. Zhao *et al* (2009) isolated a novel actinomycete, strain S187(T), from a marine sediment sample collected from Xinghai Bay, Dalian, China. On the basis of phenotypic and genotypic analyses, it is proposed that strain S187(T) represents a novel species of the genus Streptomyces, for which the name *Streptomyces xinghaiensis* sp. Hence this study aim to characterization and identification aluminum, silver and cobalt resistant actinomycete strains isolated from contaminated soils of the gold factory in the second industrial city in Riyadh region at Saudi Arabia.

2 MATERIAL AND METHODS

2.1. Soil samples

Four soil samples of a total weight of 4000g were collected from Eastern, Western, Northern and Southern directions of gold factory in second industrial city, Riyadh, Saudi Arabia mixed thoroughly. Approximately 2000 g of the mixed samples were sieved through screens with a 0.5 and 0.1 mm diameter opening to remove stones and other debris and used for isolation of actinomycetes which can tolerate the level of 1 mM /100ml concentration of aluminum, silver and cobalt ions. Samples were diluted with sterile water prior inoculation onto agar plates in three replicate.

2.2. Preparation of metals solutions

Aluminum, silver and cobalt solutions were prepared from $Al(NO_3)3.9H_2O$ (Laboratory, Rasayan), $AgNO_3$(Laba, Chemie) and $CoCl_2.6H_2O$(Shangal Chemical Reagent Works). The pH of the working solution was adjusting to 7.0. Fresh dilutions were used for each study.

2.3. Isolation and purification of actinomycetes

Isolation of aluminum, silver and cobalt resistant actinomycetes strains from the soil samples were carried out in Glycerol-asparagines agar[8]; Albumin medium[9]; Tryptone- Yeast extract broth (ISP 1) [10]Yeast extract- malt

extract agar (ISP 2) [11]and Geodermatophilus[6], Actinomycete colonies were maintained by transfer to Agar albumin slant tubes every 3 months ,incubated at 28 °C and stored at 4 oC.

2.3.1. Antagonistic activity testing

Each streptomyces isolate was cultured on different media, namely starch-nitrate agar, glycerol-asparagine agar, starch casein agar and starch-amm. sulphate agar for 7 days at 28°C. Agar discs (6 mm diameter) were prepared (cut) by using a sterile cork bores and placed on the surface of agar plates freshly seeded with bacterial, yeast or fungal test-organisms. The plates were incubated for 24 h for bacteria and yeast and for 48 h in case of fungi [12].

2.3.2. Description and identification of the actinomycetes

Actinomycetes were carring out in Yeast extract broth (ISP 1);Yeast extract- malt extract agar[11];Glycerol- asparagine agar [8]Starch-nitrate agar[13]; Inorganic salts-starch agar (starch-ammonium sulphate agar)[14]; Tyrosine agar[15]; Synthetic medium for melanin formation[16]; Peptone-yeast extract iron agar[17]; Skimmed milk medium; Carbon utilization medium[9]; Gelatin medium; Hutchinson medium;Nutrient agar, Oatmeal agar and Czapek's solution agar[6].Method describe by (Harborne,1973) used for description of cell wall and the modification method of that described by Becker *et al*. (1964) used to describe whole cell sugar composition and determination of isomers 2,6-diaminopimelic acid (DAP) of the cell wall. The micromorphology of spore chains of the isolates were determined by direct microscopic examination as described by Shirling and Gottlieb (1966). The spore print technique described by Tresner *et al*. (1961) was applied to prepare materials for electron microscopy. Cultural characteristics, the colorful appearance of mature sporulating aerial mycelium (color of colony or spore color en masse) and color of substrate mycelium as view from the reverse side of the colony and its sensitivity to pH changes, as well as the diffusible pigment other than melanoides were determined on starch-nitrate agar, starch-amm. sulphate, yeast-malt agar, glycerol-asparagine agar, oatmeal agar and Czapek's solution agar after 7, 14 and 21 day of incubation at 28°C.The production of melanin (or melanoid) pigments was tested on peptone-yeast iron agar, tyrosine agar and tryptone-yeast broth.The ability of isolates to assimilate the following carbon sources: D-glucose, D-fructose, L-arabinose, L-rhamnose, D-mannitol, D-xylose, i-inositol, raffinose and sucrose was tested(all of these sugars are chemically pure). As a negative control, no carbon source was added to the basal medium[9]. Biochemical characteristic

including Starch hydrolysis, Cellulose decomposition, Casein hydrolysis ,Gelatin liquefaction, Nitrate reductase activity and Coagulation and peptonization of milk were performed as recommended by Mansour, 1979 , Mansour & Shady, 1984and Mansour,1985.The ability of Streptomyces isolates to grow at different concentrations of NaCl was tested using starch-nitrate agar amended with 1-12% NaCl [24]and[25].

2.4. Screening for high aluminum silver and cobalt tolerance Actinomycetes

Solid albumin medium plate's with1 mM /25ml concentration of aluminum, silver and cobalt were employed to isolated high aluminum silver and cobalt tolerance actinomycetes. There were three replicates per isolate. All plates were incubating at 28 °C for bacteria and yeast and for 48 h in case of fungi [12]

5 Results and Discussion

5.1.Taxonomic identification of the Actinomycete isolates

5.1.1.Cell wall type determination

The results of the chromatographic analysis of the 6 actinomycete isolates revealed that all of these isolates contained LL-DAP, glycine and no characteristic sugars. Therefore, these isolates were classified as belonging the actinomycetes with cell wall type 1; notably Streptomycetes[19]and[26].

5.1.2.Classification and identification of the actinomycete isolate

Identification of strains has been carried out according the Identification Key of Nonomura (1974), Shirling and Gottlieb (1968a, 1968b, 1969 & 1972) and Bergey's Manual (1989). According to their morphological characteristics (micromorphology of spore chains) all isolates were found to be belonging to the genus *Streptomyces* as observed by others. Siddiq (1995); Alkahtani(2005)and Al-Kadeeb *et al*.(2009) reported that the actinomycetes isolated from Saudi Arabia soil belonging to the genus Streptomyces . Polti (2007) isolated Forty-one actinomycetes and identified as species of the genera Streptomyces (10) and Amycolatopsis (1).

5.1.3.Antagonistic characteristics and identification of isolates

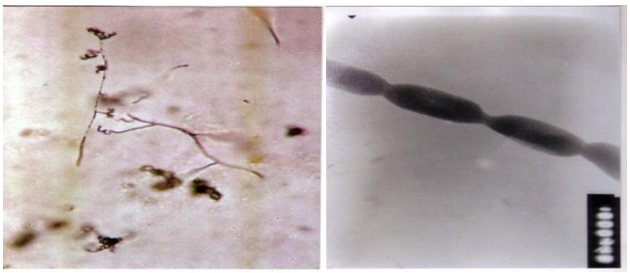
Isolates No. 1 was characteristics by its aerial mass color in the yellow series (whitish-yellow to pale greenish yellow or pale olive yellow) on all media used(Table 1). Its spore chain was spirals (Fig. 1A) with smooth surface (Fig.1 B). Reverse side of colony: Pale yellow to greyish-yellow substrate mycelium was observed on all media used. The substrate mycelium pigment was not sensitive to change in pH (Table 1). Melanoid pigments were not formed on peptone. Yeast iron agar, tyrosine agar or tyrptone yeast broth medium(Table 7). No pigment was found on any of the media used (Table 1). D-glucose, D-fructose, and D-xylose were utilized for growth. L-arabinose, L-rhamnose and D-mannitol favored slight or weak growth, whereas i-inositol, raffinose or sucrose were not utilized by this strain (Table 8). It was able to hydrolyze starch, liquefy gelatin, weakly coagulate milk and slightly reduce nitrate to nitrites, but was not able to decompose

cellulose, peptones milk hydrolyze casein or produce H_2S (Table 9). It appeared to exhibit good antimicrobial activity against Gram-negative and Gram-positive bacteria, but did affect the growth of yeast or fungal-test organisms(Table 10). It grow in presence of NaCl at a concentration of 6% (Table 11). Based on the above mentioned results, isolate No. 1 was classified as a strain belonging to *Streptomyces niveus*.

TABLE 1
CULTURE PROPERTIES OF THE ACTINOMYCETE ISOLATE NO. 1.

Medium	Color of colony	Color of substrate mycelium	Diffusible pigment
Starch-nitrate agar	Whitish yellow to pale greenish yellow	Greyish yellow, not pH sensitive	No
Starch-amm. sulphate agar	Light greenish yellow	Greyish yellow, not pH sensitive	No
Yeast-malt agar	Light greenish yellow	Greyish yellow, not pH sensitive	No
Glycerol asparagine agar	Light yellow to greenish yellow	Pale yellow, not pH-sensitive	No
Oatmeal agar	Whitish-yellow to greenish yellow	Pale yellow, not pH sensitive	No
Czapek's solution agar	Pale olive to yellow	Pale greyish olive, not pH sensitive	No

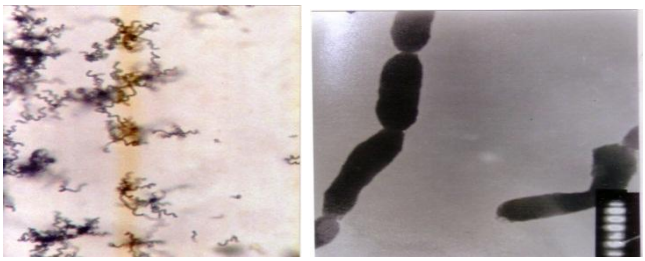
* In this table and the next tables, the following should be regarded: Colour of colony = color of aerial mycelium.
Reverse side of colony = color of substrate mycelium. Diffusible pigment = pigment (s) other than melanoid.



1A 1B

Fig. 1 *Streptomyces niveus* morphological characteristics, Light micrograph showing spore chain morphology(A,x100) and Electron image (Spore surface: Smooth (B, x4.000)

Isolate No. 2.was characteristics by its aerial mass color in the white series on all the media used(Table 2). Its spore chain was spirals (Fig. 2A) with smooth surface(Fig. 2B). Reverse side of colony, not distinctive (colorless to pale yellow substrate mycelium was produced on all of the above mentioned media (Table 2). Melanin pigments were not produced on any of peptone-yeast iron agar, tyrosine agar or tryptone-yeast broth medium (Table 7). No diffusible pigment was found in the medium on any of the media used (Table 2). D-glucose, D-fructose, D-xylose and D-mannitol were utilized for growth of this strain. L-arabinose and i-inositol favored slight or weak growth, whereas no growth was observed with L-rhamnose, raffinose or sucrose (Table 8). It was able to coagulate and peptones milk, liquefy gelatin, hydrolyze casein and reduce nitrate to nitrite but was unable to hydrolyze starch or decompose cellulose and did not produce H_2S (Table 9). It was found to exhibit pronounced antimicrobial activity against Gram-negative and Gram-positive bacteria, but did not affect the growth of yeast or fungi(Table 10). It appeared to tolerate relatively high concentration of NaCl up to 6%(Table 11) .Based on the above mentioned diagnostic features, isolate No. 2 was classified as a strain belonging to *Streptomyces albus. S. albus*,,was isolated before from Riyadh region by (Alkadeeb et al.,2009). This indicated that this species is dominant in Riyadh region.



2A **2B**

Fig. 2 *Streptomyces albus* morphological characteristics, Light micrograph showing spore chain morphology(A,x100) and Electron image (Spore surface: Smooth (B, x4.000)

TABLE 2
CULTURE PROPERTIES OF THE ACTINOMYCETE ISOLATE NO. 2.

Medium	Color of colony	Color of substrate mycelium	Diffusible pigment
Starch-nitrate agar	White	Not distinctive	No
Starch-amm. sulphate agar	White	Not distinctive	No
Yeast-malt agar	White	Not distinctive	No
Glycerol asparagine agar	White	Pale yellow	No
Oatmeal agar	White	Colourless	No
Czapek's solution agar	White	Not distinctive	No

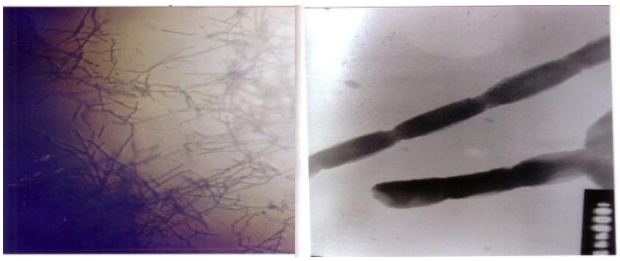
Isolate No. 3 was characteristic by aerial mass color in the red series on all of the media used(Table 3). Its spore chain was rectiflexibiles (Fig. 3A) with smooth surface((Fig. 3B). Reverse side of colony not distinctive; greyed yellow to yellowish brown on starch-nitrate agar and glycerol-asparagines agar and Czapek's solution agar (Table 3). Melanoid pigments were not formed on all of the media used (Table 7). No diffusible pigment was found on any of the media used (Table 3).
D-glucose, D-fructose, L-arabinose, L-rhamnose, D-xylose, raffinose and sucrose were utilized for growth, whereas D-

mannitol and i-inositol were not utilized by this strain (Table 8). It was able to hydrolyze starch, decompose cellulose, liquefy gelatin, coagulate and peptones milk, hydrolyze casein, reduce nitrate to nitrites and produce H_2S(Table 9).
It exhibited antimicrobial activity towards Gram-positive bacteria, but did not affect the growth of Gram-negative bacteria, yeast or fungi (Table 10). It appeared to tolerate NaCl concentration up to 8% (Table 11). On the basis of the above mentioned results, isolate No. 3 was identified as a strain belonging to *Streptomyces exfoliates*.

TABLE 3
CULTURE PROPERTIES OF THE ACTINOMYCETE ISOLATE NO. 3.

Medium	Color of colony	Color of substrate mycelium	Diffusible pigment
Starch-nitrate agar	Pale yellowish pink to red	Greyed yellow*to yellowish brown, not pH sensitive	No
Starch-amm. sulphate agar	Yellowish pink	Not distinctive to pale brown	No
Yeast-malt agar	Lilae to pink	Not distinctive or pale brown	No
Glycerol asparagine agar	Pale pink to red	Pale yellowish brown, not pH sensitive	No
Oatmeal agar	Lilae to pinkish red	Not distinctive or pale brown	No
Czapek's solution agar	Pale yellowish pink	Not distinctive or pale yellowish brown	No

* The substrate mycelium pigment was not pH sensitive when tested with 0.5N HCl or 0.5N NaOH.



3A 3B

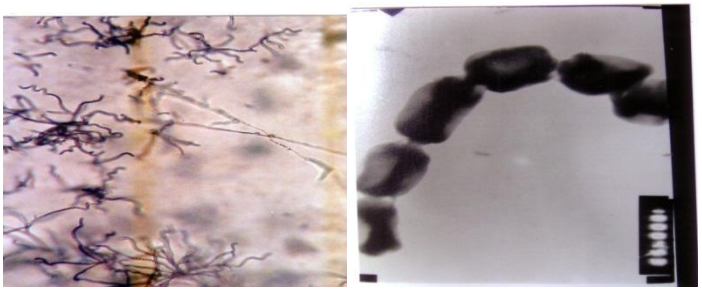
Fig (3)*Streptomyces exfoliates* morphological characteristics, Light micrograph showing spore chain morphology(A,x100) and Electron image (Spore surface: Smooth (B, x4.000).

Isolate No. 4 was characteristic by aerial mass color in the grey series on all of the media used(Table 4), its spore chains spirals, meanwhile some spore chains appeared to be straight to flexuous, i.e. rectiflexibiles (Fig. 4A)with smooth surface (Fig. 4B). Reverse side of colony, not distinctive, greyed yellow to yellowish olive or yellowish brown substrate mycelium was produced on the media used (Table 4). The substrate mycelium pigment was not pH sensitive when tested with 0.5N HCl or 0.5N NaOH. Melanin pigments were not formed all of the media used (Table 7). No pigment was found in the medium on any of the media used (Table 4). D-Glucose, D-fructose, L-arabinose, D-mannitol, D-xylose and sucrose were utilized for growth of isolate No. 4. No growth was observed with L-rhamnose, i-inositol or raffinose (Table 8). It is able to hydrolyze starch, coagulate and peptones milk, hydrolyze casein, liquefy gelatin and reduce nitrate to nitrite, but did not produce H_2S(Table 9). It exhibits a pronounced antimicrobial activity against yeast and fungi, but did not affect the growth of Gram-negative or Gram-positive bacteria(Table 10) . It could grow at NaCl concentration of 4%, but failed to grow at or above 6% (Table 11). According to the above mentioned results, isolate No. 4 was identified as a strain belonging to *Streptomyces diastaticus*.

TABLE 4
CULTURE PROPERTIES OF THE ACTINOMYCETE ISOLATE NO. 4.

Medium	Color of colony	Color of substrate mycelium	Diffusible pigment
Starch-nitrate agar	Pale yellowish grey	Greyed yellow*	No
Starch-amm. sulphate agar	Pale yellowish grey	Greyed yellow	No
Yeast-malt agar	Whitish-yellow to yellowish grey	Yellowish olive	No
Glycerol asparagine agar	Pale yellowish grey	Yellowish brown	No
Oatmeal agar	Pale yellowish grey	Yellowish brown	No
Czapek's solution agar	Whitish grey	Yellowish olive	No

* The substrate mycelium pigment was not pH sensitive when tested with 0.5N HCl or 0.5N NaOH.



4A **4B**

Fig. 4. *Streptomyces diastaticus* morphological characteristics, Light micrograph showing spore chain morphology(A,x100) and Electron image (Spore surface: Smooth (B, x4.000).

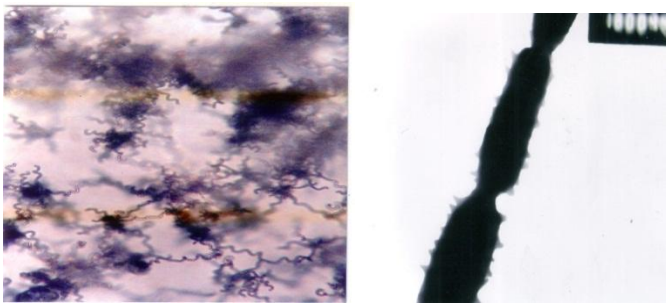
Isolates No. 5 was characteristic by aerial mass color in the red series on all of the media used(Table 5). Its spore chain was spirals (Fig. 5A), with smooth surface(Fig. 5B). Reverse side of colony was yellowish pink or yellowish orange to red substrate mycelium was produced on starch-nitrate agar, starch-amm. sulphate agar, yeast-malt agar, oatmeal agar and Czapek's solution agar, and violetish purple on glycerol-asparagine agar. This pigment appeared to be pH sensitive, changing from red in acidic pH to violet in alkaline pH (Table 5).Melanin pigments were formed on peptone-yeast iron agar and tryptone yeast broth, but not formed on tyrosine agar medium (Table 7). Meanwhile reddish orange to pink or

violetish purple diffusible pigment was produced on starch-nitrate agar, starch-amm. sulphate agar, yeast malt agar, glycerol asparagine agar, oat meal agar and Czapek's solution agar. This pigment was pH sensitive; exhibited the changes reported for the substrate mycelium pigment (Table 5).D-Glucose, D-fructose, L-arabinose, L-rhamnose, D-mannitol, D-xylose, i-inositol, raffinase and sucrose were all utilized for growth of isolate No. 5 (Table 8). It was able to hydrolyze starch, decompose cellulose, liquefy gelatin and reduce nitrate to nitrite, and weakly coagulate milk, but was unable to peptonize milk, hydrolyze casein and produce H_2S (Table 9). It exhibited pronounced antibiotic activity against Gram-negative bacteria, Gram-positive bacteria, yeast and fungi (Table 10). It could tolerate relatively high salinity up to NaCl concentration of 6%(Table 11). On the basis of the above mentioned diagnostic features, isolate No. 5 was identified as a strain belonging to *Streptomyces violaceus*.

TABLE 5
CULTURE PROPERTIES OF THE ACTINOMYCETE ISOLATE NO. 5.

Medium	Color of colony	Color of substrate mycelium	Diffusible pigment
Starch-nitrate agar	Light greyish pink	Yellowish* pink to red, pH sensitive	Reddish* orange pH sensitive
Starch-amm. sulphate agar	Greyish-yellowish pink	Yellowish pink to red, pH sensitive	Reddish orange, pH sensitive
Yeast-malt agar	Light yellowish pink	Yellowish orange to red, pH sensitive	Reddish brownish orange
Glycerol asparagine agar	Pale pink to pinkish red	Violetish purple, pH sensitive	Pink to violet, pH sensitive
Oatmeal agar	Pale reddish white	Brownish orange to red, pH sensitive	Orange-red to pinkish red pH sensitive
Czapek's solution agar	Yellowish pink	Yellowish orange to red, pH sensitive	Violetish purple, pH sensitive

* The substrate mycelium pigment and the diffusible pigment appeared to be pH sensitive, changing from red in acid pH to violet or purple in alkaline pH.



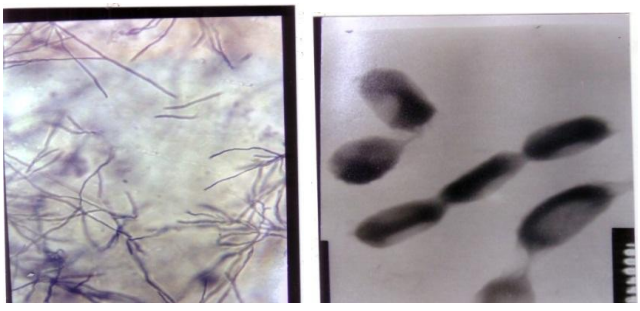
5A **5B**

Fig. 5 *Streptomyces violaceus* morphological characteristics, Light micrograph showing spore chain morphology(A,x100) and Electron image (Spore surface: Smooth (B, x4.000).

isolates No. 6 characteristic by aerial mass color in the Grey series, (light grey to cinereous on starch-nitrate agar, starch-amm. sulphate agar and oatmeal agar, whitish grey on yeast-malt agar and Czapek's solution agar, and light grey to medium grey on glycerol-asparagines agar (Table 6). Its spore chain rectiflexibles (Fig. 6A)with smooth surface(Fig. 6B).Reverse side of colony, not distinctive (grayed-yellow, grayed brown or yellowish brown) on the media used (Table 8).Melanin pigments were not formed on peptone-yeast iron agar, tyrosine agar or tryptone-yeast broth media (Table 7). No pigment was found on any of the media used (Table 6).D-Glucose, D-fructose, L-arabinose, L-rhamnose and D-mannitol were utilized for growth of isolate 6. The utilization of D-xylose, i-inositol and raffinose favored slight a weak growth, but no growth was detected with sucrose (Table 8). It could actively liquefy gelatin, coagulate milk and reduce nitrates to nitrites; weakly hydrolyze starch, decompose cellulose, peptones milk and hydrolyze casein, but did not produce H_2S (Table 9). It exhibited antagonistic activity against Gram-negative and Gram-positive bacteria, but did not affect the growth of yeast or fungal test-organisms (Table 10). It could tolerate relatively high concentration of NaCl up to 8%(Table 11). On the basis of the above mentioned description (diagnostic characteristics), isolate No. 6 was classified as a strain belonging *Streptomyces atroolivaceous*.

TABLE 6
CULTURE PROPERTIES OF THE ACTINOMYCETE ISOLATE NO. 6.

Medium	Color of colony	Color of substrate mycelium	Diffusible pigment
Starch-nitrate agar	Light grey to cinereous	Greyed yellow, not pH sensitive	No
Starch-amm. sulphate agar	Light grey to cinereous	Greyed yellow	No
Yeast-malt agar	Whitish grey	Greyed brown	No
Glycerol asparagine agar	Light grey to medium grey	Yellowish brown	No
Oatmeal agar	Light grey to cinereous	Greyed yellow	No
Czapek's solution agar	Whitish grey	Greyed yellow	No



6A 6B

Fig.6. *Streptomyces atroolivaceous* morphological characteristics, Light micrograph showing spore chain morphology(A,x100) and Electron image (Spore surface: Smooth (B, x4.000)

TABLE 7
THE ABILITY OF ACTINOMYCETES ISOLATES TO PRODUCE MELANIN PIGMENTS ON:

Isolate No.	Peptone-yeast iron agar	Tyrosine agar	Tryptone-yeast broth
1	-	-	-
2	-	-	-
3	-	-	-
4	-	-	-
5	+	-	+
6	-	-	-

TABLE 8
UTILIZATION OF DIFFERENT CARBON SOURCES BY THE ACTINOMYCETES ISOLATES.

Isolate No.	D-glucose	D-Fructose	L-Arabinose	L-Rhamnose	D-Mannitol	D-Xylose	i-Inositol	Raffinose	Sucrose
1	+	+	±	±	±	+	-	-	-
2	+	+	±	-	+	+	±	-	-
3	+	+	+	+	-	+	-	+	+
4	+	+	+	-	+	+	-	-	+
5	+	+	+	+	+	+	+	+	+
6	+	+	+	+	+	±	±	±	-

TABLE 9.
BIOCHEMICAL PROPERTIES OF ACTINOMYCETES ISOLATES

Isolate No.	Starch hydrolysis	Cellulose decomposition	Gelatin liquefaction	Coagulation of milk	Peptonization of milk	Casein hydrolysis	Nitrate reduction	H2S production
1	+	-	+	±	-	-	±	-
2	-	+	+	+	+	+	+	-
3	+	+	+	+	+	+	+	+
4	+	-	+	+	+	+	+	-
5	+	+	+	±	-	-	+	-
6	±	±	+	+	±	±	±	-

+ = Good reaction or good activity - = Weak reaction or slight activity

5.2.Effect of Media using for isolation and purification on the growth of Actinomycetes

About the effect of media using for isolation and purification on the growth of actinomycetes result in table (12) observed that the best medium for isolation of actinomycetes was albumin agar among the media screened. This result agree with these reported by Al-Kadeeb *et al.*(2009), while Alkahtani(2005)reported that the best medium for isolation of actinomycetes was starch and nitrate agar.

5.3. High aluminium silver and cobalt concentration tolerance Actinomycetes

About actinomycetes tolerance to high concentration of aluminum,silver and cobalt, the recorded results(Table12) showed that *S. diastaticus* and *S. albus* were the most tolerant Actinomycetes to the concentration 1 mM/25 ml medium of Aluminum Silver and Cobalt.

TABLE 10
ANTAGONISTIC ACTIVITY OF THE ACTINOMYCETE ISOLATES.

	Test-organisms										
	Gram-negative bacteria			Gram-positive bacteria			Yeast		Fungi		
Isolate no.	*E. coli*	*Kelbsiella pneumonia*	Pseudomonas aeruginosa	*Streptococcus pyoaenesis*	*Staphylococcus aureus*	*Bacillus subtilis*	*Saccharomyces cervisiae*	*Candida albicans*	*Aspergillus flavus*	*Fusurium solani*	*Botrytis cinerae*
1	+	+	+	+	+	+	-	-	-	-	-
2	+	+	+	+	+	+	-	-	-	-	-
3	-	-	-	+	+	+	-	-	-	-	-
4	-	-	-	-	-	-	+	+	+	+	+
5	+	+	+	+	+	+	+	+	+	+	+
6	+	+	±	+	+	+	-	-	-	-	-

TABLE 11
NACL TOLERANCE OF THE STREPTOMYCES ISOLATES OBTAINED.

Isolate no.	Concentration of NaCl% (w/v 1%	2%	4%	6%	8	10	12
1	+++	+++	+++	+++	-	-	-
2	+++	+++	+++	++	-	-	-
3	+++	+++	+++	+++	++	-	-
4	+++	+++	++	-	-	-	-
5	+++	+++	+++	++	-	-	-
6	+++	+++	+++	++	++	-	-

+++ = Good growth + = Weak growth - = No growth ++ = Moderate growth

TABLE 12
EFFECT OF MEDIA USING FOR ISOLATION AND PURIFICATION ON THE GROWTH OF ACTINOMYCETES

Isolate no.	Glycerol asparagine agar(Waksman 2)	Glycerol asparagine agar(Waksman3)	Geodermat-ophilus	Albumin medium	Tryptone-Yeast extract broth	Yeast extract-malt extract agar
1	-	-	+	-	-	-
2	-	-	-	+	-	-
3	-	-	-	+	-	-
4	-	-	-	+	-	-
5	+	+	-	-	-	-
6	-	-	-	-	+	-

+ = Growth - = No growth

TABLE 13
EFFECT OF HEAVY METAL ION ON THE GROWTH OF ACTINOMYCETES AT 1 MM/25ML CONCENTRATIONS OF ALUMINUM, SILVER AND COBALT IONS ON ALBUMINE AGAR AFTER 7 D INCUBATION AT 28°C

Al3+	Ag+	Co2+	Control	Organism
Mean(±SD) colony diameter in cm				
	.10±0.01		1.50±0.01	***S. niveus***
1.23±0.02		1.3±0.01		
		1.64±0.01		***S. albus***
±1.700.02	±0.011.47		1.56±0.01	
				S. exfoliates
1.98±0.01	2.13±0.01	2.14±0.02	2.45±0.00	
				S. diastaticus
2.20±0.02	2.19±0.01	2.21±0.02	2.12±0.01±0.00	
				S. violaceus
2.1±0.02	1.94±0.01	2±0.02	2.31±0.01	
		1.64±0.02		***S. atroolivaceous***
1.24±0.01	1.73±0.01		1.92±0.01	

Sig = 0.00

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