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Research Article

Isolation, characterization and antibacterial activity of a Rare Actinomycete: *Saccharopolyspora* sp. In Iraq

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Abstract: The aimed of the study is isolating and identifying the rare actinomycetes species in Iraq, that are capable of producing antimicrobial agent. Thirty-three colonies were found. Colonies having characteristic features such as powdery appearance with convex, concave or flat surface and color ranging from buff, brown, pink, red, white, yellow and gray were selected. Sub-cultured on ISP2 were supplemented with 12 % (w/v) NaCl for growth, and incubation of plates for 10-12 days at 28°C, only five isolates demonstrated cultural characteristics similar to that of genus *Saccharopolyspora* sp. Five isolates were selected and purified by pure culture techniques. All isolates were given a number as AW12, AW 4, MK 7, AW 9 and AW 2. The isolates were identified as *Saccharopolyspora* sp. based on their morphological, physiological and biochemical characteristics. All *Saccharopolyspora* isolates were screened for their antibacterial activity on malt extract yeast extract agar medium (ISP2) using scross-streak technique. The highest activities were shown by AW12 isolate against *Staphylococcus aureus* with inhibition zone diameters 17 mm.

Keywords: rare actinomycetes, *Saccharopolyspora*, antibacterial, Iraq.

INTRODUCTION

Actinomycetes are filamentous bacteria, (Ventura *et al*., 2007). The soil actinomycetes produce a volatile compound called geosmin, which literally translates to "earth smell" of healthy soil (Gust *et al.*, 2003; Sprusansky *et al*., 2005). Actinomycetes are Gram-positive bacteria. Although *Streptomyces* species still promise to remain fruitful sources of new antibiotics (Amin *et al.*, 2016; Risan *et al.*, 2016; Risan *et al.*, 2017; Risan *et al.*, 2018; Al-Rubaye *et al.*, 2018a, b). The Recent tendency has shown more frequent detection of a number of new antibiotics from micro-organisms other than *Streptomyces* as rare Actinomycetes. Rare actinomycetes once isolated from soils are much easier to die than *Streptomyces* species. Rare actinomycetes, show much slower growth than Streptomyces species. Some require vitamin and biotin for growth and most of them have more complex nutritional requirements (Lechevalier and Lechevalier 1981). The characteristics of rare actinomycetes are slower growth, more complex nutritional requirements, poorer sporulation and less stable as for preservation (Kroppenstedt and Goodfellow 2006). The family pseudonocardiaceae belong to rare actinomycetes. This family currently contains seven genera, *Actinopolyspora*, *Amycolata*, *Amycolatopsis*, *Kibdelosporangium*, *Pseudonocardia*, *Saccharomonospora* and *Saccharopolyspora*. It includes several cultures with industrial interest as antibiotic producers (erythromycin, vancomycin, rifamycin) or in bioconversion processes (Okazaki *et al.*, 1983). All of pseudonocardiaceae members are aerobic and catalase-positive, but exhibit a wide range of different physiologies (autotrophic, thermophilic, halophilic, etc.) (Embley 1992). Pseudonocardiaceae family includes genus *Saccharopolyspora* (Lazzarini *et al*., 2000; Tiwari and Gupta, 2013). The genus *Saccharopolyspora* was first described by Lacey and Goodfellow (1975). The genus *Saccharopolyspora* includes species: *Saccharopolyspora erythraea* (Labeda, 1987), *Saccharopolyspora gregorii* and *Saccharopolyspora hordei* (Goodfellow *et al.*, 1989), *Saccharopolyspora rectivirgula* and *Saccharopolyspora taberi* (Korn-Wendisch *et al.*, 1989), *Saccharopolyspora spinosa* (Mertz and Yao, 1990), *Saccharopolyspora hirsuta*

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(Lacey and Goodfellow, 1975), *Saccharopolyspora spinosporotrichia* (Zhou *et al.*, 1998), *Saccharopolyspora flava* and *Saccharopolyspora thermophila* (Lu *et al.*, 2001), *Saccharopolyspora antimicrobica* (Yuan *et al.*, 2008), *Saccharopolyspora cebuensis* (Pimentel-Elardo *et al.*, 2008), *Saccharopolyspora shandongensis* (Zhang *et al.*, 2008), *Saccharopolyspora endophytica* (Qin *et al.*, 2008), *Saccharopolyspora halophila* (Tang *et al.*, 2009a), *Saccharopolyspora jiangxiensis* (Zhang *et al.*, 2009), *Saccharopolyspora qujiaojingensis* (Tang *et al.*, 2009b), *Saccharopolyspora rosea* (Yassin, 2009), *Saccharopolyspora tripterygii* (Li *et al.*, 2009) and *Saccharopolyspora patthalungensis* (Duangmal *et al.*, 2010).

Members of this genus are aerobic, Gram-positive, non-acid-fast organisms with substrate hyphae that either fragment into rod-shaped elements, do not fragment or are transformed partially into chains of spores (Korn-Wendisch *et al.*, 1989). The combination of a green to bluish green color of the aerial and substrate mycelia and monosporic morphology appears to be a good indication that an isolate belongs to the genus *Saccharomonospora*. The genus encompasses 27 recognized species of which 17 were identified in the last 10 years. Most of these species were isolated from soil samples (Cheng *et al*., 2013), saline lakes (Tang *et al*., 2009) and endophytic associations (Qin *et al*., 2008). Furthermore, some members of *Saccharopolyspora* have been recovered from symbiotic associations with marine sponges. So the aim of the study is isolating and identifying the rare actinomycetes species in Iraq, that are capable of producing antimicrobial agent.

MATERIALS AND METHODS

Sample Collection

Soil samples were collected from two provinces in 2018, Baghdad city (Al-Jadriya) and Wasit province (Al - Noamaniya). The samples were taken at a depth of 5-7 centimeters below the surface, where most of the microbial activity takes place, and thus where most of the bacterial population is concentrated. Soil samples (100 g sample of each soil type) were collected by using clean, dry and sterile Polyethylene bags along with sterile spatula, marking pen, rubber band and other accessories. The site selection was done by taking care of the point where widely varying characteristics as possible with regard to the organic matter, moisture content, and particle size and colour of soil and to avoid contamination as far as possible. Samples were stored in boxes and transported to the laboratory where they were kept in a refrigerator at 4^0C until analysis.

Tested Microorganisms

Three pathogenic bacteria (*Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*) were used for the antibacterial tests. Pathogenic bacteria were obtained from Central Public Health Labrotory in Baghdad city. They were maintained on Mueller-Hinton (MH), plates at 4ºC. Stock cultures were kept in 20% glycerol at -70ºC.

Isolation of Actinomycetes

Each soil sample was air dried at room temperature and sieved through a 2 mm pore size sieve to get rid of large debris. The sieved soil was used for the isolation purpose. One g was suspended in 99 ml sterile distilled water and incubated at 28°C with shaking at 180 rpm for 1 h. (Oskay *et al.*, 2004), 0.1 ml of each solution, at dilutions of 10^{-4}-10^{-5}, then spread on Glycerol yeast extract agar medium (GYEA) (2g Yeast extract, 10ml Glycerol, 20 agar and 1 L water) supplemented with tetracycline 50 µg/ml and 50 µg/ml nystatin, to prevent growth of other bacteria and fungi, respectively. (The pH value of the medium was adjusted to 7-7.2 and then sterilized in an autoclave at 121°C for 15 minutes), then incubated at 28°C for 8-12 days still actinomycetes colonies appear. (El-Nakeeb and Lechevalier, 1963; Kuster and Williams, 1964; Qasim and Risan, 2017). After the incubation period, the plates were examined for typical actinomycetes colonies, which had regular round, small, opaque, compact, frequently pigmented with white, brown, gray-pink, or other colors, the colonies were examined under a light microscope to observe their morphology and distinguished from fungi colonies. The isolated actinomycetes were re-cultured and stored at 4°C for further study (Venkateswarlu *et al*., 2000; Gesheva et *al.*, 2005).

Purification And Identification Of Actinomycetes spp

After incubation, plates were examined for the appearance of actinomycetes colonies. Colonies of actinomycetes were recognized by their microscopic characteristics (optical microscopy, aerial, substrate mycelium colour and Gram's stain). The stock cultures were maintained and transferred on International *Streptomyces* project Medium slants (ISP-2) (4g Yeast extract, 10g Malt extract, 4g Dextrose, 20 agar and 1 L water) to obtain pure colonies used for identification (Nonoh et al., 2010 ; Whitman et al., 2012). Once in four months and stored at 4°C.

Identification by Cultural Characterizations:

The identification of *Saccharopolyspora* isolates were based largely upon the morphological observations. It includes some basic observations, Gram's stain, produced Substrate mycelia (Branched or Fragments), spores on substrate mycelia, colors of the substrate mycelia, aerial mycelia and colour of soluble pigment (Prauser, 1964; Aghighi *et al.*, 2004).

Identification by Biochemical Characterizations

Biochemical characterization, various biochemical tests were performed for the identification of *Saccharopolyspora* isolates. These tests, including,

Carbon Source Utilization (several sugar types were used such as L-Arabinose, D-Fructose, D-Galactose, Glycerol, D-Lactose, D-Maltose, D-Mannitol, D-Mannose, D-Raffinose, L-Rhamnose , Sucrose and D-Xylose), Catalase production test, Urea test and Starch Hydrolysis (Shirling EB, Gottlieb; Korn-Wendisch and kutzner., 1992 ; Macfaddin, 2000).

Identification by physiological Characteristics

Physiological properties of all isolates of *Saccharopolyspora* species. According to (Khan and Patel, 2011), growth was tested at 20- 40°C on ISP2 medium and pH range for growth was investigated between pH 5.0 - 8.0, and 12–16% NaCl.

Growth At Different Temperature

Growth at in different temperatures was tested by incubating ISP2 slants inoculated with bacterial suspension of the test isolates at 20, 28, 37 and 40°C in an incubator for 12 days (James *et al*., 1991).

Growth at different pH

According to Khan and Patel (2011) locally isolated *Saccharopolyspora* were grown on yeast extract malt extract broth (ISP2) supplemented with different pH 5, 6 and 8 in order to obtain an optimum pH of the isolates.

Effect of salt concentration

Sodium chloride (NaCl) tolerance of locally isolated *Saccharopolyspora* was evaluated by growing them in malt extract yeast extract broth (ISP2) medium supplemented with graded doses of sodium chloride (12, 14, 16 % w/v), after 12 days of incubation at 28°C, the results were observed by reading their absorbance at 600nm (Tresner *et al.,* 1968).

Screening for Actinomycetes activity

Primary screening

Primary screening for antimicrobial activities was, according to (Kumar *et al.,* 2012), by using cross-streak technique, in which the *Saccharopolyspora* isolates were used against three pathogenic bacteria, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. The *Saccharopolyspora* were streaked as across lines in the center of plates poured with malt extract yeast extract agar medium (ISP2) and inoculated plates were incubated at 28°C for 5 days to secrete antibiotics into the medium. Each streaking was situated near the edge of the plates and streaked toward the *Saccharopolyspora* growth line. The positive results were observed by the naked eye.

Secondary screening (well plate method)

The *Saccharopolyspora* isolates which showed higher production of bioactive compounds during primary screening, were submitted to secondary screening for antimicrobial activity. The most active isolate was chosen for identification and characterization of antimicrobial metabolites. Supernatant that prepared in last step were collected and separated from the crude precipitation for each isolate by centrifugation at 10,000 rpm \ 2 min. After solidification of 20 ml of sterilized muller-Hinton agar, spread 100 microliters of pathogenic activated bacteria by L shape spreader. Wells (6 mm in diameter) were prepared in each seeded agar plate and each well was filled with 100 microliters of filtered supernatant (0.45µm) and screened via agar well diffusion procedure mentioned previously against the reference strains, the same was done to the crude precipitation by separation the supernatant and leaving a little amount of the broth to mix them very well, then each well was filled with 100 microliters of each crude precipitation, the plates incubated at 37 °C and the zone of inhibition was determined after 24 hours overnight. (Deshmukh and Sridhar, 2002; Al-Rubaye *et al*., 2018b).

RESULTS AND DISCUSSION

Isolation of Actinomycetes

The serial dilution technique was used to isolate actinomycetes from ten different soil sources after inoculating the plates with soil suspension on glycerol yeast extract agar (GYEA) supplemented with tetracycline 50 µg/ml and 50 µg/ml nystatin, and incubation of plates for 10-12 days with a dilution of 10^{-4}, 10^{-5}. Collection and isolation of actinomycetes from soil samples. In this study, ten soil samples were collected from two different sites of Baghdad city (Al-Jadriya) and Wasit province (Al – Noamaniya). Colonies of actinomycetes were recovered from soil samples. These isolates have been cultured and purified on GYEA. Thirty-three colonies were found. Colonies having characteristic features such as powdery appearance with convex, concave or flat surface and color ranging from buff, brown, pink, red, white, yellow and grey were selected, table (1). Glycerol yeast extract agar medium is specific and sensitive for actinomycetes since it contains glycerol, more actinomycetes use as a sole carbon source (Oskay *et al.,* 2004). Nystatin reduces fungal growth, whereas tetracycline reduces other bacteria. Colony size varied, powdery, colour varied from chalky white, buff, brown, pink, red, white, yellow and grey. They were grown on a glycerol yeast extract agar, and this was in agreement with that described by (Saadoun *et al.,* 2015; Risan *et al.,* 2016).

Table (1): Actinomycetes colonies appear on glycerol yeast extract agar (GYEA) for 10- 12 days

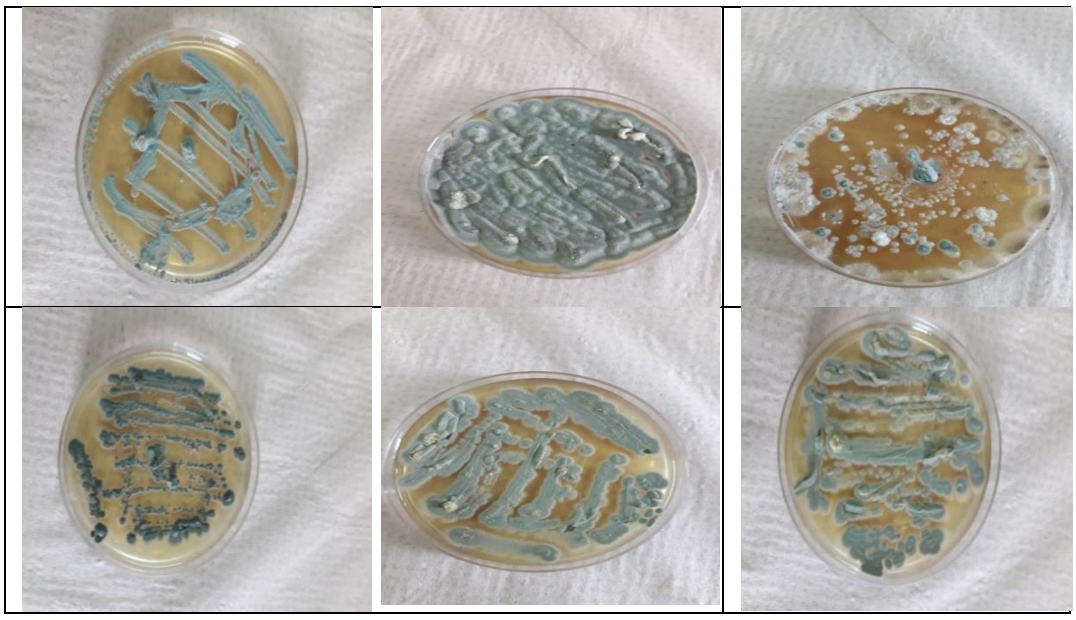
Soil samples sites	Actinomycetes colonies	Total colonies
Al – Noamaniya / Wasit province	3	13
Al – Noamaniya / Wasit province	1	
Al – Noamaniya / Wasit province	3	
Al – Noamaniya / Wasit province	2	
Al – Noamaniya / Wasit province	4	
Al-Jadriya / Baghdad city	2	20
Al-Jadriya / Baghdad city	4	
Al-Jadriya / Baghdad city	6	
Al-Jadriya / Baghdad city	5	
Al-Jadriya / Baghdad city	3	

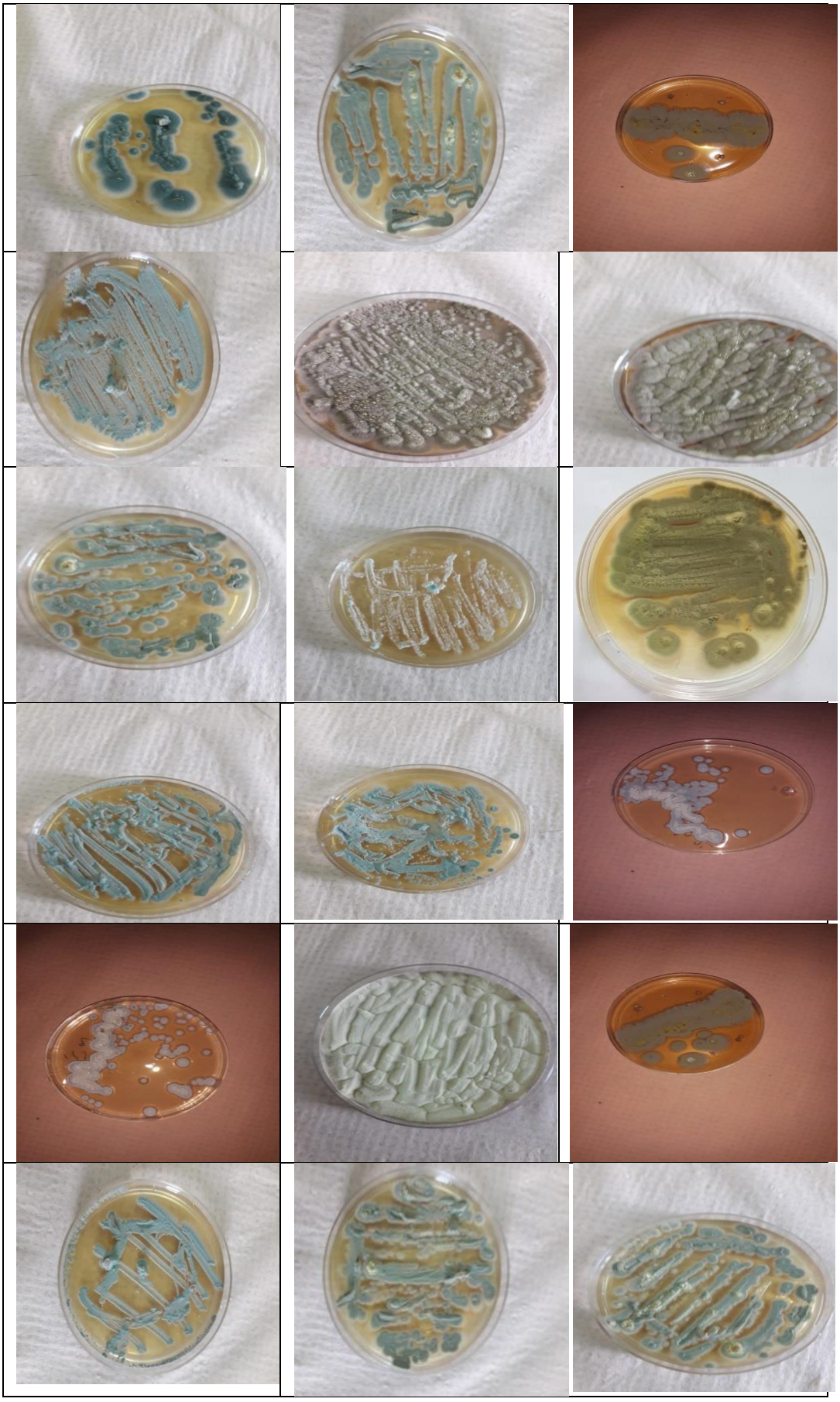
The morphology and size of the colonies were about 1-12 mm in diameter with a relatively smooth surface at the beginning of the growth, buff, brown, pink, red, white, yellow and grey, it was developed to an aerial mycelium that appeared as granular, powdery and soft. (Stackebrandt and Goebel, 1994) and Ramazani *et al.* (2013) described actinomycetes colonies being slow growing, glabrous or chalky, aerobic, piled, as well as with different color of aerial and substrate mycelium. In addition, all isolated colonies possess an earthy odor.

From 10 soil samples, Thirty three colonies were obtained (Fig. 1). Colonies selected from each plate were five to seven based on colony appearance. Colonies having characteristic features such as powdery appearance with convex, concave or flat surface and color ranging from white, buff, brown, pink, red, white, yellow and grey were selected.

Out of the thirty three actinomycetes colonies. Sub-cultured on ISP2 were supplemented with 12 % (w/v) NaCl for growth, and incubation of plates for 10-12 days at 28°C, only five isolates demonstrated cultural characteristics similar to that of genus *Saccharopolyspora* sp. Five isolates were selected and purified by pure culture techniques. All isolates were given a number as AW12, AW 4, MK 7, AW 9 and AW 2 (Table 2), (Fig. 2). Five of the isolates were recovered from soil samples 4 isolates from Al – Noamaniya and 1 isolate from Al-Jadriya. All of the isolates were considered as *Saccharopolyspora* sp to tolerate the isolates for NaCl.

The results were in agreement with the finding of both Zhou *et al.* (2007), (Kim and Goodfellow, 2015), (Whitman *et al.*, 2012) (Bergey's Manual of Systematic Bacteriology, V 5: The Actinobacteria, concerning the isolation process that each plate was often contained one or few colony types ranging from two to four colonies, and from similar habitats the actinomycetes diversity exhibited few different colony types.





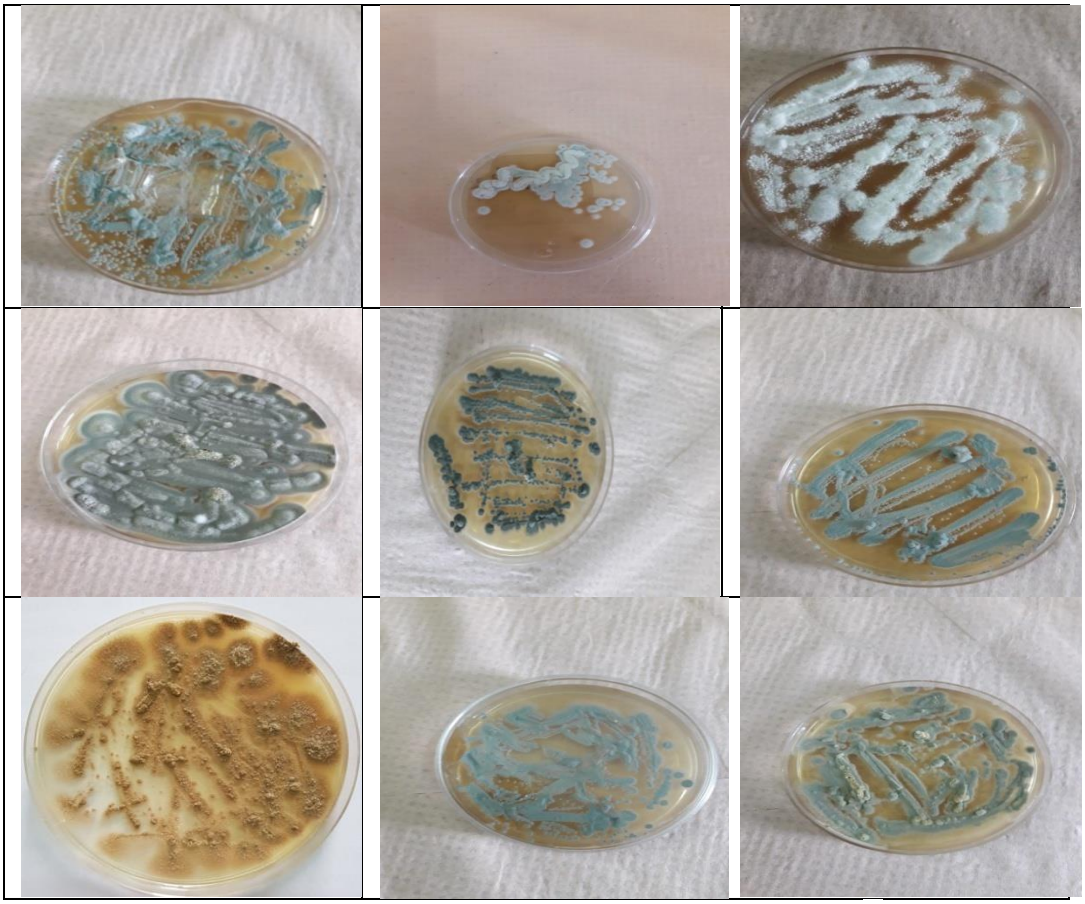


Fig (1): Local Actinomycetes spp. isolates grow on glycerol yeast extract agar (GYEA) for 10- 12 days

Table (2): The Growth characteristics of colony on medium ISP2with 12 % (w/v) NaCl

Medium	Isolates	Growth
ISP2	AW12	++
	AW 4	++++
	MK 7	+
	AW 9	+++
	AW 2	++++
+ : less ++: moderate +++ : good ++++: very good		

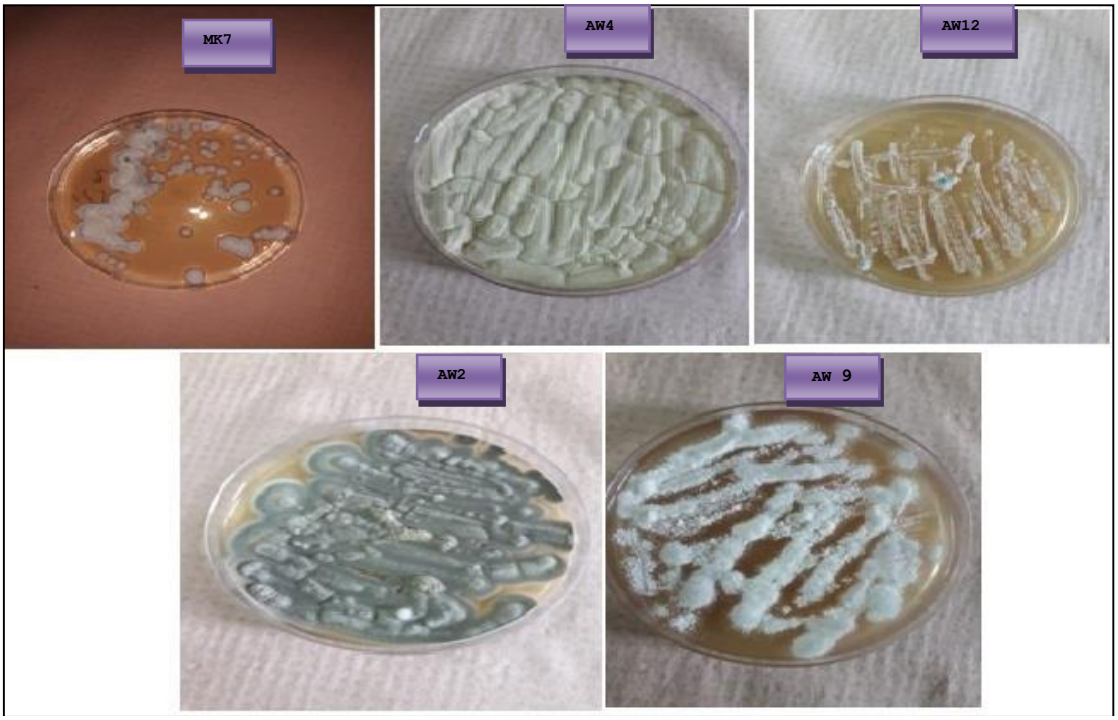


Fig (2): Local Actinomycetes Spp. Isolate grow on on glycerol yeast extract agar (GYEA) for 10- 12 days

Purification and identification of *Saccharopolyspora* sp

The colours of substrate and aerial mycelia and any soluble pigments produced were determined. Five isolates grew well on ISP 2 agar and potato dextrose agar (PDA) and showed no growth on nutrient agar, table (3). The colour of aerial and substrate mycelia was white, orange, brown, pink, yellow and grey. Growth characteristics of isolates were observed by light microscopy, after 12 days growth on ISP 2 and PDA agar medium containing 12 % (w/v) NaCl. Growth was tested at 280C on ISP2, nutrient agar and PDA medium . Rare actinomycetes, show much slower growth than *Streptomyces* species. Some require vitamin and biotin for growth and most of them have more complex nutritional requirements. Sporulation of rare actinomycetes is much poorer than *Streptornyces* species. Partly because of poor sporulation, it is often difficult to maintain rare actinomycetes in viable form for a long period of time unless kept in lyophile tubes or in liquid nitrogen. Rare actinomycetes once isolated from soils are much easier to die than *Streptomyces* species. These characteristics are good reasons why rare actinomycetes have not been isolated so frequently as *Streptomyces* species. In addition, rare actinomycetes occur in nature ecologically in much less frequently than *Streptomyces* species according to Lechevalier and Lechevalier (1981).

Collection of rare actinomycetes from natural sources and river water (Hayakawa, 2008) were reported as good sources for *Saccharopolyspora*, *Micromonospora* and *Streptosporangium.* The characteristics of rare actinomycetes are, slower growth, more complex nutritional requirements, Poorer sporulation and less stable as for preservation (Kroppenstedt, 1982). *Saccharopolyspora* spp. is anaerobic, Gram-positive, non-acid-fast actinomycete belong to family *Pseudonocardiaceae* that also includes the genera *Pseudonocardia* and *Saccharomonospora*. *Saccharopolyspora* contain profusely branched substrate hyphae (fragmenting into rod-shaped elements), and aerial hyphae (forming bead-like chains of spores (Zhou *et al.*, 1998). A number of species have been reported with the ability to produce antibacterial (Oliynyk *et al.*, 2007; Yuan *et al.*, 2008)

Table (3). Growth characteristics of *Saccharopolyspora* isolates after 12 days growth on ISP 2, nutrient agar and PDA agar medium containing 12 % (w/v) NaCl

Characteristic	*Saccharopolyspora* AW12	*Saccharopolyspora* AW 4	*Saccharopolyspora* MK7	*Saccharopolyspora* AW 9	*Saccharopolyspora* AW 2
Growth on:PDA	Moderate	abundant	moderate	abundant	abundant
ISP2	Moderate	abundant	Weak	good	abundant
nutrient agar	-	-	-	-	-

- : no growth

Cultural and Microscopical Characterization of *Saccharopolyspora* isolates

All Saccharopolyspora sp isolates were Gram's stain (Table 4). Young cultures (6 to 8 days old) produced substrate mycelia, Branched or Fragments, no spores on substrate mycelia of all isolates, like those described by Lacey (1989). The colors of the substrate mycelia and aerial mycelia of the five isolates. (AW12, AW4, MK7, AW9 and AW2), varied from colorless to white, orange, brown, pink, yellow and grey on ISP2 (Table 4). The color of soluble pigment of *Saccharopolyspora* sp AW12, *Saccharopolyspora* sp AW4 and *Saccharopolyspora* sp AW2 were grey-violet, grey and dark brown-green respectively on ISP2 supplemented with 12% NaCl, in contrast, *Saccharopolyspora* sp MK7, AW no produce soluble pigment.

Table (4). Cultural and microscopical characteristics of *Saccharopolyspora* isolates after 12 days growth on ISP 2 medium containing 12 % (w/v) NaCl

	Characteristic	*Saccharopolyspora* AW12	*Saccharopolyspora* AW 4	*Saccharopolyspora* MK7	*Saccharopolyspora* AW 9	*Saccharopolyspora* AW 2
1	Gram's stain	+	+	+	+	+
2	Substrate mycelia	Branched	Fragments	Fragments	Fragments	Branched
3	Spores on substrate mycelia	-	-	-	-	-
4	Colour of aerial mycelia	white - pink	Grey – yellow- white	White- orange	white	Grey- brown
5	Colour of substrate mycelia	white - brown	Grey	orange	white	Grey- White
6	Colour of soluble pigment	grey-violet	grey	None	None	dark brown-green

Abbreviations: +, positive; - , negative;

Biochemical Characteristics

The biochemical properties are summarized in (Table 5). All of the isolates belonging to the *Saccharopolyspora* sp. Biochemical studies in the taxonomy of rare actinomycetes are indispensable not only for taxonomy itself, but also for the recognition, during screening of most of the genera belonging to rare actinomycetes which usually lack distinctive morphological features (Kroppenstedt and Goodfellow,2006). Results agreed with (Goodfellow *et al.*, 1989; Lu *et al.*, 2001; Tang *et al.*, 2009a; Duangmal *et al*., 2010).

Table (5). Biochemical characteristics of Saccharopolyspora isolates after 12 days growth on ISP 2 medium containing 12 % (w/v) NaCl

	Characteristic	*Saccharopolyspora* AW12	*Saccharopolyspora* AW 4	*Saccharopolyspora* MK7	*Saccharopolyspora* AW 9	*Saccharopolyspora* AW 2
1	Catalase test	+	+	+	+	+
2	Utilization of carbohydrates as sole carbon source: L-Arabinose	-	+	+	-	+
	D-Fructose	+	+	+	+	+
	D-Galactose	+	+	+	+	+
	Glycerol	+	+	+	+	+
	D-Lactose	-	-	-	+	+
	D-Maltose	+	+	+	+	+
	D-Mannitol	+	+	+	+	+
	D-Mannose	+	+	ND	+	+
	D-Raffinose	+	+	+	+	ND
	L-Rhamnose	+	+	+	+	+
	Sucrose	+	+	+	+	+
	D-Xylose	+	+	+	+	+
3	Degradation of: Starch Urea	+ +	+ -	+ +	+ -	+ -

Abbreviations: +, positive; -, negative; ND, not determined.

Physiological Characteristics

Physiological properties of all isolates of the five *Saccharopolyspora* species (Table 6). Growth was tested at 20, 28, 37 and 40°C on ISP2 medium and pH range for growth was investigated between pH 5.0 - 8.0, and 12–16% NaCl. All isolates *Saccharopolyspora* sp. grew at temperatures ranging from 20 to 40°C, except isolate AW 4 no grow at 20°C and some isolates

Saccharopolyspora sp. were grew weak at 20°C. All isolates of *Saccharopolyspora* were moderately thermophilic, growing at temperatures ranging from 37 and 40°C. And all isolates *Saccharopolyspora* sp were mesophilic, growing at temperatures ranging from 20 and 30°C. The isolates of *Saccharopolyspora* sp grew in a wide range of NaCl concentrations (12–16% NaCl w/v on ISP2 medium) and were strictly halophilic. Isolates *Saccharopolyspora* MK7 and *Saccharopolyspora* AW 2 no grow in 16% NaCl (%, w/v). The isolates of *Saccharopolyspora* sp grew in a range of pH 5.0 - 8.0. Isolates *Saccharopolyspora* MK12, *Saccharopolyspora* AW 9 and *Saccharopolyspora* AW 2 no grow at pH 5.0. The results of the current study agree with the studies conducted by (Mertz and Yao, 1990 ; Zhou *et al.*, 1998; Lu *et al.*, 2001; Yuan *et al.*, 2008), who reported that their *Saccharopolyspora* isolates were grows at NaCl concentrations ranging from 12% to 18% (w/v), and temperature for growth is between 20 and 45°C, and PH 6- 8.5.

Table (6). Physiological characteristics of Saccharopolyspora isolates after 12 days growth on ISP 2 medium.

	Characteristic	*Saccharopolyspora* AW12	*Saccharopolyspora* AW 4	*Saccharopolyspora* MK7	*Saccharopolyspora* AW 9	*Saccharopolyspora* AW 2
1	Growth at: 20°C 28°C 37°C 40°C	(Weak) + + + +	- + + +	(Weak) + + + +	+ (Weak) + + +	+ (Weak) + + +
2	Growth on: 12%NaCl (%, w/v) 14% NaCl(%, w/v) 16% NaCl(%, w/v)	+ + +	+ + +	+ + -	+ + +	+ + -
3	pH: 5.0 6.0 8.0	- + +	+ + +	+ + +	- + +	- + +

Abbreviations: +, positive; -, negative;

Screening for *Saccharopolyspora* isolates activity

All *Saccharopolyspora* isolates (AW12, AW4, MK7, AW 9 and AW2) were screened for their antibacterial activity on Yeast extract-malt extract agar medium (ISP2) using scross-streak technique against three pathogenic bacteria include Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) and Gram-positive (*Staphylococcus aureus*). Among five *Saccharopolyspora* isolates that obtained from two different sites of Baghdad city (Al-Jadriya) and Wasit province (Al-Noamaniya), one isolate (MK7) didn't show any antibacterial activity against any type of pathogenic bacteria (Gram-negative and Gram-positive bacteria), while one *Saccharopolyspora* isolates (AW2) showed antibacterial activity against only Gram positive bacteria. However the others three *Saccharopolyspora* isolates (AW12, AW4 and AW9) showed antibacterial activity against both Gram negative and Gram positive bacteria. A broad spectrum of antibacterial activity was observed in 80% (4 out of 5) (AW12, AW4, AW9 and AW2) of the total *Saccharopolyspora* isolates (Table 7).

Table (7): Primary screening of *Saccharopolyspora* isolates using scross-streak technique on Yeast extract-malt extract agar medium

Isolates	Gram-positive	Gram-negative		Note
	S. aureus	*P. aeruginosa*	*E. coli*	
AW12	+ve	+ve	+ve	Selected
AW 4	+ve	+ve	+ve	Selected
MK 7	-ve	-ve	-ve	Neglected
AW 9	+ve	+ve	+ve	Selected
AW 2	+ve	-ve	-ve	Selected

Screening was performed by Agar-Well Diffusion method and growth inhibition zones were measured in millimeters for each of the *Saccharopolyspora* isolates (AW12, AW4, AW9 and AW2), the results are shown in Table (8). Tested isolates have shown potent *in vitro* antibacterial activities against all tested pathogens. The highest activities were shown by isolate AW12 against S. *aureus* 17 mm, *Pseudomonas aeruginosa* 9mm, *Escherichia coli* 9.8 mm. It is also evident that isolates AW 4 and AW 9

have shown activities against all pathogenic bacteria with inhibition zone diameters ranging between 11 and 16 mm. Isolate AW 4 have shown moderate inhibitory effect against pathogenic bacteria with inhibition zone diameters in the ranging between 11 and 13.7mm, and isolate AW 9 with inhibition zone diameters in the ranging between 12 and 16 mm, while isolate AW 2 shown moderate inhibitory effect against only pathogenic bacteria *S. aureus* with inhibition zones 11 mm.

Table (8): Inhibition zones (mm) by different *Saccharopolyspora* isolates against pathogenic bacteria

Isolates	Zone of inhibition (mm)		
	S. aureus	***P. aeruginosa***	***E. coli***
AW12	17.0	9.0	9.8
AW 4	13.7	11.0	12.5
AW 9	12.0	14.2	16.0
AW 2	11.0	-	-

- No test

Saccharopolyspora is a well-documented producer of antibiotics (Bhattacharyya *et al.*, 1998), with *Saccharopolyspora* erythraea well known for the industrial production of the antibiotic erythromycin. Sibanda *et al.*, (2010), found Crude extracts of actinomycetes species belonging to *Saccharopolyspora* TR 046 and TR 039 were screened for antibacterial activities against a panel of several bacterial strains. The extracts showed antibacterial activities against both gram-negative and gram-positive test bacteria with inhibition zones ranging from 8 to 28 mm (TR 046) and 8 to15 mm (TR 039). Minimum inhibitory concentrations ranged from 0.078 to 10 mg/mL (TR 046) and 5 to >10 mg/mL (TR 039). The extract was however weakly bactericidal against two environmental bacterial strains (*Klebsiella pneumoniae* and *Staphylococcus epidermidis*); and against *Pseudomonas aeruginosa* (ATCC 19582): the extract showed bacteriostatic activities at all concentrations tested. Study Pandey *et al.*, (2004) Antibacterial activity of actinomycetes isolated. A total of 106 actinomycetes were subjected to primary screening by perpendicular streak method against Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram-negative (*Enterobacter aerogens*, *Escherichia coli*, *Klebsiella* species, *Proteus* species, *Pseudomonas* species, *Salmonell typhi* and *Shigella* species) test bacteria. It was observed that 2 isolates were active against only Gram-negative bacteria, 8 against Gram-positive and 26 against both Gram-positive and Gram-negative bacteria. Also found antibacterial substances were extracted with ethyl acetate from isolate-inoculated starch-casein broth fermented for 7 days at 28°C by solvent extraction method. Minimum bactericidal concentration (MBC) of ethyl acetate extract against *Staphylococcus aureus* was 1.25 mg/ml for *Saccharopolyspora* species

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