Journal of Applied Science Research, 2(10): 809-815, 2006

A Biological and Biochemical Studies of Actinomycetes Isolated from Kuwait Saline Soil-Kuwait.

Ibrhaim H. Abbas

Department of Botany, Faculty of Science-Benha University. Egypt.

Abstract: The present investigation aimed at the isolation of actinomycets from saline soil (total five samples) from different parts of Kuwait saline soils which is a newly investigation in Kuwait. The isolates were obtained and characterization using the following criteria: Color of aerial vegetative mycelia, morphology of spore surface, melanin production, utilization of carbon and nitrogen sources, degradation of some complex insoluble compounds, sensitivity to antibiotics, fatty acid analysis, physical and chemical analysis for each soil sample of soil was done. Three genera of mesophilic actinomycetes were isolated from the soil samples of Kuwait: *Actinopolyspora spp., Microbispora Spp*. and *Amyclatopsis spp.*

Key word: *Actinomycetes, Actinopolyspora, Microbispora and Amyclatopsis.*

INTRODUCTION

Actinomycetes are gram-positive bacteria, which show marked chemical and morphological diversity but form a distinct evolutionary line of organisms[9].

Bacteial systematic has undergone revolutionary techniques during the past 3 decades. The application of new and reliable biochemical, chemical, genetical, numerical and molecular biology techniques have been responsible for rapidly changing views on how bacteria could be classified and identified[1,7].

The methods used in the ISP have been described in detail by Shirling and Gottlieb[25]. The following characters were considered important and are now commonly used in the keys for classification of the species of the genus *Streptomyces.*

Morphology of spore chains and the spore surfaces, color of aerial and vegetative mycelia, melanin production water soluble pigment production and utilization of carbon an nitrogen sources[5].

Halophilic microorganisms can be conveniently grouped according to their requirements for NaCl for growth Larsen[18]. Slightly halophilic organisms in marine environements can grow in the presence of 2 to 3% NaCl. The moderate halophilic grow over a much wider NaCl concentration range (5 to 20%, w/v). The extreme halophiles, including the well-known halobacteria and halococci, are able to grow in saturated NaCl and unable to grow in the presence of NaCl concentratios lower than 12%. The occurence of actinomycetes in high saline environements and the tolerance of these organisms to high concentrations of salts have been described by Tresener *et al*.,[31] and Gottileb[13].

MATERIALS AND METHODS

Soil samples were collected from five sites, fresh fields, El-Keran, Kuwait.

Soils from both five sites have been studied in previous investigation[4]. Soil samples were collected by removing the surface loose litter layer and collecting from the underlying 10 cm depth. Mean values of some physico-chemical properties of soil were determined according to Jackson[14].

Bacterial strains: Bacterial strains were isolated by plating serially diluted samples on to Bennett medium (2 g glucose, 1g yeast extract, 1g malt extract, 2g peptone, 20g agar, 100g NaCl, pH 7.2). After incubation at 30°C for 2 weeks, a visible colony, was transferred and subcultured until pure culture was obtained. Starch nitrate agar (starch 20g, K_2Hp0_4 1g, $KN0_3$ 2g, $MgSo_4$ 0.5g, $CaCo_3$ 3g, agar 20g, 100g NaCl, pH7.2). Was used for growing isolates.

Morphology and pigmentation: The cultures was grown on ISP medium 2(ISP) medium 3 (ISP) medium 4 (ISP) medium 5, as described by Shirling and Gottlieb (1966) and was examined for pigmentation, aerial mycelium and other morphological features. Cultures were grown for 4 weeks and observation were made at weekly intervals. The morphological properties of colonies, cells and spores were determined using light and scanning electron microscopes (JEM-1200 Ex II electron microscope). Samples of 14-days old colonial growth on ISP medium 4 supplemented with 10% NaCl were prepared by cutting agar blocks from the growth medium, fixing them with glutaraldehyde and dehydrating them by using a graded

Corresponding Author: Ibrhaim H. Abbas, Department of Botany, Faculty of Science-Benha University. Egypt.

methoxyethanol series and finally 100% acetone. The dehydrating sections were critical point dried, mounted on aluminium stubs and then suputter coated with gold-palladium.

Physiological tests: The physiological testes used to characterize the isolates were those of Gordon[11,12] and Gordon and Mihm[10]. The testes used to determine carbohydrate utilization and the test used to determine melanoid pigments were those of Shirling and Gottlieb[25]. The degradation of tyrosine, xylane, urea, DNA and RNA was determined by the method of Gordon[11,12].

The hydrolysis of tween was determined by a modification of method of Sierra[29]. Starch degradation was determined using both starch-casin agar and nutrient agar supplemented with 1% (w/v) of soluble starch hydrolysis was detected by the presence of zones of clearing around the colonies. Cellulose degradation was determined by the method of Goodfellow and Pirouz[8].

Tolerance to salt was determined by growing the organisms on glucose-nutrient agar plates supplemented with 0 to 20% (w/v) NaCl. The susceptibilites of the organisms to various antibiotics were studied on nutrient agar plates containing various antibiotics such as triacylolandomycin, lincomycin and rifampicin .

The antibiotics were aseptically mixed with sterile molten agar and the preparations were maintained at 45°C and poured into plates. After inoculation, the plates were incubated at 30°C for 1 week . Growth on the media was compared with growth of a control and was recorded as negative or positive (no growth or growth respectively)

Chemotaxonomy: Freeze-dried biomass was prepared from ISS broth grown at 30°C for 7 day using a shaking incubator. Analysis for diaminopimilic acid and major sugar were carried out using the procedures described by Staneck and Roberts[30] and Lechevalier and Lechevalier[20] respectively . Isoprenoid quinine analysis was performed by the method of Collins[3], the isoprenoid quinines were identified by high-performance liquid chromatography[23]. Phospholipidis were extracted and identified by the methods of Lechavalier and Lechvalier[21]. Occurrence zof mycolic acids was determined by the procedure of Minnikin *et al*.,[23].

RESULT AND DISCUSSION

From 60 isolates of halotolerant actinomycetes which isolated from soil samples collected from El-Keran region of Kuwait, the perilminary investigation from morphological and chemical analysis we can differentiated the isolates into 3 groups:

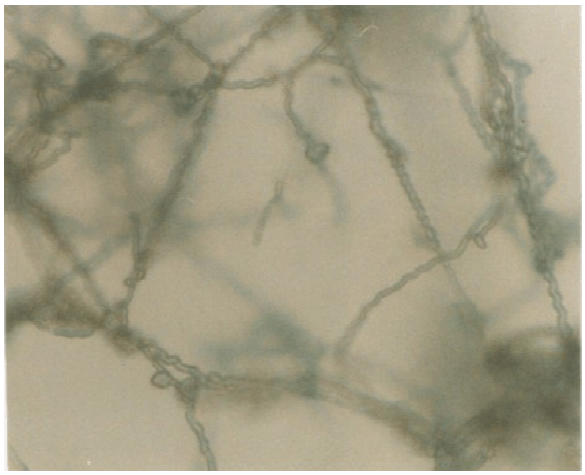

Fig. 1: Light micrograph of branched aerial mycelium in a 14 days old culture X 160.

Fig. 2: Electron micrograph of stain A-I spores in 14-days old culture X 2055.

Group 1: This group include 25 isolates of halotolerant actinomycetes, the representative isolate is A-1, Fig. (1 and 2) show that the isolates of this group produce, on solid media well developed colonies, that consist of extensively branched nonfragmenting substrate mycelium that carries rarely branching flexous to curled straight rarely branching aerial hyphae. The aerial hyphae fragment into spores. The spores appear cylindrical to oblong and of unequal length, spore surface is smooth. The aerial mass color of isolate A-1 was white to pale yellow on oat meal agar,

glycerol asparagin agar and inorganic salts starch agar (all media fortified with 10% NaCl) Table 2. No diffusible pigment are produce on the used media . The isolate did not produce melanin pigments, when cultivated

Table 1: Soil analysis and particle size distribution.

	pH	Course sand	Fine sand	oil	Clay	Texture
		Granulometeric analysis (%)				
1	7.4	8	87	3.5	1.5	Sandy
2	7.3	6.8	90.7	1.9	0.6	Sandy
3	7.5	4.8	91.2	3.2	0.8	Sandy
4	7.5	5	90	1.0	4.0	Sandy
5	7.5	9	88.4	2.0	0.6	Sandy

Table 2: Chemical analysis

			(mg/eg/L)				ppm				
	Fe	Ca	mg	Na	K	d-	Hco3	Caco3	N	p	K
1	2.3	5.5	3	10.5	1.2	7	2	11.5	20	1.2	8.3
2	0.5	2.4	1	0.3	0.3	1	1	5.4	30	1.2	33
3	0.63	3.0	1.6	1.2	0.5	2	1.5	10.6	100	10	2.5
4	1.4	6.6	3	9.5	2.2	5	4	11.5	21	1.2	7.2
5	2	5.83	3	12	2	5.5	2	13	30	1.6	29

Table 3: Physiological characteristics of strain A-1.

Characteristics	Strain A-1	Characteristics	Strain A-1
Carbon utilization		Growth in pressure of:	
L-arabinose	-ve	Gentamycine	-ve
D-fructose	+ve	Neomycin	-ve
D-galactose	+ve	Vancomycin	+ve
Glucose	+ve	Streptomycin	-ve
Sucrose	+ve	Triacylolandomycin	-ve
Strach	+ve	Lincomycin	-ve
D-xylose	-ve	Pencillin G	+ve
Rahmnose	-ve	Tobramycine	-ve
Raffinose	-ve	Rifampicin	-ve
Cellobiose	+ve	Chlorotetracyclin	+ve
Sodium acetate	+ve	Degradation of:	
Sodium pyruvate	+ve	Tyrosine	+ve
Sodium citrate	+ve	Tween 80	+ve
Sodium tartarate	+ve	Strach	+ve
Nitrogen utilization		Xylan	-ve
Potassium nitrate	+ve	Urea	-ve
Cysteine	+ve	Testosterone	+ve
Valine	+ve	Esculin	+ve
Serine	+ve	Guanine	+ve
Methinine	+ve	Adenine	-ve
Histidine	+ve	DNA	-ve
Therionine	-ve	RNA	-ve
Growth at:			
Optimum temperature	25-28°C		
Optimum pH:	7-8		
Optimum NaCl:	0-20%		

on either peptone-yeast iron-agar or tyrosine agar (Table 2).

The optimum growth temperature was 25°C to 28°C, but did not grow at 4°C, 10°C, 40°C and 50°C . The isolate failed to grow at pH4.3 but grow well at pH 7-8 (Table 3). The isolate of this group were found to be resistant to the inhibitory effect of a wide range of NaCl concentrations (0-20%). Isolate A-1 grow well at NaCl concentrations from 0 to 20%.

Isolate A-1 was sensitive to gentamycin, neomycin, streptomycin, tobramycin, rifampicin, oleandomycin, lincomycin, but resistant to, tetracycline and pencillin G. The isolate able to utilize as sole carbon acetate, sodium, sodium citrate and sodium tartarate but not able to utilize arabinose, xylose, rhamnose and raffinose. Isolate of this group utilized potassium nitrate, valine, serine, methionine, histidine, but not utilize therionine as sole of nitrogen source (Table 3).

Isolate A-1 able to degrade tyrosine, starch, tween 80, aesculine, testosterone, guanine, but not able to degrade xylan, urea, adenine, DNA and RNA.

The cell wall contains primarily meso-DAP, the whole-cell hydrolsate contain arabinose, galactose and ribose. The predominant menaquinone is MK-9(H4). The phospholipids include phosphatidylcholin, lysophosphatidyl glycerol, phosphatidyl glycerol and diphosphatidyl glycerol. Mycolic acid are absent.

Identification of isolate of group 1: The cell chemistry and cultural, morphological and physiological characteristics of strain A-1 which summarized in Table (2 and 3).

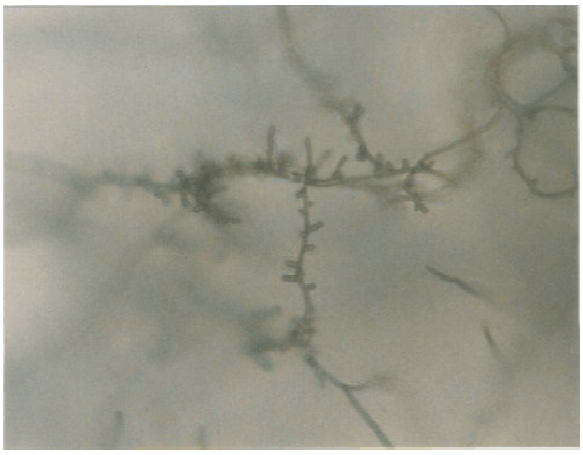

Fig. 3: Light micrograph of aerial hyphe longtidinal of spores isolate 15-1 X 1600

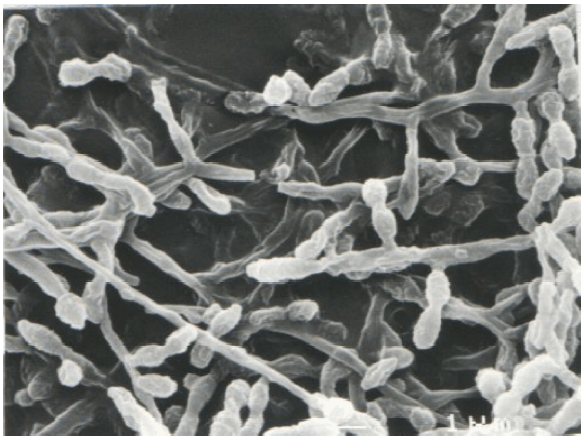

Fig. 4: Electron micrograph of strain B-1 showing lateral spore parts carried on aerial hyphe X2500.

The development of spore chains on aerial myceliumand the un-fragmentedvegetative myceliumare shown in Fig. (1 and 2) . The strain had a type IV cell wall (meso-DAP, arabinose and galactose, whole-cell wall sugar pattern A) and type III phospholipids, Mycolates were absent. The predominant menaquions were MK-9 (H4-H6). On the basis of properties above, the strain showed be placed in the genus *Actinopolyspora* Gochnauer, *et a l.*,[6]

Group 2: This group includes 15 isolates of which isolate No.B-1 is the representaiveone. Isolate B-1 produces very small round colonies with powdery texture. Strain B-1 produce branched and un-fragmented substrate mycelia and monopodially branching aerial mycelia . The aerial hyphae bore non-motile spore on short sporophores in characteristic longitudinal pairs (Fig. 3 and 4). Each spore was oval and its surface was smooth. The cultural characteristics of isolate B-1 are shown in Table 4. The representative isolate of group 2 gave a white aerial mycelium on inorganic salt starch agar, glycerol-asparagine agar, oat-meal extract agar and yeast malt extract agar, but no diffusible pigment are produced. The isolate did not produce melanin pigments when cultivated on either peptone yeast iron agar or tyrosine agar.

The optimum temperature was 30°C, good growth was found at 37°C but no growth at 4°C, 40°C and 50°C. Isolate B-1 failed to grow at pH 4 and 11 but grow well at pH 7 and 8. The isolate B-1 gave growth in inorganic salt starch agar fortified NaCl on concentrations from 0.0 to 14% w/v. The isolate showed positive ability to assimilation the following carbon sources on carbon utilization medium; glucose, starch arabinose, sucrose, xylose, fructose, galactose, sodium pyruvate and sodium tartarate. Isolate are also able to utilized potassium nitrate, cystein, methonine, therionine and valine but not utilized serine and histidine as sole of nitrogen source Table 5. The sensitivity of isolate against antibiotics indicated that it is sensitive against gentamycin, tobramycin, rifamipcin and pencillin G, tetracyclin but resistant against oleandomycin, lincomycine, vancomycin, streptomycin and neomycin. It coulde able to degrade tyrosine, tween 80, starch, xylan, urea, testosterone, aesculine but not guanine, adenine DNA or RNA.

The cell wall contain meso-DAP, the whole-cell hydrolysate contain glucose, mannose, ribose but galactose, arabinose and xylose were not (whole-cell sugar pattern B of Lechevalier and Lechevalier[20]. The predominant menaquinones were MK-9 (H2) and MK-9 (H8). The cellular fatty acid compositions of the isolate

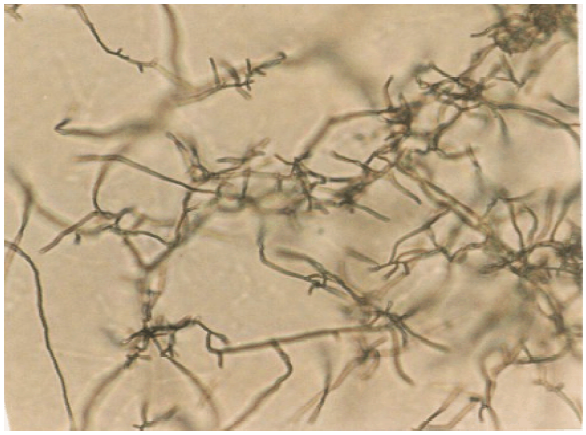

Fig. 5: Light micrograph of branched aerial hyphe in 14-days old culture R-1 X 1600

Table 4: Cultural characteristics of isolte B-I of 14 days old cultue on media , media fortified with 10% NaCl.

Media	Growth	Aerial mycelium	Substrate mycelium	Diffusible pigments
Nutrient agar	Moderate	White	Light yellow	nonpegmented
Glycerol aparagin agar ISP*	Intense	White	Pale yellow	nonpegmented
Inorganic salt starch agar ISP*5	Intense	Pale yellow	Pale yellow	nonpegmented
Yeast-extract malt exteract agar ISP*5	Intense	White	Pale yellow	nonpegmented
Oat meal agar ISP*5	Moderate	White	Pale yellow	nonpegmented
Peptone yeast extract iron agar	Moderate	White	Light yellow	nonpegmented
Tyrosine agar	Intense	White	Pale white	nonpegmented

Table 5: Physiological characteristics of strain B-1.

Characteristics	Strain B-1	Characteristics	Strain B-1
Carbon utilization		Growth in pressure of:	
L-arabinose	+ve	Gentamycine	-ve
D-fructose	+ve	Neomycin	+ve
D-galactose	+ve	Vancomycin	+ve
Glucose	+ve	Streptomycin	+ve
Sucrose	+ve	Triacylolandomycin	+ve
Strach	+ve	Lincomycin	+ve
D-xylose	+ve	Pencillin G	-ve
Rahmnose	-ve	Tobramycine	-ve
Raffinose	-ve	Rifampicin	-ve
Cellobiose	-ve	Chlorotetracyclin	-ve
Sodium acetate	-ve	Degradation of:	
Sodium pyruvate	+ve	Tyrosine	+ve
Sodium citrate	-ve	Tween 80	+ve
Sodium tartarate	+ve	Strach	+ve
Nitrogen utilization		Xylan	+ve
Potassium nitrate	+ve	Urea	+ve
Cysteine	+ve	Testosterone	+ve
Valine	+ve	Esculin	+ve
Serine	-ve	Guanine	-ve
Methinine	+ve	Adenine	-ve
Histidine	-ve	DNA	-ve
Therionine	+ve	RNA	-ve
Growth at:			
Optimum temperature	30-37°C		
Optimum pH:	7-8		
Optimum NaCl:	0-14%		

B-1 was characterized by the presence of phosphatidylethanolamine and glycophospholipids.

Identification of isolate of group 2: On the basis of microbiological properties and chemical composition, including the type of cell wall diaminopimilic acid, the sugar pattern, the phospholipids pattern, the predominant
species of menaquione and the lacke of mycolic acids, we concluded the strain B-1 should be placed in the genus *Microbispora*, Kroppensted[17] miyadoh *et al*[24].

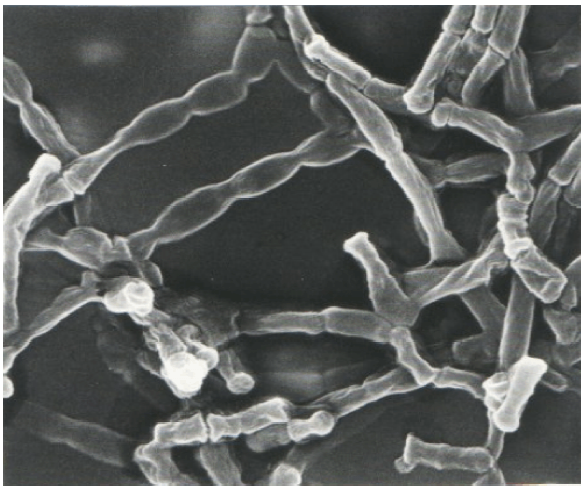

Fig. 6: Electron micrograph of strain R-1 showing smooth spores in 14-days old culture X 2500

Group 3: This group includes 20 isolates of which the isolate R-1 is the representative one. The isolate R-1 forming branching vegetative hyphae with a diameter o.5 to 1.5Mm which break down into squarish elements. White to gray aerial hyphae are produced. The aerial hyphae are fragment into spores (Fig 5).

Spores appear oblong or oval shaped. Spore surface is smooth (Fig. 6). The aerial hyphae was white on starch salt agar and glycerol asparagines agar but it produce gray color on oat meal agar (Table 6). No diffusible pigment are produced . The isolate did not produce melanin pigments, when cultivated on either peptone-yeast iron agar or tyrosine agar.

The optimum growth temperature was 10°C to 37°C but did not grow at 4°C or 45°C. The isolate produced good growth at pH 7.2 but failed to grow at pH 4.3 or 11. The isolate R-1 gave growth in inorganic salt starch agar fortified with NaCl on concentration 5-10% (w/v). Isolate R-1 was sensitive to penicillin, vancomycin, tetracyclin gentamycin but resistant to neomycin, oleandomycin,

Table 6: Cultural characteristics of isolte R-I of 14 days old cultue on media , media fortified with 10% NaCl.

Media	Growth	Aerial mycelium	Substrate mycelium	Diffusible pigments
Nutrient agar	Poor	White	Light yellow	nonpegmented
Glycerol aparagin agar ISP*	Intense	White	Pale yellow	nonpegmented
Inorganic salt starch agar ISP*5	Intense	Pale gray	Pale yellow	nonpegmented
Yeast-extract malt exteract agar ISP*5	Intense	Pale gray	Pale yellow	nonpegmented
Oat meal agar ISP*5	Moderate	Gray	Pale yellow	nonpegmented
Peptone yeast extract iron agar	Intense	White	Light yellow	nonpegmented
Tyrosine agar	Intense	Pale gray	Pale white	nonpegmented

Table 7: Physiological characteristics of strain R-1.

Characteristics	Strain R-1	Characteristics	Strain R-1
Carbon utilization		Growth in pressure of:	
L-arabinose	+ve	Gentamycine	-ve
D-fructose	+ve	Neomycin	+ve
D-galactose	+ve	Vancomycin	-ve
Glucose	+ve	Streptomycin	+ve
Sucrose	+ve	Triacylolandomycin	+ve
Strach	+ve	Lincomycin	+ve
D-xylose	+ve	Pencillin G	-ve
Rahmnose	-ve	Tobramycine	+ve
Raffinose	-ve	Rifampicin	+ve
Cellobiose	-ve	Chlorotetracyclin	-ve
Sodium acetate	+ve	Degradation of:	
Sodium pyruvate	-ve	Tyrosine	-ve
Sodium citrate	-ve	Tween 80	-ve
Sodium tartarate	+ve	Strach	+ve
Nitrogen utilization		Xylan	+ve
Potassium nitrate	+ve	Urea	-ve
Cysteine	+ve	Testosterone	-ve
Valine	-ve	Esculin	-ve
Serine	-ve	Guanine	+ve
Methinine	-ve	Adenine	+ve
Histidine	+ve	DNA	+ve
Therionine	+ve	RNA	+ve
Growth at:			
Optimum temperature	30-37°C		
Optimum pH:	7-8		
Optimum NaCl:	0-14%		

melanin pigments, when cultivated on either peptone-yeast iron agar or tyrosine agar.

The optimum growth temperature was 10°C to 37°C but did not grow at 4°C or 45°C. The isolate produced good growth at pH 7.2 but failed to grow at pH 4.3 or 11. The isolate R-1 gave growth in inorganic salt starch agar fortified with NaCl on concentration 5-10% (w/v). Isolate R-1 was sensitive to penicillin, vancomycin, tetracyclin gentamycin but resistant to neomycin, oleandomycin, rifampicin, streptomycin, tobramycin and lincomycin . The representative isolate are able to utilize arabinose, fructose, galactose, raffinose, rhamnose, glucose, sucrose, starch, sodium tartarate, sodium acetate and xylose but did not able to utilize cellobiose, sodium citrate and sodium pyruvate as sole carbon sources .

Isolate R-1 of group 3 are able to utilize cystein, histidin, potassium nitrate and therionin but not utilize valine, serin and methionin (Table 7). The isolate R-1 able to degrade starch, xylan, tyrosein, guanine, adenine, DNA, and RNA but not able to degrade tween 80, urea, testosterone and esculin. The cell wall analysis indicate the isolate contains major amounts of meso-diaminopimilic acid, the whole cell hydrolysate contain arabinose and galactose . The organism containdiphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and phosphatidylinositol as major polar lipids (PII). Mycolic acids are absent. The predominant menaquinons is MK-9 (H2-H4).

Identification of isolate of group 3: On the basis of the data described above, morphological and chemical analysis the isolate R-1 should be placed in the genus *Amycolatopsis* as *Amycolatopsis* sp.[22].

REFERENCES

1. Berkeley, R.C.W. and M. Goodfellow, 1981 The aerobic endospore forming bacteria: Classification and Identification . Acad. Press. London
2. Bergey, s Mannual of Systematic Bacteriology Vol.4 1989. S.T Williams, ed. Editonal board. Murray. Brenney Holt Krieg. Moulder. Pfennig. Sneath. Staley and William. The Williams and Wilkins Comp.Batim..
3. Collins, M.D., 1985. Isoperenoid quinine analysis in bacteria classification and identification, P. 267-278. In Goodfellow and D.E Minnikin (ed)., chemical methodsin bacterial systematic. Society for applied Bacteriology, Technica Series no.20Academic Press, London.
4. Diab and A. Al-Zaidan, 1976. Actinomycetes in desert of Kuwait. Zbl.Bakt. 11 Abt., 131: 545-554.
5. Diab, A., 1982. *Streptomyces spinoverrucous*, a new species from the air oKuwait Int. J. Bacteriol., pp: 327-331.

6. Gochnauer, M.B., G.G. Leppared. P. Komaratat, M. Kates T. Novitsky and D.J. Kuchner, 1975. Isolation and characterization of *Actinopolyspora halophila*. Gen.et sp. Nov. an extremely halophilic actinomycetes. Candian J. Microbiol., 21: 1500-1511.
7. Goodfellow, M. and R.G. Board, 1980. Microbiological classification and identification. Acad. Press, London.
8. Goodfellow, M. and T. Pirouz, 1982. Numerical classification sporoactinomycetes containing meso-diaminopimilic acid in the cell wall. J. Gen. Microbiol., 128: 503-527.
9. Goodfellow, M. and A.G. O'Donnell, 1989. Search and discovery of significant actinomycetes. In microbial productes. New approach. 44 the symposium of the society for General Microbiology ed. Baumberg, S.Hunter, I. and Rhodes, M. Chambridge. U.K: Cambridge University Press. pp: 343-383.
10. Gordon, R.E. and J.M. Mihm 1962. The type species of the genus *Nocardia.* J.Gen. Microbiol., 27: 1-10.
11. Gordon, R.E., 1966. Some strains in search of a genus *Corynebacterium, Mycobacterium, Nocardia*, or what ? J.Gen. Microbiol., 43: 329-343.
12. Gordon, R.E., 1967. The taxonomy of soil bacteria. In: The biology of soil bacteria, Ed. Liverpool University Press. pp: 239-321.
13. Gottlieb, D., 1973. General consideration and implication of the Actinomycetales In: Actinomycetales: Characteristics and Practical importance (G-Sykos and F.A. Skinner, eds), Academic Press. pp: 1-10.
14. Jackson, D., 1967. Soil chemical analysis prentice Hall.
15. Jahnson, L.E, E.A. Curl, J.H. Bond and H.A. Fibourg, 1960. Methods for studying soil microflora. Burgess Publishing Co. Minneapolis, U.S.A, p.
16. Kuster, E., 1972. Simple working key for the classification and identification of named taxa included in the international *Streptonyces* Project: Intl. J. Syst Bacteriol., 22: 139-148.
17. Kroppenstedt, R.M., E. Stackebrandt and M. Goodfellow, 1990. Taxonomic revision of the actinomycet genera Actinomadura and Microtetraspora.Syst. Appl. Microbiol., 13: 148-160.
18. Larsen, H., 1986. Halophilic and halotolerant microorganisms: an overview historical perspective, F EMS Microbial Rev., 39: 3-7.
19. Lechevaier, H.A., 1968. Status of the generic names *Micropolyspora* Lechevalier *et al*., 1961 and *Micropolyspora shchepkina* (Actinomycetales). I. J. of systematic Bacteriol., 18: 203-206.
20. Lechevalier, M.P. and H.A. Lechevalier, 1970. Chemical composition as an criterion in the classification of aerobic actinomycetes. Intl. J. Syst. Bacteriol, 20: 435-443.
21. Lechevalier, M.P. and H.A. Lechevalier, 1980. The chemotaxonomy of actinomycetes. In A. Dietz and D.W. Thayer (ed). Actinomycetes taxonomy. Special Publication no. 6. Society for Industerial Microbiology. Arlington, Va. pp: 227-291.
22. Lechevalier, M.P., H. Prauser, D.P. Labeda and J.S. Ruan 1986. Two new genera of nocardioform actinomycetes; *Amycolata* gen. nov. and *Amyclatopsis* gen.nov. Intl. J. Syst. Bacteriol., 36: 29-37.
23. Minnikin, D.E., I.G. Hutchinson, A.B. Caldicontt and M. Goodfellow, 1980. Thin layer chromatography of methanolsates of mycolic acid-containing bacteria. J. Chromatography, 188: 221-233.
24. Miyadoh, S., S. Amano, H. Tohyama and T. Shonura, 1990. A taxonomic review of the genus *Micropolyspora* and a proposal to transfer two species to the genus *Actinomadura* and to combine ten species into Microbispora rosea J. Gen. Microbiol., 136: 1905-1913.
25. Shrilling, E.B. and D. Gottlieb. (1966. Methodes of characterization of *Streptomyces* species. Intl. J. Bacteriol., 61: 313-340.
26. Shrilling, E.B. and D. Gottlieb, 1968a. Cooperative description of type cultures *of Streptomyces* II. Species description from first and second studies. Intl. J. Syst. Bacteriol., 18: 69-189.
27. Shrilling, E.B. and D. Gottlieb, 1968b. Cooperative description of type cultures of *Streptomyces* III from first and second studies. Intl. J. Syst. Bacteriol., 18: 279-392.
28. Shrilling, E.B. and D. Gottlieb, 1969. Cooperative description of type culture *Streptomyces* IV species description from the second, third and forth studies Intl. J. Syst. Bacteriol., 19: 391-512.
29. Sierra, G., 1957. A simple method for the detection of lipolytic activety of microorganisms and some observations on the influence of the contact between cells and fatty substrates. Antoni Van leeuwenhoeck 23: 15-22.
30. Staneck, J.L. and G.D. Robert, 1974. Simplified approach to identification of aerobic actinomycetes by thin layer chromatography. Appl. Microbiol., 28: 226-231.
31. Tresner, H.D., J.A. Hayes and E.J. Backus, 1968. Differential tolerance of *Streptomycetes* to sodium chloride as a taxonomic aid. J. Appl. Microbiol., 16: 1134-1136.