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Streptomyces Waksman and Henrici (1943) species and their applications anti-pathogenic bacteria and study its compatibility with plant extracts

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Abstract

Twelve samples were collected from Baghdad city. From 12 samples, 17 colonies were obtained. Out of the 17 Actinomycetes colonies. sub-cultured on ISP2 for growth, and incubation of plates for 7 days, only three isolates demonstrated cultural characteristics similar to that of *Streptomyces* sp. three isolates were selected and purified by pure culture techniques of *Streptomyces* sp. All isolates were given a number as B1, B2, and B3. All *Streptomyces* sp isolates were screened for their antibacterial activity on Yeast extract-malt extract agar medium (ISP2) using scross-streak technique against two pathogenic bacteria include Gram-negative (Two pathogenic bacteria, including Gram-negative (*Pseudomonas aeruginosa*) and Gram-positive (*Staphylococcus aureus*). Among three *Streptomyces* sp isolates that obtained from Baghdad city (Al-Jadriya), one isolates (B2) didn't show any antibacterial activity against any type of pathogenic bacteria (Gram-negative and Gram-positive bacteria), while two *Streptomyces* sp isolates (B1 and B3) showed antibacterial activity against Gram-negative (*Pseudomonas aeruginosa*) and Gram-positive (*Staphylococcus aureus*. Screening was performed by Agar-Well Diffusion method and growth inhibition zones were measured in millimeters for each of the *Streptomyces* isolates (B1 and B3). Tested isolates have shown potent *in vitro* antibacterial activities against all tested pathogens. The highest activities were shown by isolate B1 against *S. aureus* 19.5 mm, *Pseudomonas aeruginosa* 14 mm. It is also evident that B3 isolate have shown activities against all pathogenic bacteria with inhibition zone diameters ranging between 17 and 13 mm against *S. aureus* and *Pseudomonas aeruginosa* respectively. Effect of Pomegranate peel and *Matricaria chamomilla* extracts (200µl, 300µl and 400µl) on the growth of *Streptomyces* sp were initially determined by the agar well-diffusion method, showed there is no diameters of inhibition zones exerted by the extract towards *Streptomyces* in different concentrations of Pomegranate peel and *Matricaria chamomilla* extracts.

Keywords: *Streptomyces*; Pomegranate peel; *Matricaria chamomilla*; Extracts

1. Introduction

Actinomycetes produce about two-thirds of the known antibiotics and among them 80% are made by members of the genus *Streptomyces,* with other genera trailing numerically. Actinomycetes also account for 60% of secondary metabolites with biological activities other than antimicrobial, and again *Streptomyces* species account for 80% of these [1-8]. *Streptomyces* are Gram positive aerobic bacteria belonging to the phylum Actinobacteria [9]. They have a DNA G+C content of 69-78 % [10]. At least 7000 different secondary metabolites have been discovered in *Streptomyces* isolates [11]. *Streptomyces* synthesize an amazing variety of chemically distinct inhibitors of many different cellular processes. These include antibiotics, fungicides, modulators of the immune response, and effectors of plant growth (Hopwood, 2007). Several studies have stated the efficacy of extracts from different tree parts, such as bark, leaves and

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fruit to hinder the growth of Gram positive and Gram negative bacteria, which are foodborne and human pathogens [12]. [13] showed that pomegranate extracts inhibit and delay *Staphylococcus aureus* growth and subsequent enterotoxin production at 0.01, 0.05 and 1% v/v concentrations. Chamomile has exhibited both positive and negative bactericidal activity with *Mycobacterium tuberculosis, Salmonella typhimurium* and *Staphylococcus aureus.* About 120 chemical constituents have been identified in chamomile as secondary metabolites, including 28 terpenoids, 36 flavonoids and 52 additional compounds with potential pharmacological activity [14]. The present work was aimed to study effect of antibiotic types and some plant extracts on the growth and morphology of *Streptomyces* bacteria.

2. Material and methods

2.1 Actinomycetes Isolation

Out of 12 soil samples were collected from Baghdad city (Al-Jadriya) on December 2020; soil samples were collected from 10 cm depth of the upper surface. The samples were placed in sterile plastic containers separately, tightly sealed and transported to the laboratory. The collected soil samples were dried in a hot air oven at 60-65 °C for about three hours for reducing the vegetative bacterial. Subsequently, the soil samples which containing spores of actinomycetes were transferred to sterile tubes separately and store at 4 °C until the screening performed. The starch-casein-nitrate-agar medium was used to isolate of actinomycetes. The pH value of the medium was adjusted to 7-7.2 and then sterilized in an autoclave at 121 °C for 15 minutes. The medium was then allowed to cool to around 45-50 °C, and with tetracycline 50 µg/ml and 50 µg/ml Nystatin,were added before pouring into plates. Then, the medium was poured into the plates with different thick to prevent drying during the incubation period [15].

One gram of dried soil sample was suspended in 99 ml sterile distilled water. Serial dilutions (10^{-1}-10^{-4}) were made from the stock suspension. Three petri dishes containing isolation medium were cultured by transferring 0.1 ml of the spore suspension from each dilution and spreading on the surface of agar medium using a sterile glass spreader. Then, the plates were incubated at 28 °C for 7 days. 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 in nutrient broth and nutrient agar slants and stored at 4°C for further study [15].

2.2 Pathogenic Bacteria Used in The Study

Two pathogenic bacteria, including Gram-negative (*Pseudomonas aeruginosa*) and Gram-positive (*Staphylococcus aureus*), were used as test microorganisms for evaluation the antibacterial activity of actinomycetes. All the tested pathogenic bacteria were obtained from Laboratory Microbiology/Mycology – college of Biotechnology.

2.3 Primary Screening for Antibacterial Activity

Primary screening of antagonism was performed on Muller Hinton agar using the perpendicular streak plate method against two test organisms, including Gram-negative (*P. aeruginosa*) and Gram-positive pathogenic bacteria (*S. aureus*). The medium was prepared according to manufacturer company instructions. The actinomycetes isolates was streaked across the surface of the agar medium at the middle position of the plate and incubated at 30 °C for 7 days, in triplicate. After that, the test organisms were streaked perpendicularly with actinomycetes growth and the space of 2-3 mm between each two streaks. Then, the plates were incubated at 37 °C for 2 days for the test organism growing. After that, the plates were then examined, and the presences of the clear zone between the actinomycetes growth and test microorganism indicate growth inhibition of test organisms.

2.4 Cultural Characterization of Actinomyctes Isolates

Cultural characteristics of the strainwere determined in accordance with the method described by [16]. Cultural characteristics are best made on a variety of International *Streptomyces* Project such as ISP2.

2.5 Effect of Pomegranate peel extract on the growth of *Streptomyces* sp

Petri dishes contained 20 ml of Nutrient agar (N.A) have been used for well-diffusion assay. Wells have been prepared in the N.A plates. In agar well diffusion 200µl, 300µl and 400 µl of Pomegranate peel extract plates were inoculated of a bacterial suspension containing 10^7 c.f.u. ml and incubated at 37 °C at 48 h. Diameters (in mm) of growth inhibition zones were measured after incubation at 37 °C for 48 h.

2.6 Effect of *Matricaria chamomilla* extract on the growth of *Streptomyces* sp

Effect of *Matricaria chamomilla* extract (200 µl, 300 µl and 400 µl) on the growth of *Streptomyces* sp were initially determined by the agar well-diffusion method. Petri dishes contained 20 ml of N.A have been used for well-diffusion assay. Wells have been prepared in the N.A plates. In agar well diffusion 200µl, 300 µl and 400 µl of *Matricaria chamomilla* extract plates were inoculated of a bacterial suspension containing l0^{7} c.f.u. ml and incubated at 37 °C at 48 h. Diameters (in mm) of growth inhibition zones were measured after incubation at 37 °C for 48 h.

3. Results and discussion

3.1 Isolation of Actinomycetes

Twelve soil samples were collected from Baghdad city. The serial dilution technique was used to isolate actinomycetes from ten different soil sources after inoculating the plates with soil suspension on the starch casein nitrate agar medium supplemented with tetracycline 50 µg/ml and 50 µg/ml Nystatin, the plates were incubated at 28°C for 7 days with a dilution 10^{-4}. The data presented in Table (1) summarize all suspected actinomycetes obtained from the above soil sources on the basis of forming pinpoint colonies with inhibitory or clear zone of inhibition around them as recommended by [17]. Nystatin reduces fungal growth, whereas tetracycline reduces other bacteria. Colonies size varied, powdery, colour varied from chalky white, buff, brown, pink, red, white, yellow and grey. this was in agreement with that described by [18].

Table 1 Actinomycetes colonies appear on starch casein nitrate agar medium for 7 days

No.	Soil samples sites	Actinomycetes colonies	Total colonies
1	Al-Jadriya	0	17
2	Al-Jadriya	1	
3	Al-Jadriya	2	
4	Al-Jadriya	2	
5	Al-Jadriya	3	
6	Al-Jadriya	1	
7	Al-Jadriya	0	
8	Al-Jadriya	3	
9	Al-Jadriya	0	
10	Al-Jadriya	2	
11	Al-Jadriya	0	
12	Al-Jadriya	3	

The morphology and size of the colonies was about 10 mm in diameter with a relatively smooth surface at the beginning of the growth, white, yellow and grey, it was developed to an aerial mycelium that appeared as granular, powdery and soft. [19] 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 12 soil samples, 17 colonies were obtained. Colonies having characteristic features such as powdery appearance with convex, concave or flat surface and color ranging from white, brown, and grey were selected.

Out of the 17 Actinomycetes colonies. sub-cultured on ISP2 for growth, and incubation of plates for 7 days, only three isolates demonstrated cultural characteristics similar to that of *Streptomyces* sp. three isolates were selected and purified by pure culture techniques of *Streptomyces* sp. All isolates were given a number as B1, B2, and B3 (Table 2). The Growth characteristics of colony on medium ISP2 as (very good) were a prerequisite for isolates selection of *Streptomyces* sp. The results were in agreement with the finding of both [20-22].

Table 2 The Growth characteristics of *Streptomyces* colonies on medium ISP2

Medium	Isolates	Growth
ISP2	B1	++++
	B2	++++
	B3	++++

++++: Growth very good

The results were in agreement with the finding of both [20] 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. [23], mentioned that because of their stringent aerobic metabolisms, actinomycetes.

Cultural and Morphology characteristics of *Streptomyces* sp. The all *Streptomyces* sp isolates were Gram's stain (Table 3). Young cultures (5-7 days old) produced Substrate mycelia, Branched or Fragments. The colors of the substrate mycelia and aerial mycelia of the isolates, varied from colorless to white, brown, and grey on ISP 2 (Table 3-3).

Table 3 Cultural and Morphology characteristics of Streptomyces isolates after 7 days growth on ISP 2 medium

No.	Characteristic	*Streptomyces* isolates		
		B1	B2	B3
1	Gram's stain	+	+	+
2	Substrate mycelia	Fragments	Fragments	Fragments
3	Colour of aerial mycelia	white	White - Grey	White- orange
4	Colour of substrate mycelia	white – brown	Grey	brown
5	Colour of soluble pigment	grey-violet	grey	grey

3.2 Biochemical characteristics

The biochemical properties are summarized in (Table 4). All of the isolates belonging to the *Streptomyces* sp.

Table 4 Biochemical characteristics of Streptomyces sp isolates after 7 days growth on ISP 2 medium

No.	Characteristic	B1	B2	B3
1	Catalase production	+	+	+
2	Hydrogen sulfide production	-	-	-
3	Nitrate reduction	+	+	+
4	Citrate utilization	-	-	-
5	Oxidase production	-	-	-
6	Casein hydrolysis	+	+	+
7	Indole production	-	-	-
8	Melanine reaction	-	-	-
9	Starch	+	+	+

+ positive, - negative

3.3 Screening for *Streptomyces* sp isolates activity

All *Streptomyces* sp isolates (B1, B2 and B3) were screened for their antibacterial activity on Yeast extract-malt extract agar medium (ISP2) using scross-streak technique against two pathogenic bacteria include Gram-negative (Two

pathogenic bacteria, including Gram-negative (*Pseudomonas aeruginosa*) and Gram-positive (*Staphylococcus aureus*). Among three *Streptomyces* sp isolates that obtained from Baghdad city, one isolates (B2) didn't show any antibacterial activity against any type of pathogenic bacteria (Gram-negative and Gram-positive bacteria), while two *Streptomyces* sp isolates (B1 and B3) showed antibacterial activity against Gram-negative (*Pseudomonas aeruginosa*) and Gram-positive (*Staphylococcus aureus*). (Table 5).

Table 5 Primary screening of *Streptomyces* isolates using scross-streak technique on Yeast extract-malt extract agar medium

Isolates	Gram-positive	Gram-negative	Note
	S. aureus	***P. aeruginosa***	
B1	+	+	Selected
B2	-	-	Neglected
B3	+	+	Selected

B1, B3: + antibacterial activity

Screening was performed by Agar-Well Diffusion method and growth inhibition zones were measured in millimeters for each of the *Streptomyces* isolates (B1 and B3), the results are shown in Table (6). Tested isolates have shown potent *in vitro* antibacterial activities against all tested pathogens. The highest activities were shown by isolate B1 against *S. aureus* 19.5 mm, *Pseudomonas aeruginosa* 14 mm. It is also evident that B3 isolate have shown activities against all pathogenic bacteria with inhibition zone diameters ranging between 17 and 13 mm against *S. aureus* and *Pseudomonas aeruginosa* respectively.

Table 6 Inhibition zones (mm) by different Streptomyces isolates against pathogenic bacteria

Streptomyces Isolates	Zone of inhibition (mm)	
	S. aureus	***P. aeruginosa***
B1	19.5	14
B2	17	13

Study [24] Antibacterial activity of actinomycetes isolated from Lobuche area (5000-5300 meter in height) and Lukla area (2660 meter in height) in Khumbu region has been studied. 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.

Altogether 36 putative isolates were subjected to secondary screening by agar well method to further test the capabilities of primarily screened organisms. Selected isolates (20) from the secondary screening belonged to the genera *Streptomyces* (10 isolates). Finally one isolate (*Streptomyces* species) was selected for further study on the basis of (a) broad spectrum activity and (b) larger zone of inhibition in comparison to others. The 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* were 5 mg/ml for *Streptomyces* species. Thin layer chromatography (TLC) of the ethyl acetate extracts were carried out in duplicate using Chloroform: methanol (4:1) as solvent system and Tetracycline as reference antibiotic. Under UV light they gave greenish yellow spots with Rf value 0.88 for the antimicrobial from *Streptomyces* species. In bioautography (using *Staphylococcus aureus* as test organism) inhibition zones were obtained and they were associated with the yellowish green spots of the chromatogram as detected under UV light. This may indicate the same compounds were responsible for the antibacterial activity of those actinomycetes isolates.

4. Effect of antibiotic types on the growth of *Streptomyces* sp

4.1 Effect of Pomegranate peel extract on the growth of *Streptomyces* sp

Effect of Pomegranate peel extract (200 µl, 300 µl and 400 µl) on the growth of *Streptomyces* sp were initially determined by the agar well-diffusion method. Table (7), showed there is no diameters of inhibition zones exerted by the extract towards *Streptomyces* in different concentrations of Pomegranate peel extract. [25], show that pomegranate peel is rich in tannins, high-molecular weight plant polyphenols, which can be categorized into two chemically and biologically separate groups, condensed hydrolysable tannin and tannin, the latter composed of glycosyl esters and phenolic acids. Hydrolyzable tannins are parted into gallotannins containing gallic acid and ellagitannins, containing ellagic acid. [26], reported that only water-methanol extract of peels has marked inhibition (12–20 mm inhibition zones) and the water extract was inactive against eleven microorganisms tested, such as *S. aureus* (2 strains), *B. subtilis, E. coli, Listeria monocytogenes, Pseudomonas aeruginosa, Klebsiella pneumoniae, Yersinia enterocolitica, Candida utilis, Saccharomyces cerevisiae* and *Aspergillus niger.*

Table 7 Effect of Pomegranate peel extract on the growth of *Streptomyces* sp

Treatments		Inhibition zone diameter (mm)
Pomegranate peel extract + *Streptomyces*	200 µl	0
	300 µl	0
	400 µl	0

Effect of *Matricaria chamomilla* extract on the growth of *Streptomyces* sp Effect of *Matricaria chamomilla* extract (200 µl, 300 µl and 400 µl) on the growth of *Streptomyces* sp were initially determined by the agar well-diffusion method. Table (8), showed there is no diameters of inhibition zones exerted by the extract towards *Streptomyces* in different concentrations of *Matricaria chamomilla* extract.

Table 8 Effect of *Matricaria chamomilla* extract on the growth of Streptomyces sp

Treatments		Inhibition zone diameter (mm)
Matricaria chamomilla extract + *Streptomyces*	200 µl	0
	300 µl	0
	400 µl	0

Chamomile has exhibited both positive and negative bactericidal activity with *Mycobacterium tuberculosis, Salmonella typhimurium* and *Staphylococcus aureus.* About 120 chemical constituents have been identified in chamomile as secondary metabolites, including 28 terpenoids, 36 flavonoids and 52 additional compounds with potential pharmacological activity [27].

Components such as alpha-bisabolol and cyclic ethers are antimicrobial. Umbelfiferone is fungistatic while chamazulene and alpha-bisabolol are antiseptic [28].

Known, since ancient times, by the Egyptian, Greeks and Romans for their medicinal, cosmetic, decorative and aromatic properties, Chamomile is widely used in traditional medicine due to analgesic properties, anti-allergic, antispasmodic, antibacterial, anti-inflammatory, sedative, healing, anti-proliferative cancer cells, cytotoxic against cancer cells. [29]. The essential oil is mentioned as a bactericide against gram positive microorganisms such as *Staphylococcus duwus* and a fungicide against *Candida albicans* [30]. The mouthwash with chamomile was considered satisfactory in reducing gingival inflammation, showing great performance in reducing bacterial plaque index [31].

[32-34]. The most characteristic constituents of chamomile are unstable oil, sesquiterpene lactones, ascorbic acid, and phenol compounds, primarily the flavonoids, apigenin, quercetin, patulin, luteolin, and glycosides. Flavonoids are

chemical phenyl benzopyrones which are usually observed in all vascular plants. The benzopyran ring system is a molecular scaffold which can be seen in flavonoid-inherent products and has weak aromatase inhibitory activity.

5. Conclusion

Morphology and growth of *Streptomyces* sp, were not affected by different concentrations of Pomegranate peel and *Matricaria chamomilla* extracts.

Compliance with ethical standards

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