

Studies on the Antimicrobial Activity of *Streptomyces* sp. Isolated from Jazan

Salha H.M Al-Zahrani

College of Training Teachers, Jeddah, Saudi Arabia

Abstract. Antimicrobial activity of *Streptomyces* sp. (J12) isolated from soil sample collected from Jazan was studied. The isolate was classified according to the color of its mature sporulated aerial mycelia. It showed antimicrobial activities on solid and liquid media against some Gram positive (*Bacillus subtilis* and *Staphylococcus aureus*), Gram negative bacteria (*Escherichia coli* and *Salmonella typhi*), as well as fungi (*Fusarium oxysporum* f. sp. *melongenae*, *Rhizoctonia solani* and *Candida albicans*). Results showed that oat meal, starch casein and Sabouraud media improved antibiotic biosynthesis more than yeast malt extract medium. Biosynthesis of antibiotics by the species under agitated culture conditions was decreased. The influence of different cultural conditions on antibiotic biosynthesis by *Streptomyces* J12 was studied. It showed that the highest antimicrobial activities were obtained after 6 days of growth at pH 7.2 and a medium composition of 10g/l starch as the sole carbon source, 2.5g/l of potassium nitrate as a nitrogen source and 0.3g/l casein.

Introduction

Actinomycetes are Gram positive, free living, saprophytic bacteria, widely distributed in soil, water and colonizing plants. Their population has been identified as one of the major group of soil population^[1]. The actinomycetes are noteworthy known as antibiotic producers, making three quarters of all known products; the streptomycetes are especially prolific and can produce many types of antibiotics and other class of biologically active secondary metabolites^[2]. Some *Streptomyces* spp. produce antibiotics at the same time of cell sporulation^[3]. In general, *Streptomyces* spp. grow best in media containing carbon and nitrogen sources, including chitin, starch, glycerol, arginin, asparagine, casein and nitrate^[4].

The aim of this work was to study the antimicrobial activity and characterization of *Streptomyces* sp. (isolate J12) isolated from Jazan soil of Saudi Arabia.

Materials and Methods

Isolation of Streptomyces

Soil samples were collected from various locations in Jazan region at Saudi Arabia. The samples were dried for 3 days at room temperature to reduce the bacterial flora with no harms to the growth of *Streptomyces* [5]. One gram of air dried soil was shaken in a flask containing 100 ml of distilled water, and serial dilutions were plated on starch casein solid media [6]. The plates were incubated at $28 \pm 2^\circ\text{C}$ until the sporulation of *Streptomyces* colonies occurred. The colonies (where the mycelia remained intact and the aerial mycelia and long spore chain were abundant) were picked up and transferred to a starch nitrate medium [7]. Pure cultures were obtained from selected colonies for repeated sub-culturing.

Characterization of the Isolate

The *Streptomyces* isolate was cultivated on nutrient agar (Oxoid), inorganic salts/starch agar, glycerol/asparagine agar, tyrosine-glycerol agar [8] and Czapek's Dox agar (Difco). Some diagnostic characteristics were determined using the criteria recommended by many workers [8-10]. The proteolytic activity was tested using milk and gelatin liquefaction [11].

Antimicrobial Screening

Antagonistic activity of *Streptomyces* J12 was tested by diffusion plate method. It was grown on agar media of starch casein, yeast and malt extract [12], oat meal [13] and Sabouraud dextrose, containing (g/l): 40 dextrose, 10 peptone and 15 agar, pH 7.2 ± 0.2 . The plates were incubated at $28 \pm 2^\circ\text{C}$ for 7 days. After growth, 5 mm in diameter of agar discs were transferred to the surface of nutrient agar plates, previously inoculated with the test bacteria (*Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*) or to the surface of Sabouraud agar plates, previously inoculated with test fungi (*Fusarium oxysporum* f. sp. *Melongenae*, *Rhizoctonia solani* and *Candida albicans*).

The antibiotic potentiality of the isolate in broth media was biologically determined by the diffusion method. The isolate was grown in submerged culture in 250 ml flasks containing 50 ml of the liquid test media. The flasks were inoculated with 1 ml of active *Streptomyces* culture and incubated at $28 \pm 2^\circ\text{C}$ for 7 days under static and shaken conditions. After growth, 0.2 ml aliquots

of the cell-free filtrate were transferred to wells pored in agar plates previously inoculated with the test organisms. The plates were incubated at 35°C for 24 h and examined. The diameter of complete inhibition zone was measured to the nearest whole millimeter.

Influence of Culture Conditions on the Biosynthesis of Antibiotics by Streptomyces J12

This includes studies of the effects of pH values, incubation period, and composition of growth media leading to optimum biosynthesis of antibiotics. Different carbon and nitrogen sources were also tested using starch casein medium with some modifications. To test the effect of initial pH value on biosynthesis of antibiotics, 100 ml aliquots of medium were adjusted to initial pH values (4-8), using 5mM HCl or NaOH. After inoculation, cultures were incubated at $28 \pm 2^\circ\text{C}$ under static and shaken conditions. The effect of incubation period was studied after adjusting the media at the best pH value, and 100 ml aliquots of sterile media were inoculated and incubated at $28 \pm 2^\circ\text{C}$ under static and shaken conditions for different incubation periods. At the end of each incubation period, the biomass and antibiotic activity were determined. 0.2 ml of the broth was transferred aseptically to wells of agar plates inoculated with the test organism *Bacillus subtilis*. The diameter of clear inhibition zone was measured after 24 h of incubation at 35°C.

To test the effect of different carbon sources on antibiotic biosynthesis by *Streptomyces J12*, starch was replaced, in starch casein medium with an equivalent carbon of glucose, fructose, sucrose, xylose, lactose, arabinose and raffinose. Influence of various levels of best carbon source on the antibiotic biosynthesis was examined.

Different nitrogen sources were supplemented to the basal medium containing the best carbon source and level. The suitability of nitrogen source supporting antibiotic production was examined. In addition to yeast extract, urea and peptone at a concentration of (0.2g/100 ml) were tested. This experiment was carried out in the absence or presence of 0.03g/100 ml casein. Influence of various levels of best nitrogen source was also examined.

Results

The ability of *Streptomyces* isolate to produce antibiotics is not consistent, but could be increased or decreased remarkably under different cultural conditions. The isolate had different antibacterial activities against the test bacteria grown on solid media (Table 1) and in liquid media (Table 2).

Table 1. Antibiotic activity of 7-days old cultures of *Streptomyces* isolate J12 grown on different solid media.

Media	Bacterial species (Inhibition zone in mm)				Fungal species (Inhibition zone in mm)		
	<i>E. coli</i>	<i>S. typhi</i>	<i>S. aureus</i>	<i>B. sub.</i>	<i>F. oxysporum</i> f. sp. <i>melongenae</i>	<i>R. Solani</i>	<i>Candida albicans</i>
S/C	–	–	26 ± 0.6	25 ± 0.6	7 ± 0.9	8 ± 1.2	–
YME	12 ± 0.6	13 ± 0.0	11 ± 1.0	13.7 ± 0.9	12 ± 1.2	11 ± 1.2	–
S/D	30 ± 1.0	29 ± 0.6	22 ± 0.0	20 ± 1.2	8 ± 0.6	9 ± 1.5	–
O/M	20 ± 1.5	15.33 ± 0.9	29 ± 1.5	20.7 ± 0.7	15 ± 0.6	10.7 ± 1.2	–

S/C: Starch casein medium, YME: Yeast-malt extract medium, S/D: Sabouraud/Dextrose medium, O/M: Oat meal medium.

Table 2. Antagonistic properties of 7-days old cultures of *Streptomyces* J12 in different liquid media, under static culture conditions.

Media	Bacterial species (Inhibition zone in mm)				<i>Candida albicans</i>
	<i>E. coli</i>	<i>S. tyfi</i>	<i>s. aureus</i>	<i>B. sub.</i>	
S/C	19 ± 0.6	20 ± 1.2	23 ± 1.5	25 ± 1.0	–
YME	19.7 ± 0.3	13 ± 0.6	18 ± 1.0	21 ± 0.6	–
S/D	11 ± 1.0	–	14 ± 0.6	11 ± 0.6	–

S/C: Starch casien medium, YME: Yeast -malt extract medium, S/D: Sabouraud /dextrose medium.

Streptomyces J12 showed no antimicrobial activity against *Candida albicans*, but it showed different activities against other test organisms (Table 1). The antibacterial activity of isolate J12 against Gram negative bacteria was high when grown on Sabouraud dextrose medium. The lowest activity was exhibited against Gram negative bacteria when grown on yeast-malt extract medium. The isolate J12 showed no antibacterial activity against *E.coli* and *S. typhi* when grown on starch casein. The diameters of inhibition zones produced by isolate J12 ranged between 11-30 mm against tested bacteria and 7-15 mm against tested fungi.

Various antibacterial activities were recorded by *Streptomyces* J12 when grown in different liquid media, under static condition. The highest antibacterial activity was found against all tested bacteria when grown on starch casein medium. The diameters of inhibition zones ranged between 19 mm against *E. coli* and 25 mm against *B. subtilis* when grown in starch casein;

between 13 mm against *S. typhi* and 21 mm against *B. subtilis* when grown yeast-malt extract medium; and finally between 0 mm with *S. aureus* and 14 mm with *S. aureus* when grown in sabouraud medium.

Under the shaken condition, the isolate varied in its antibacterial activities (Table 3). The inhibition zones produced ranged between 9 and 20 mm. The lowest activity was found against *S. aureus* when grown in starch casein and yeast malt extract media, while the highest inhibition was recorded against *B. subtilis*.

Table 3. Antagonistic properties of 7-days old cultures of *Streptomyces* J12 in different media, under shaking conditions.

Media	Bacterial species (Inhibition zone in mm)			
	<i>E. coli</i>	<i>S. tyfi</i>	<i>S. aureus</i>	<i>B. sub.</i>
S/C	15 ± 0.0	12 ± 1.15	–	20 ± 1.15
YME	15 ± 0.58	11 ± 0.15	–	12 ± 1.15
S/D	10 ± 0.0	–	9 ± 0.58	9 ± 1.0

S/C: Starch casein medium, YME: Yeast-malt extract medium, S/D: Sabouraud /dextrose medium.

Influence of Different Culture Conditions on Biomass Yield and Anti-microbial Activity

The data given in Fig. 1 revealed the increasing of growth at the second day of cultivation, and the maximum growth was achieved at the sixth day of incubation. The antibacterial activity was detected starting from the second day, and reaching its maximum activity in the sixth day of incubation.

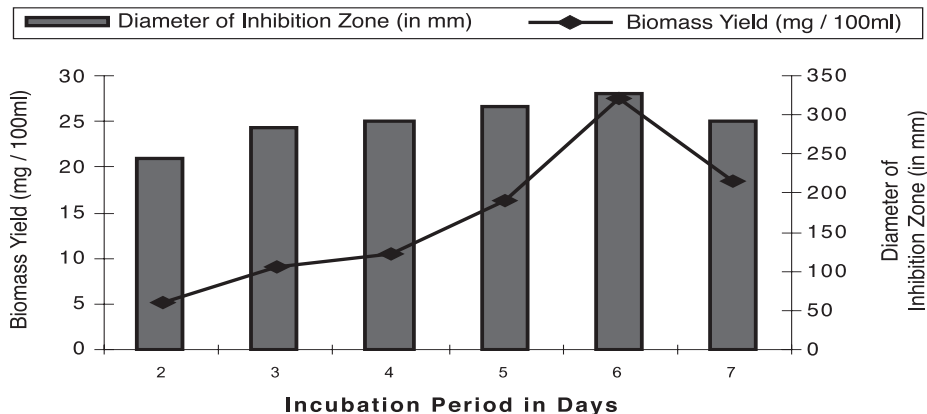


Fig. 1. Effect of incubation period on biomass yield and antibiotic biosynthesis by *Streptomyces* isolate J12.

The initial pH value of the fermentation (Fig. 2) indicated that the production of biomass and antibacterial agents by *Streptomyces* J12 were strongly dependent on the pH value of culture broth. The optimum pH value for biomass production was 7, and 7.2 for the antimicrobial activity. Results in Fig. 3 showed that the isolate J12 was able to grow in all tested carbon sources. However, maximum antibacterial agents biosynthesis was obtained in medium supplemented with starch as a sole carbon source followed by glucose. Although the maximum growth of J12 isolate was obtained in media supplemented with starch, xylose and raffinose, respectively.

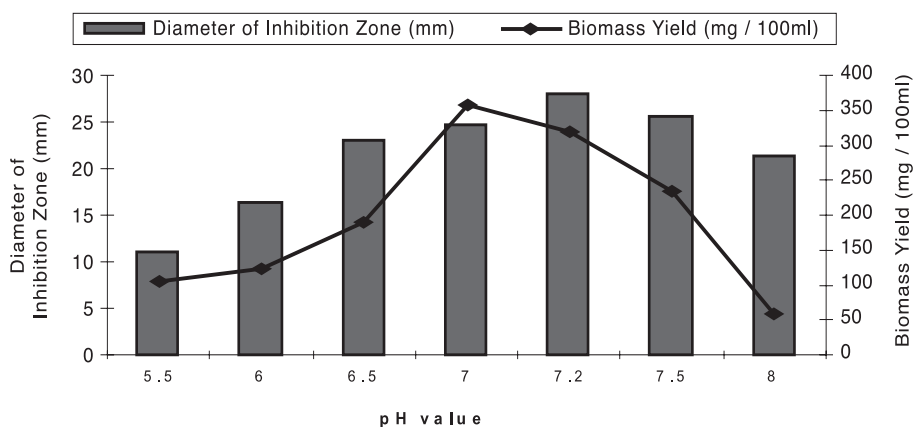


Fig. 2. Effect of different initial pH values on biomass yield and antibiotic biosynthesis by *Streptomyces* isolate.

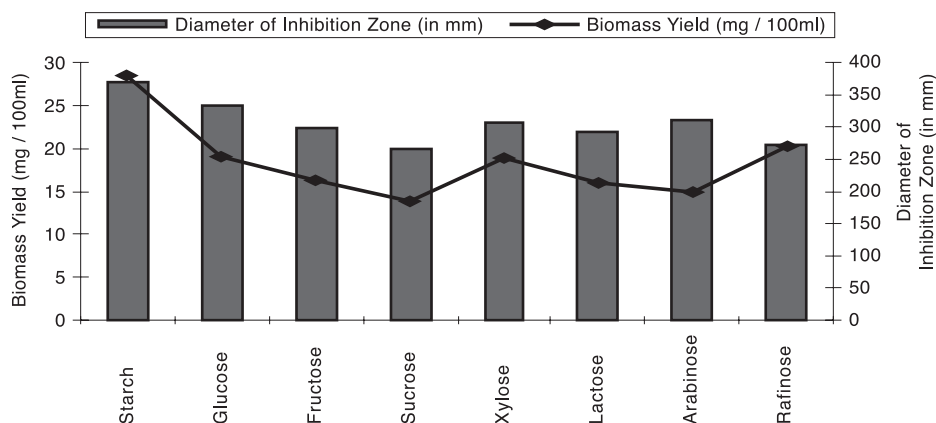


Fig. 3. Effect of different carbon sources on biomass yield and antibiotic biosynthesis by *Streptomyces* isolate J12.

When starch concentrations increased from 2 to 10g/l, the maximum biomass and antibacterial agents were attained (Fig. 4). An approximately linear relationship between the biomass yielding and the antibiotic production was observed. However, increasing starch concentrations above 10g/l was concomitant with decreased antibacterial activity and biomass yield.

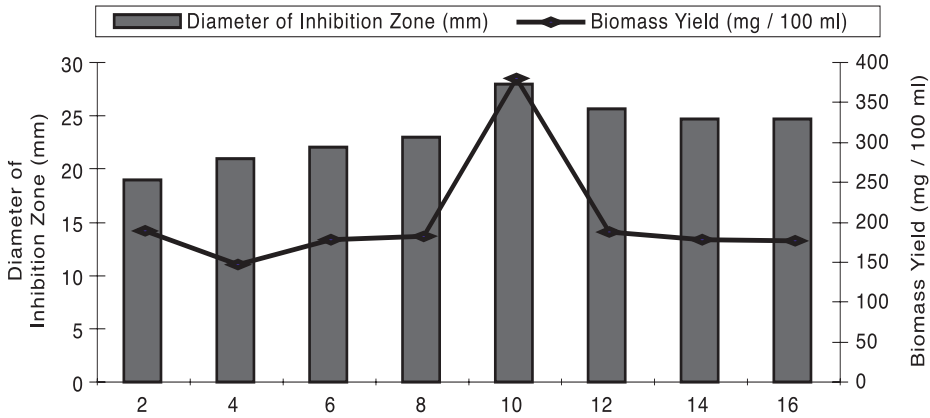


Fig. 4. Effect of different concentrations of starch on biomass yield and antibiotic biosynthesis by *Streptomyces* isolate J12.

Influence of nitrogen sources utilization, without casein is shown in Fig. 5. The highest antibacterial activity was obtained in culture medium containing potassium nitrate followed by yeast extract and then peptone. The addition of casein at concentration of 0.3g/l, was accompanied with the highest antibacterial activity in culture containing potassium nitrate followed by sodium

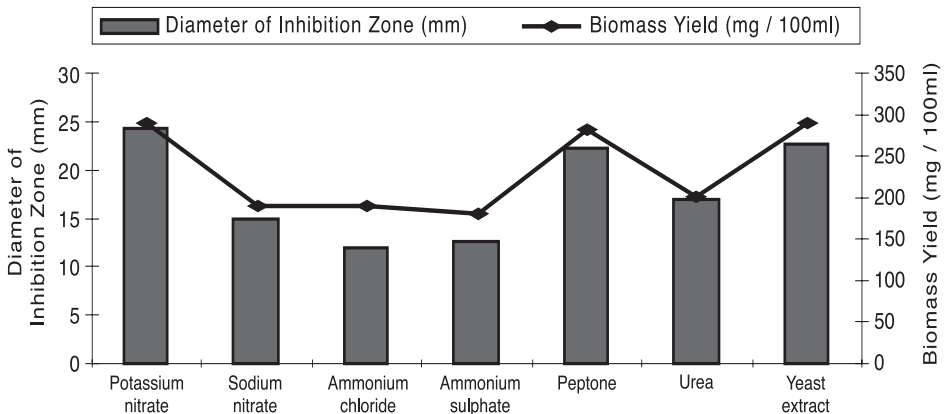


Fig. 5. Effect of different nitrogen sources on biomass yield and antibiotic biosynthesis by *Streptomyces* isolate J12.

nitrate and ammonium sulphate (Fig. 6). The concentration of potassium nitrate was greatly influenced the biosynthesis of antimicrobial activity (Fig. 7). The maximum antibacterial activity was obtained in cultures supplemented with 2.5 g/l of potassium nitrate in the presence of 0.3g/l casein.

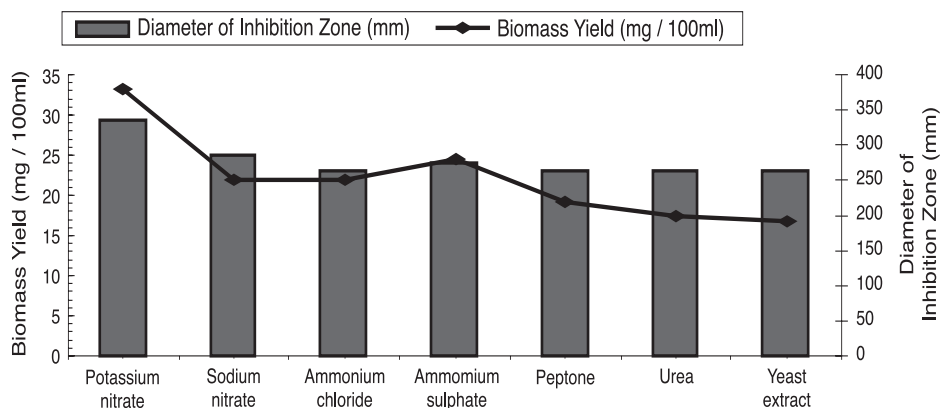


Fig. 6. Effect of different nitrogen sources in presence of casien on biomass yield and antibiotic biosynthesis by *Streptomyces* isolate J12.

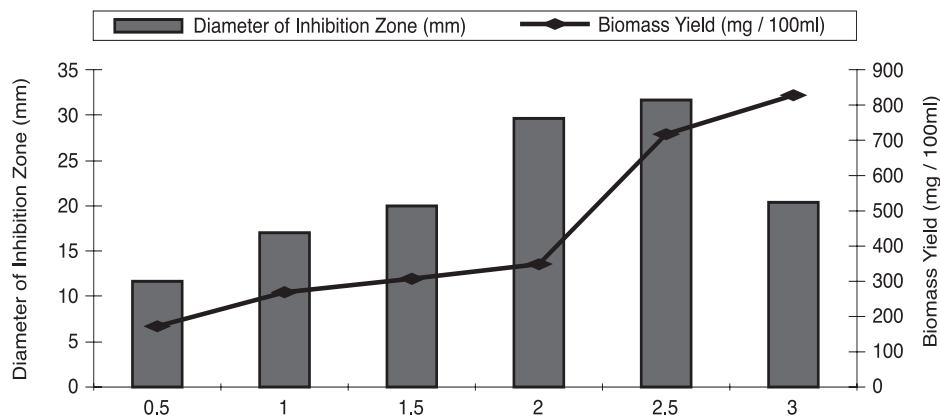


Fig. 7. Effect of different concentrations of potassium nitrate in presence of casien on biomass yield and antibiotic biosynthesis by *Streptomyces* isolate J12.

The maximum production of antimicrobial agents by *Streptomyces* J12 was obtained in a medium of the following composition (g/l): starch, 10; KNO₃, 2.5; K₂HPO₄, 2.5; casein, 0.3; CaCO₃, 2.0; MgSO₄·7H₂O, 0.5; FeSO₄·5H₂O, 0.01; NaCl, 0.5; at pH 7.2, after six days of incubation.

The characteristic properties of *Streptomyces* J12 are shown in Table 4. The aerial mass color was grey in the diagnostic media, with yellow reverse side of colony on most media. It was green on glycerol asparagin agar medium, aerial mycelium with long spore chains formed its open loop, hooks, with spiny spore surface (Fig. 8). This isolate produced a yellow pigment diffused on all media (organic and salts media). This pigment was not pH indicator, and the melanin pigment was not produced. Starch hydrolysis and proteolytic activities were positive on gelatin and milk agar media.

Table 4. Morphology and pigmentation of *Streptomyces* J12.

Spore chain	Spore mass	Color of aerial spore mass	Pigment of substrate mycelia	Diffused pigment	Biosynthesis of melanin on tyrosine agar	Starch hydrolyses	Proteolytic activity
Long-open-loop	Smooth	Grey	Red-orange	Strong red to brown	-	+	+

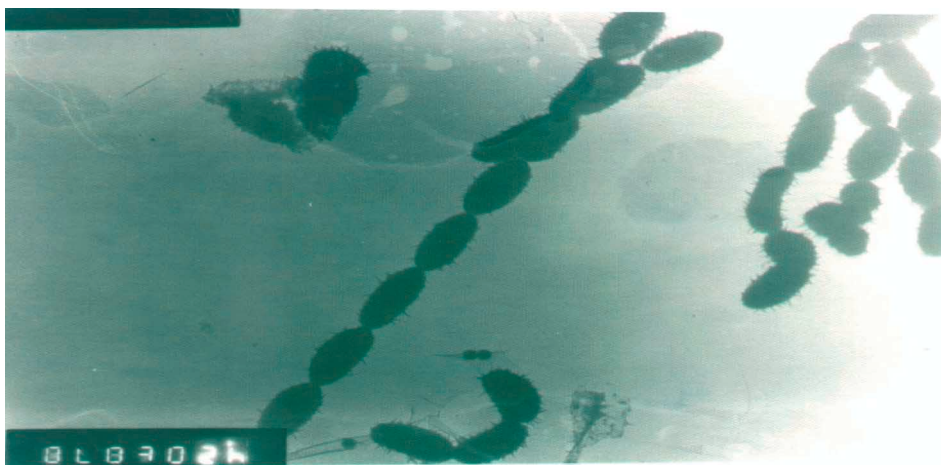


Fig. 8. Spores of *Streptomyces* isolate J12.

Discussion

Oat meal, starch casein and Sabouraud dextrose media appeared to be more favorable for biosynthesis of antimicrobial compounds by *Streptomyces* J12. The higher biosynthesis of antibiotics was on oat meal solid media. *Streptomyces* species are heterotrophic feeders and they can utilize simple molecules as nutrients^[14]. The results showed that some isolates produce antibiotics on solid cultures but its activity against the test organisms of bacteria became lower, higher or negative on liquid media. These results depend on the isolate of

Streptomyces and the type of medium. It was reported that clear elucidation of the antagonistic properties is largely influenced by the quality of the medium or type of organisms^[15,16]. The biosynthesis of antibiotic substances was found to be largely influenced, both quantitatively and qualitatively, by the composition of the medium^[17]. Supplementation of medium with hydrolyzed casein improved growth and antibiotic biosynthesis more than yeast-malt extract. Casein hydrolysate improves growth and antibiotic titre but yeast extract exhibits marked inhibition^[18]. The growth rate and antibiotic biosynthesis were increased on media supplemented with sponge extract by *Streptomyces* sp. (BTL7) which was isolated from the marine sponge *Dendrilla nigra*^[19]. Results revealed that the condition of incubation influenced quantitatively the biosynthesis of antibiotics. Nutritional and cultural conditions for biosynthesis of antibiotics by *Streptomyces psammoticus* under shake-flask conditions have been optimized and resulted in 1.82-fold increase in antibiotic yield^[20]. The ability of microbes to form antibiotics is not a fixed property but can be greatly increased or completely lost under different conditions of nutrients and/or cultivation^[21]. Glucose and ammonium nitrate were found to be the best carbon and nitrogen sources, respectively, for growth and antibiotic SBR-22 biosynthesis by *Streptomyces psammoticus*. Similarly, initial pH of 7.2, incubation time of 96 hours was found to be optimal^[20]. The maximum antibiotic biosynthesis by *Streptomyces* isolate J12 was obtained in medium supplemented with 10g/l starch as a sole carbon source and 2.5g/l potassium nitrate in addition to 0.3g/l casien as nitrogen sources at pH 7.2 after six days of incubation. A study of the influence of different nutritional compounds on antibiotic biosynthesis by *Streptomyces* sp. US24 strain showed that the highest antibiotic biosynthesis that have the highest antibacterial activities were obtained when starch at 1% (w/v) was used as a sole carbon source in the presence of traces of mineral oligoelements^[22].

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دراسات على النشاط التضادي للاستربتومييسس المعزولة من جازان

صالحة حسن مستور الزهراني

كلية إعداد المعلمات، جدة - المملكة العربية السعودية

المستخلص. الهدف من هذا العمل هو دراسة القدرة التضادية لعزلة من الاستربتومييسس عزلت من عينة تربة أخذت من جازان. صنفت العزلة تبعاً للون الميسيليوم الهوائي وتبين أن لها قدرة تضادية في البيئات الصلبة والسائلة ضد البكتيريا الموجبة *Bacillus subtilis*, *Staphylococcus aureus* والسالبة لصبغة جرام *E. coli*, *Salmonella typhi* والفطريات (*Fusarium oxysporum* f. sp. *melongenae* and *Rhizoctonia solani*) وتتأثر هذه القدرة بتركيب البيئة الغذائية، حيث وُجد أن منابت الشوفان المغذي، النشا-الكازين والدكستروز-البيتون أكثر تشجيعاً على إنتاج مضادات الحيوية من المنبت المكون من مستخلص الخميرة - مستخلص الشعير. ووجد أيضاً أن استخدام المزارع المهترزة يخفض من إنتاج مضادات الحيوية بواسطة العزلة J12.

وبدراسة أفضل الظروف للبناء الحيوي لمضاد الحيوية، تبين أن أعلى كمية تم إنتاجها كانت بعد ستة أيام من التحضين، درجة حموضة الوسط الغذائي المناسبة ٢, ٧. كذلك كان أفضل مصدر للكربون هو النشا بتركيز ١٠ جم/ لتر من المنبت الغذائي، أما المصدر النيتروجيني فكانت نترات البوتاسيوم أفضل المصادر سواء بإضافة أو عدم إضافة الكازين ولكن ازداد بناء مضاد الحيوية عند تركيز ٥, ٢ جم / ل من نترات البوتاسيوم مع إضافة الكازين بتركيز ٣, ٠ جم/ل.