Antibacterial Activity of *Streptomyces* sp. Isolated from High Tolerant Ecosystem in Babylon Province, Iraq

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Abstract

The goal of this research is producing antibiotic from two Actynomycites species isolated from the high tolerant ecosystem, isolate from high salty soil and high alkaloid soil, used whey and yeast extract medium as a perfect medium to culturing (1% glucose, 1% trypton, 0.092% glycine, 0.004% HCl and 8.5 PH), perfect incubation circumstances are 30 C° in 4 days, the infiltrate of crude antibiotic concentrate by using a rotary evaporator, then extract the antibiotic from concentrate infiltrate with ethanol: methanol: ethyl acetate methods (50:30:20 Vol), drying the extract at 30 C° to dissolve both antibiotic in distilled water with three concentration (50, 75, 100 µg/ml) and disinfectant by 0.45 Millipore.

Antibiotic activity from two isolates of *Streptomyces sp*. were measured against positive and negative control bacteria (*Escherichia coli, Bacillus subtilis, streptococcus pyogenes and staphylococcus aureus*) by well diffusion method, the results show the antibiotic produced by isolating No. 2 active against *E. coli, S. aureus and S. pyogenes* while the isolating No. 1 were active only against *E. coli* and no activity against gram positive bacteria.

Extract component was separated with TLC technique (thin layer chromatography) by using silica gel 20*20cm with high polar mix (7 water: 5 acetic acid: 3 biotanol vol.) according to relative factor R_f for antibiotic, isolate No.1 give 0.6 R_f for one antibiotic and isolate No.2 give 0.15 R_f for two antibiotic against *S. aureus* and the other one with 0.45 R_f against *E. coli* and *S. pyogenes*, means these antibiotic has synergism activity.

Introduction

Actinomycetes are positive to gram stain grow in aerobic condition and some obligate anaerobic, chemo-organic nutrition, some grow in extreme circumstances like acidophil or alkalophil [1].

Morphemically is varied between cocci like *Micrococcus* to branched mycelium hold long threads attached to long chains of spores [2].

Actinomycetes very important source of antibiotics which also produce secondary metabolism active against microorganism and as anticancer, antibiotic number produced by *Actinomycetes* reaches 2500 antibiotic, 73% of this antibiotic secreted by *Streptomyces* [3].

Actinomycetes secondary metabolism not just briefing on antibiotic it's also secreted enzyme like catalase, its mycelium are grounded and aero and its branched spores are fragmented tend to form bulges, these spores vary in size with smooth and forked wall [2]. because of its ability to produce 73-75% of antibiotic chemicals compound so it's important economically, its produced broad spectrum of antibiotic like glycosides, aminoglycosides, glycopeptides, betalactamase, peptides and tetracycline [3], and as increasing to antibiotic resistant pathogens there is necessary to developing and find new chemicals compounds may produce by un-isolated *Streptomyces*.

Materials and methods

Preparing mediums

Soluble whey –yeast extract medium prepared from 1% glucose, 1% trypton,0.092% glycine and 0.004% HCl, nutrient broth prepared by dissolving amount of broth in amount of distilled water according to instruction write on medium cane sterilized by autoclave.

Bacterial activation

Streptomyces isolates was collected from an advanced biotechnology lab at Babylon university / college of science activated by culturing on casein-starch agar medium incubate at 30 C^o for 14 days, then studying colony formation and diagnosis bacterial cell form to culturing Soluble whey –yeast extract medium (ISP1) in 30 C^o for 7 days, the solution was calibrated with McFarland3 tube.

Antibiotic production and harvesting

Sterile implant medium (100 ml) was inoculate with antibiotic bacteria producer under study, three replicate for each isolate of bacteria antibiotic producer by adding 2 ml of bacteria (match to McFarland tube No. 3), incubate at 30 C^o for 5 days, then estimate activity using Whatman filtered paper No. 1 to filtering medium then 0.45 Millipore to sterile solution, antibiotic solution was concentrated by rotary evaporator with 60 C^o preparing to dissolve it with different types of solvents like ethyl acetate, ethanol and methanol by adding 5 ml of solvent to 1 ml of concentrate antibiotic solution, more concentration of solution by evaporator to condense volume to 4 ml and then saved at 4 C^o, by using agar well diffusion the results was read by measuring diameter of inhibition zone [4].

Then layer chromatography (TLC)

This method used to measure relative factor (R_f) and also for detecting purity of antibiotics, in this technique we used glass plates coated with silica gel after activating it (activation is done with 120 C^o of heating for 1 hour), the mobile phase is biotanol: acetic acid: water (3:5:7) high polarity, loading 5 µl of partially purified antibiotic solution about 1cm far away from the edge of plate soaked in TLC jar that contain mobile phase then left for 2 hours, results showed as spots after exposing the plate to iodine vapor R_f measured by calculate :

$$R_f = \frac{distance\ made\ by\ antibiotic}{distance\ made\ by\ solvent}$$

Results and discussion

Activity of *Streptomyces* sp.1 and *Streptomyces* sp.2 to produce Secondary metabolites inhibit negative and positive gram bacterial growth was tested by inoculate bacteria in soluble whey-yeast extract medium under perfect circumstances (30C°, 4 days) then extract antibiotic and dissolved in high polarity mix ethanol :methanol :ethyl acetate (50:30:20) followed by drying and prepare three concentration (100, 75, 50 µg), results showed *Streptomyces* sp.1 have ability to inhibit *E.coli* while show no result of inhibition to gram positive pathogenic bacteria as shown in table (1-1), same results showed of *Streptomyces* isolated from soil samples from different localities of Punjab and Himachal Pradesh (India) but more effective against *E.coli* and other pathogenes [5] .

Streptomyces sp.2 showed ability to inhibit gram positive and negative pathogenic bacteria except *Bacillus subtilis* as shown in table (2-1) and that's match with results in actinomycetes isolated from nepal soil which showed no inhibition zone against *Bacillus subtilis* in both primary and secondary metabolites[6], that's mean *Streptomyces* sp.2 have broad inhibition ability while antibiotic extracted from *Streptomyces* sp.1 effect only negative bacteria, antibiotics extracted from different bacteria its ability to inhibit is varying to inhibit positive and negative bacteria Consistent with what came with[7] they found 17 from 50 *Actynomycites* isolates have vary ability to inhibit gram positive and negative pathogens, some inhibit both (- and + gram bacteria) and some (- or + bacteria), also local research showed the same, that's *Actynomycites* varying its inhibition ability to (- and + gram bacteria in adding to produce secondary metabolites inhibit fungi and anticancer materials in vevo[8] [9].

Table (1-1): Antibiotic activity for partial purified extract of *Streptomyces* sp.1

Antibiotic Concentration / Pathogens	Inhibition zone (melemeter)		
	100 µg/ml	75 µg/ml	50µg/ml
E. coli	18	14	11
B. subtilis	0	0	0
S. pyogenes	0	0	0
S. aureus	0	0	0
control	0	0	0

Table (2-1): Antibiotic activity for partial purified extract of *Streptomyces* sp.2

Antibiotic Concentration / Pathogens	Inhibition zone (melemeter)		
	100 µg/ml	75 µg/ml	50 µg/ml
E. coli	16	13	10
B. subtilis	0	0	0
S. pyogenes	12	10	0
S. aureus	13	12	10
control	0	0	0

Component of two antibiotics from both isolates was separated by TLC technique by using silica gel 20*20cm with biotanol:acetic aced:water (7:5:3) high polarity mix as mobile phase to measure R_f, results showed 0.6 R_f for *Streptomyces* sp.1 while *Streptomyces* sp.2 showed relative factor 0.15 to *S. aureus* and the other antibiotic extract show 0.45 R_f to *S. pyogenes* and *E. coli* as shown in figure (1-1) and table (3-1).

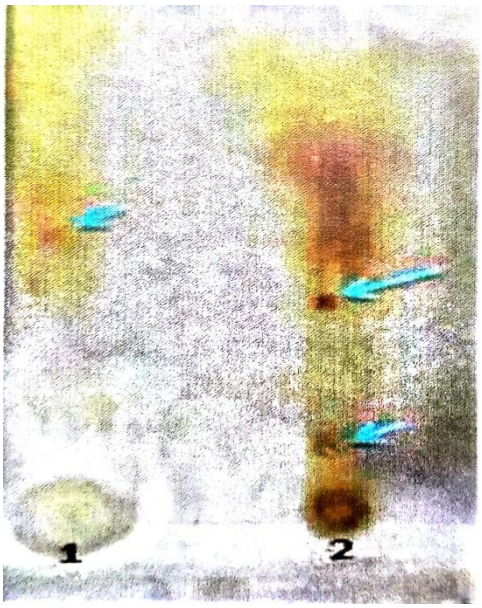

Figure (1-1): Thin layer chromatography for antibiotic extracted from *Streptomyces* sp.1 & sp.2

Table (3-1): Flow rate of R_f antibiotic extracted from *Streptomyces* sp.1 & sp.2

Partial purified antibiotic	Relative factor (R_f)	Polarity	Inhibited pathogens
from *Streptomyces* sp.1	**0.6**	**Moderate polar**	***E. coli***
from *Streptomyces* sp.2	**0.15** **0.45**	**High polarity** **Moderate polar**	***S. aureus*** ***S. pyogenes*** ***E. coli***

Antibiotic produced by different microorganism have vary chemical compound depending on its pathway of metabolism also depend on microorganism, some of them has glycosidic some peptide some steroid or beta-lactam etc. and each one of these antibiotic from different microorganism have specific relative factor R_f , the higher polarity much more closer to start line of TLC, which mean R_f lowest than 0.3 while nonpolar compounds are closer to the end line of TLC , R_f more than 0.7 [4].

Streptomyces sp.2 is the perfect source to produce active antibiotic against gram negative and positive pathogenic bacteria, needs more study to characterize these antibiotic and tested on other pathogenic bacteria.

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الخلاصة

تم عزل البكتريا الخيطية .*Streptomyces* sp من تربة ذات ملوحة جدا عالية واخرى من تر بة ذات ذات درجة قاعدية جدا عالية وتم استعمال الوسط الامثل لانتاج المضادات الحيوية من بكتريا .*Streptomyces* sp وهو وسط خلاصة الخميره والشعير السائل والمتكون من ١% كلوكوز و ١% تربتون و ٠.٠٩٢ كلايسين و ٠.٠٠٠٤% هيدروكسيد الصوديوم, ومن ثم تم استخلاص المضاد من الراشح المركز باستعمال النظام (ايثانول: ميثانول: اثيل اسيتات) بنسبة (٢٠:٣٠:٥٠ حجم) على التوالي ثم تجفيف المستخلص بدرجة حرارة ٣٠م وتاذابة بالماء المقطر وتحضير ثلاث تراكيز (٥٠مايكروغرام و٧٥ مايكروغرام و١٠٠مايكروغرام).

تم فحص فعالية المضادات المنتجهة من سلالتين من بكتريا .*Streptomyces* sp ضد بكتريا الاختبار الموجبه والسالبه لصبغه كرام والتي شملت *Escherichia coli* و *Bacillus subtilis* و *Streptococcus pyogenes* و *Staphylococcus aureus* , باستعمال طريقة الانتشار من الحفر (well diffusion method) واظهرت النتائج بان المضاد المنتج من السلالة رقم (٢) فعالة ضد بكتريا *S. pyogenes* و *S. aureus* و *E. coli* وغير فعالة ضد بكتريا *B. subtilis* في حين المضاد المنتج من السلالة رقم (١) فعالا ضد بكتريا E. coli فقط وغير فعال ضد البكتريا الموجبة لصبغة كرام.

الكلمات المفتاحية: البكتريا الخيطية, ظروف نمو متطرفة, فعالية تضادية لنمو البكتريا, مستخلص كحولي , تقنية كروماتوغرافيا الطبقه الرقيقة