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Determination the Optimum Conditions for Antibiotics Production from Streptomyces albus that Locally Isolated

Marwa H. Abdull Wahaab

Depart. Of biology, college of science, Tikrit university, Tikrit , Iraq.

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***Corresponding author :**

Email : dr.marwa2017@tu.edu.iq

Mobile : 009647719297999

Contact To Journal

E-mail: tjops@tu.edu.iq

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Abstract

A total of 31 different Streptomyces isolates were recovered from 25 samples of soil collected from different sites in Tikrit University gardens. There were 14 isolates showed as activity against pathogenic bacterial isolates, 6 (43)% isolates were effected against Staph. aureus. and E. coli, while 3 (21)% isolates showed impact high against Staphylococcus aureus only, and did not show any effect against Escherichia coli, while 5 (36)% isolates showed impact against E. coli only, Streptomyces albus were identified which gave the high effectiveness against pathogenic bacteria on the bases of morphological and biochemical tests and sensitivity to some antibiotics. This isolate was gave high production at 30 C for 7 days and pH 7, the best carbon source when used glucose at concentrations 15-20 g / l and the level of salt was at 1.5 g / l and with the use of Cysteine as a nitrogen source oncluded that AEA has a therapeutic activity against nephrotoxicity induced by gentamicin in male rats.

تحديد الظروف المثلى لإنتاج المضادات الحيوية من بكتريا Streptomyces albus المعزولة محلياً

مروة حسن عبدالوهاب

الخلاصة

تم الحصول على (31) عزلة تابعة إلى جنس Streptomyces من (25) عينة ترابية جمعت من مناطق مختلفة من محافظة صلاح الدين تميزت 14 عزلة ذات فعالية تضادية ضد بكتريا الاختبار حيث أظهرت 6عزلات (43)% تأثيراً ضد النوعين Staph. aureus و E. coli، في حين أظهرت 3 عزلات (21)% تأثيراً تثبيطيا عالياً ضد النوع Staph. aureus فقط ، ولم تظهر أي من العزلات تأثير على النوع E. coli في حين أظهرت 5عزلات (36)% تأثيراً مثبطاً ضد النوع E. coli فقط. شخصت العزلة المنتخبة Streptomyces albus التي أعطت اعلي فعالية تثبيطية ضد بكتريا الاختبار على أساس الاختبارات الظاهرية والكيموحيوية وحساسيتها لبعض المضادات الحيوية. التي تبين ان اعلى إنتاج للمضاد الحيوي كان عند 30 م لمدة 7 ايام ورقم هيدروجيني 7 وعند استعمال الكلوكوز بتركيز 15 -20 غم / لتر ومستوى من ملح الطعام عند 1.5 غم /لتر واستعمال الحامض الاميني Cysteine كمصدر نيتروجيني.

Introduction

Streptomyces is genus of aerobic gram-positive bacteria, belong to streptomycetaceae family of actinomycetales order and actinobacteria class (1). Streptomyces genus has a distinctive and special appearance, production multi-branch mycelium, followed by the formation of vertical aerial mycelium and formation of a multi-nuclei mycelium to form chains of three or more arthrospores. The appearance of this chain may be straight, flexous, hooked, looped or spiral. The surface of arthrospores may be it is hairy, knob, ridged, smooth, spiny or warty while the appearance of colonies, the surface is smooth in the beginning and then become floccose, granular, powdery or velvety. The members of this genus has a complex life cycle, and its colonies are multicellular with distinct individuals showing temporary control on gene expression, synthesis, metabolism, and flow of metabolic substances (2). Antibiotics are known as the secondary metabolism products during the log phase after the cellular growth of Streptomyces has been completed and reached the stationary phase (3). Antibiotics produced from Streptomyces have different compositions and effects on other microorganisms and organisms, some of them are antibacterial, antifungal, antiparasitic, antitumor and antiviral (4). As a result of the increased resistance of pathogenic bacteria to the synthesis antibiotics and limited pharmacological effectiveness, which led to the need to find new compounds against microorganisms could be produced by isolates of the genus streptomyces not isolate. The aim of the research is to isolate and diagnose some species of Streptomyces genus from different soil samples and to determine optimal conditions for the production of antibiotics, which may show anti-efficacy in isolated bacteria from different sites in Tikrit University gardens.

Materials and methods

Collection of samples: Twenty-five samples were collected at the weights of 2 kg of soil from Tikrit University gardens and the residential area for the period from 1 March to 1 April 2018, at a depth of 5-15 cm after removal of 3 cm from the surface of the soil. Then drying in oven at 37^{o} C for 4 days with the addition of calcium carbonate $CaCo_3$ (1:10

W/W). Calcium carbonate was added because the drying of the soil leads to reduction of the number of bacteria and the addition of calcium carbonate lead to increase the value of pH, thus promoting the growth of Actinomycetes(5).

Isolation of Streptomyces: Series of dilutions were carried out by adding 25 g of each sample to 225 ml of the normal saline and reaching the dilution needed to obtain isolated colonies. Then cultivating on the Yeast Extract (Malt Extract agar medium) and incubating at 30 ° C for 7 days (6). The ability of Streptomyces bacteria to grow was tested with sodium azide (0.01%) and phenol (0.1%) using the basal medium containing glucose (1%), yeast extract (0.5%) and agar (1.5), then part of the bacteria colony was inoculated and incubated at 30 ° C for 7 days, the negative result was recorded in the absence of growth or very weak growth (7).

Diagnosis of Streptomyces.

Streptomyces isolates were identified according to the form of colonies and their color on the middle of mineral salts, Starch mineral salt agar, the shape of the aerial and terrestrial mycelium and the arrangement of chains of arthrospres by using the technique of the slide culture technique and for the identification of the bacterial strain of Streptomyces which gave the highest inhibitory effect against the test bacteria to the species level, was adopted in the working methods to test its ability to produce melanin on Tyrosine agar medium, Carbon utilization, gelatin melts, starch analysis, nitrate reduction, casein degradation, urease production, DNase, haemolysin production lipase, lecithinase, catalase and oxidase, with different concentrations of food salt, sodium azide (0.01%) and phenol (0.1) on growth, citrate utilization and growth capacity at 45^{o}C (5).

The bacterial test for antibiotic production

Bacterial isolates *Staph aureus* and *E. coli* that used in the test of the production of antibiotics were obtained from the laboratories of Tikrit General Hospital from 1-2-2018 to 1-7-2018. The diagnosis of these isolates was confirmed by a number of diagnostic tests and by the use of the necessary culture of diagnosis such as MacConkey agar, mannitol salt agar, phosphatase agar, peptone broth, glucose and phosphate

Test the inhibitory efficacy of Streptomyces isolates

For testing the ability of Streptomyces to the production of antibiotics, the agar disk diffusion method was used as previously described (7),

Preparation of inoculation and antibiotic extraction

spores suspension of Streptomyces were prepared as mentioned (8). The best production medium was used (yeast extract, malt extract, Gauza broth, or aspargine and Glycerol), which was pourced in conical glass flasks (250 ml) (1) ml for each flask and at a repeat twice rate for each treatment. the flasks were sealed with cotton clamps tightly and covered with foil and then sterilized by autoclave. the flasks were left to cool and then inoculated with the spore suspension by 3% volume / volume. Temperature is 30^{o} C and rotational speed (150) rpm for 7 days. The antibiotic extraction produced by isolation was carried out using the method (9).

Determination of some optimal conditions for antibiotic production

A number of factors and optimal conditions were studied for producing the highest amount

antibiotic After their growth in 250 mL conical flasks with 50 mL and repeated twice for each treatment and then 3% of *Streptomyces albuls* was inoculated at pH, carbonate, nitrogen sources and studied factors (10).

Results and discussion

The isolation Thirty-three of the Streptomyces species were isolated from 25 soil samples collected from different areas of Saladin Governorate. The isolates were selected based on the chalky appearance of growthing colonies on the isolation culture media and their production of wetland odor (11). It was observed that soil treatment with calcium carbonate ($CaCo_3$) and drying at 37 ° C for 4 days had a significant role in increasing the number of Streptomyces bacteria in primary isolation, because soil drying reduced the number of vegetal bacteria and fungi. This is similar to what has been reported in a number of studies. (12) noted that the addition of calcium carbonate to soil samples gives better results in isolation (13). The results of the glass slide examination showed that the arrangement of the spores chains of the Streptomyces isolates ranged from the spiral shape to the straight-shaped with curved end. The studied isolates showed a marked difference in the colors of the aerial mycelium when cultured and growth on the mineral salts and Starch mineral salt agar. The colors of the aerial mycelium are varied, including gray, light green, red and white chalk, the most common color was gray. Table 1.

Table (1):- Diagnostic tests for isolating *Streptomyces albus*

Tests	Result
Gram stain	+
Production of melanin on tyrosine media	-
Spore chain form	RF
Catalase	+
Oxidase	-
Gelatin lysis	+
Starch lysis	-
Nitrate reduced	-
Gasein lysis	+
Urea decomposition	-
Blood lysis	+
DNase production	+
H_2S production	+

Lecithin lysis	+
Lipase lysis	-
Citrate utilization	+
NaCl (1.5)%	+
NaCl (5)%	+
NaCl (7)%	+
NaCl (15)%	-
NaCl (20)%	-
Sodium azide 0.01%	-
Phenol 0.1%	-
Growth at 45^{o} C	-
Sugars fermentation tests	
Glucose	+
Fructose	+
Sucrose	-
Mannitol	+
Raffinose	-
Rhamnose	-
Inositol	+
Melibose	+
Melezitose	+
Lactose	+
Maltose	+

(+) positive result, (-) negative result, (RF) Radio frequency

Production of antibiotics

Thirty-one of streptomyces isolates obtained in this study were tested for antibiotic production (antibacterial activity against positive and negative Gram-positive bacteria). Fourteen isolates of Streptomyces showed an antibacterial effect against the test bacteria as shown in Table (2). The study showed that 6 of isolates at rate (43%) had an effect against the two species *Staphylococcus aureus* and *E. coli*. This result was consistent with the findings of Ceylan and others (2008). Streptomyces isolated from the soil showed an effect on the positive and negative gram species, while 3 (21%) of isolates showed a high inhibitory effect against *Staph.aureus* and there was no effect on *E.coli*. Five of isolates (36%) showed an effect on *E. coli*, this is consistent with (15) as soil isolates of streptomyces isolated from the soil

showed a postive effect on *Staph. aureus* but showed no effect on *E. coli* and *Bacillus subtilis*. The reason for the variation in the effectiveness of Streptomyces against the test bacteria may be due to the nature of the culture media and its components. Many studies have pointed to the effect of carbon and nitrogen content in the production media on the effects of Streptomyces and the role of incubation conditions in detecting the effectiveness of antibiotics (16).

Table (2):- The ability of the isolates of the genus Streptomyces to produce antibiotics against some bacterial species

Streptomyces isolates	The diameter of the inhibition zone (mm)	
	E. coli	***Staph. aureus***
1	14	17
5	-	18
6	18	20
11	16	19
15	16	-
16	15	16
	17	-
	14	-
	18	-
	-	18
	13	-
	15	18
	12	17
	-	15

(-) There is no antimicrobial effect

Optimal conditions for the production of antibiotics from *Streptomyces .albus* bacteria

1 - Effect of the type of culture media

For the purpose of studying the effect of culture media types in antibiotic production, the isolated bacteria was cultured on three liquid media, Yeast Extract-malt extract broth, Glycerol aspargine broth and Gauza broth. The results showed that the Yeast Extract-malt extract broth was the best medium of productivity, after incubation for 7 days at a temperature of 30^{o}C and by measuring the diameter of the inhibition area against the test bacteria as shown in Table 4. The isolates of *Streptomyces albus* showed an inhibition ability (21, 17) mm against *Staph aureus* and *E. coli*, respectively. It was observed by determining the optimal culture media for antibiotic production in our study that the liquid media is better in production antibiotics from the solid media by obtaining higher inhibition areas in the liquid media compared to the inhibition zones of the isolates produced on the steel medium and this result is consistent with the (17)

suggests that liquid media is better in producing antibiotics than solid media.

Table (3):- Effect the type of culture media on antibiotics production

Culture media	The diameter of the inhibition zone (mm)	
	E.coli	*Staph. aureus*
Yeast Extract-malt extract broth	17	21
Glycerol aspargine broth	13	16
Gauza broth	12	14

2.Effect of incubation period

Antibiotics production from *Strep.albus* bacteria were monitored during different incubation periods and using the best production media (Yeast Extract-malt extract broth). A sample is pulled daily to estimate the inhibitory effect of antibiotics. The results showed increased in antibiotic production by increasing the duration of incubation, and found that the isolates were able to produce antibiotic after 48 hours of incubation, and the highest production of the antibiotic after 7 days of incubation and with evidence diameter of inhibition zone (21, 17) mm against bacteria *staph.aureus* and *E. coli* respectively (Table 4). This finding was agreed with the study of (18) that the maximum antibiotic production of Streptomyces bangladeshiensis was obtained after 7 days of incubation using a culture media.

Table (4):- Effect of incubation period on antibiotics production on antibiotics production

Incubation period/days	The diameter of the inhibition zone (mm)	
	E. coli	***Staph. aureus***
1	0	0
2	6	9
3	9	12
4	12	15
5	14	18
6	15	20
7	17	21

3 - Study the effect of different values of pH

On this study pH of the culture media is determined before the start of the center inoculation. The results showed that *Streptomyces .albus* bacteria were able to produce antibiotic in a range of pH (6-9) and that the highest yield of the antibiotic in terms of diameter of the inhibition zone was obtained at pH 7(Table 5). The change of pH has a big and important effect on enzymatic activity in microorganisms. A number of antibiotic microorganisms live in a neutral pH of about 7, and most types of antibiotic-derived Streptomyces grow in pH ranging from 6.7 to 7.8 (7).

Table (5):- Effect of different values of pH on antibiotics production

pH	The diameter of the inhibition zone (mm)	
	E. coli	***Staph. aureus***
5	0	0
5.5	0	0
6	7	9
6.5	12	14
7	14	16.5
7.5	14	16
8	11	13
8.5	8	11
9	6.5	10

4- Studying the effect of incubation temperature

The results in Table (6) showed that the temperature 30^{O} C is the best temperature for the production of antimicrobial with evidence of diameter of the inhibition zone (20, 16) mm against Staph bacteria. aureus and E.coli, respectively. The result of our study confirms (7) that the appropriate temperature gradients for Streptomyces that producing antibiotics range between 26-30^{o} C, that pointed out that any deviation in the values of the optimal temperature scores affects the growth and reduces its production of antibiotics, This can be explained by the strong influnce of temperature on the activity of enzymes and the efficiency of the transport system, as well as on the important biochemical and phylogenetic functions for the cell of microorganism.

Table (6):- The effect of incubation temperature on antibiotics production

Temperature ^{O}C	The diameter of the inhibition zone (mm)	
	E. coli	***Staph. aureus***
24	0	0
26	9	10
28	12	15
30	16	20
32	11	14
35	10	12

5-Study the impact of different carbon sources

Various sources of carbon were studied at a concentration of 1% to the culture media to show their effect on antibiotic production and bacterial growth. The results in Table (7) showed that glucose gave the highest productivity of the antibiotic and the diameter of the inhibition zone were 13, 11 mm. The other sugars used varied carbon sources in their effect on the production of antibiotic and were less productive of antibiotics when using sucrose, Galactose and Lactose as sources of Carbon. This

result was agreed with the study of (20 and 21) that glucose glucose was given the highest productivity of the antibiotics Kanamycin and Anthracycline of the strain M27 *Streptomyces kanamyceticus* and strain *Streptomyces peucetius*.

Table(8):- Impact of different carbon sources on antibiotics production

Carbone source	The diameter of the inhibition zone (mm)	
	E. coli	***Staph. aureus***
Glucose	11	13
Fructose	6	8
Sucrose	6	8
Maltose	9	11
Galactose	5	8
Lactose	7	9
Raffinose	6	9

6-Study the effect of different concentrations of glucose

Based on the fact that glucose is the optimal carbon source for antibiotic production from the selected isolation. In this study, different concentrations of glucose were used to show their effect on antibiotic production as in Table (8). The highest antibiotic yield in terms of diameter of the inhibition zone is the isolation of *Streptomyces albus* bacteria when glucose is used at a concentration of 15, 20 g / L. Productivity varied for other concentrations of glucose glucose, with the lowest antibiotic production at concentration (40 g / L). The results of our study agree with (22) that the optimal concentration of glucose to produce Nastamycin from Streptomyces natalensis is 20 g / L.

Table (8):- Effect of different concentrations of glucose on antibiotics production

Glucose concentration g/L	The diameter of the inhibition zone (mm)	
	E. coli	***Staph. aureus***
5	6.5	8
10	31	16
15	71	20
20	51	81
25	11	14
30	11	13
35	9	10
40	8	9

7 - Effect of different concentrations of sodium chloride on the production of antibiotic

The production of antibiotics is influenced by concentrations of NaCl because this salt affects the work of enzymes that may be sensitive or salt resistant. Different concentrations of sodium chloride were added from (0.5-4 g / L). For example, sodium chloride affects the action of the malate dehydrogenase enzyme, which affects the production of antibiotics in *Streptomyces rimosus* (23). The results in Table 8 showed that the highest antibiotic yield in terms of diameter of the inhibition zone (18, 14 mm) for the isolation of *Streptomyces albus* against *Staph aureus* and *E. coli* respectively when sodium chloride is added to the food medium at a concentration of 1.5 g / L.

Table (9):- Effect of different concentrations of sodium chloride on the production of antibiotic.

Sodium chloride concentration g/L	Inhibition zone diameter (mm)	
	E. coli	***Staph. Aureus***
0.5	11	13
1	13	15
1.5	14	18
2	14	16
2.5	11	12
3	10	10
3.5	7	9
,4	6	8

8- Effect of using different nitrogen sources on antibiotic production

The results in Table (10) showed the contrast in the different sources of nitrogen in their support for the production of antibiotic. The highest antibiotic yield was obtained from the selected isolation in terms of the diameter of the inhibition zone when using the citric acid Cysteine. The diameter of the inhibitor (18, 15) against *Staph. aureus* and *E. coli* respectively, and this result agreed with what (24) indicated that the amino acid cysteine promotes the production of antibiotic in large quantities.

Table (10):- Using different nitrogen sources on antibiotic production

Nitrogen source	Inhibition zone diameter (mm)	
	E. coli	***Staph. aureus***
Cysteine	15	18
$NaNo_3$	7	12
Asparagine	12	14

NH_4Cl	12	15
Methionine	9	11
Glycine	9	13

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