Effect of Carbon and Nitrogen sources on the antibiotic production by local isolate of Streptomyces sp.Z1

Ayhan R. Mahmoud
College of Dentistry- University of Tikrit-IRAQ
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Abstract:

The genus Streptomyces consists of sporulating Gram-positive soil bacteria with a mycelia growth habit, and a life cycle with complex morphological and physiological differentiation, also produces antibiotics which are the secondary metabolite.

The objective of the present study is to study of an ability of a local isolate of Streptomyces Z1 to produce antibacterial substance, and effect of different carbon and nitrogen sources on antibacterial production. Results in present study showed that Streptomyces Z1 have ability to produce antimicrobial agent against Escherichia coli, Shigella dysentery, Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis which indicated by diameter of inhibition zone that reached to 16.0, 19.3, 18.1, 25.0, 9.2, 11.2, 12.0 mm, respectively, results in this study showed that antimicrobial agent produced by Streptomyces Z1 was more effective against gram negative organisms rather than gram positive.

Production of antibiotics from microorganisms varies with the constituents of the media. In present study we have investigated the influence of medium constituents such as carbon and nitrogen sources on the antibiotic production from Streptomyces Z1, different carbon sources were used in this study included the following: maltose, glycerol, galactose, glucose, mannitol, lactose, starch, and dextrose, the samples were assayed against K. pneumonia, the highest antibacterial activity was obtained when dextrose at 4% was used as carbon sources. We have also tested a number of nitrogen sources (Yeast extract, L-valine, sodium glutamate, Na2NO3, NH4SO4, NH4NO3) on antibiotic production by local isolate of Streptomyces sp.Z1, results showed that Yeast extract at 1.5% was the most suitable nitrogen source, though L-valine, sodium glutamate, Na2NO3, NH4SO4, NH4NO3 gave moderate antimicrobial activity.

Results:

The considered carbon sources were investigated for their influence on the production of the antibiotic by Streptomyces Z1. It was found that Glucose, Maltose, Mannitol and Galactose stimulate the antibiotic production, whereas the production of antibiotic was reduced in the case of Glycerol, Lactose and Starch. The results showed that maximum antibacterial activity against K. pneumonia was obtained with Glucose as a carbon source.

The effect of different nitrogen sources on the antibiotic production was studied. It was found that Yeast extract, L-valine, and sodium glutamate stimulate the production of the antibiotic, whereas Na2NO3, NH4SO4, and NH4NO3 gave moderate antibacterial activity.

Conclusion:

The study revealed that Streptomyces Z1 isolate produces an antibacterial agent against K. pneumonia. The production of the agent was significantly influenced by the type of carbon and nitrogen sources used. Glucose and L-valine were found to be the most suitable sources for the production of the agent.

References:

Introduction:-

*Streptomyces* genus is filamentous bacteria of the family *Streptomycetaceae*; belong to the order Actinomycetales that includes more than 500 species occurring in soil and water. Members of the genus *Streptomyces*, have remarkable capacity to produce a wide variety of secondary metabolites that include about half of the known microbial antibiotics in use today. Many of these compounds have been important in medicine, such as aminoglycosides, anthracyclines, chloramphenicol, β-lactams, macrolides, tetracycline's etc.¹,²,³.

Antibiotics are the secondary metabolites produced by microorganisms, classified into several families, such as polyketides, polyethers, macrolides, and β-lactams, based on chemical structure similarity and common biosynthetic pathways, and they are often produced from the same primary metabolic precursors.⁴ The secondary metabolites accumulate only after the growth phase "tropophase" when the culture attains a specific growth rate. Secondary metabolites are often called "idiolites" usually produced in the stationary phase "idiophase".⁵,⁶

A study on the production of antibiotics usually involves a search for optimal media. This is achieved by a systematic study of the suitability of a large number of carbon and nitrogen sources. Earlier experiments have shown the effects of carbon source, nitrogen, and other culture medium variables on antibiotic production in *Streptomyces*.⁷,⁸,⁹ glucose repression of a variety of streptomyces promoters is known to occur.¹⁰,¹¹,¹²

The wide occurrence of multiple antibiotic-resistant bacteria pathogens of humans has made it urgent to develop new antibiotics. Although over 6,000 different antibiotics have been identified from Actinomycetes, these microorganisms are still considered likely to be an important source of further new antibiotics. Therefore, intensive search for new antibiotics is going on worldwide.⁴,¹³

The present study focuses on the production of an antibiotic from local isolates of *Streptomyces sp. Z1*, and to determine how carbon source and nitrogen source could be manipulated to enhance production of the antimicrobial agent by *Streptomyces sp. Z1*.

Materials and methods:-

Microorganisms:

Local isolate of *Streptomyces sp. Z1* was used in this study for antibiotic production. To determine antibiotic activity, test organisms *Escherichia coli, Shigella dysentery, Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis* were used. *Streptomyces sp. Z1*, and all test organisms were obtained from Genetic Engineering and Biotechnology Institute / University of Baghdad.

Stock Culture:

Local isolate of *Streptomyces sp. Z1* were grown on R2YE media, and incubated for 14 days at 28 °C to allow for sporulation. Sterile distilled water (5 ml) was added to each plate, and the surface was gently scraped to release the spores. Suspensions were collected by centrifugation and washed twice with distilled water.

Production Media:

1 ml of the stock cultures of local isolates of *Streptomyces sp. Z1* inoculated into 100 ml of sterilized seed medium in 500 ml conical flasks consisting of 0.5% tryptane, yeast extract 0.3%; pH adjusted to 7.2, and incubated with shaking at 28°C for 48 h, 8 ml of the culture was transferred in to a 500-ml Erlenmeyer flask containing 100 ml modified production medium. The modified production medium was composed of maltose 4%, sodium glutamate 1.2%, K₂HPO₄ 0.01%, MgSO₄.7H₂O 0.05%, CaCl₂.2H₂O 0.01%, FeSO₄.7H₂O 0.005%, ZnSO₄.7H₂O 0.0005%; pH adjusted to 8.0. The cultures were then fermented on a rotary shaker at 200 rpm for 8 days at 30 °C, and antibiotic accumulation monitored daily.

Antimicrobial activity:

Antimicrobial activity in cultures of *Streptomyces sp. Z1* against test organisms was determined. 5 ml of samples were withdrawn from fermented production media, and centrifuged at 5000 rpm for 15 min to separate the mycelia biomass, then all extracts of
Streptomyces sp. Z1 were assayed for their antimicrobial activity by using agar well diffusion method (17).

Culture conditions:
To enhance antibiotic production by Streptomyces sp. Z1 in production medium, different carbon and nitrogen sources were used which included the following:

Effect of carbon source on antibiotic production:
To detect the effect of carbon-source on antibiotic production of Streptomyces sp. Z1, various carbon sources were used, include the following: maltose, glycerol, galactose, glucose, mannitol, lactose, starch, and dextrose, also effect of optimal carbon source concentration (1%, 2%, 3%, 4%, 5%, 6%) on production of the antibiotic was studied.

Effect of nitrogen source on antibiotic production:
Effect of different nitrogen sources (Yeast extract, L-valine, sodium glutamate, Na2NO3, NH4SO4, NH4NO3) on antibiotic production by local isolate of Streptomyces sp.Z1 were studied, and the effect of optimal nitrogen source at varied concentrations (0.6%, 0.9%, 1.2%, 1.5%, 1.8%, 2.1%) on antibiotic production were also studied.

Results:
The biosynthesis of antimicrobial substance by Streptomyces sp. Z1 was studied during fermentation in modified production media, results showed that Streptomyces sp. Z1 have ability to produce antimicrobial substance against test organisms (Escherichia coli, Shigella dysentery, Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis) used in this study. After incubation test organisms with extracts of Streptomyces sp.Z1 for 24 hr at 37 ºC, the zone of inhibition was measured for each test organisms. The diameter of inhibition zone for gram negative bacteria: Escherichia coli, Shigella dysentery, Pseudomonas aeruginosa, Klebsiella pneumoniae was 16.0, 19.3, 18.1, 25.0 mm, respectively, where for gram positive bacteria: Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis was 9.2, 11.2, 12.0 mm, respectively, as shown in figure (1).

According to results in figure (1), the Gram-negative organisms were more sensitive than Gram-positive organisms to the antibiotic produced by Streptomyces sp. Z1, also result showed that the antibiotic was more effective against Klebsiella pneumoniae so it was chosen for further studies.

Antibiotic production varies with the constituents of the media, we attempted in present study to enhance of Streptomyces sp.Z1 for antibiotic production by optimizing the carbon and nitrogen sources in production media. The antimicrobial activity of Streptomyces sp.Z1 against test organism K. pneumoniae was influenced by addition of different carbon sources such as maltose, glycerol, galactose, glucose, mannitol, lactose, starch, and dextrose, it was observed that antimicrobial agent production was greatly increased by addition of dextrose, followed by starch, maltose, glycerol, mannitol, lactose, and glucose, where no antimicrobial activity was detected using galactose, as presented in Figure (2). Results in this study showed that antimicrobial activity of Streptomyces sp. Z1 against K. pneumonia was induced by dextrose, starch, maltose and less activity found with glucose, as shown in Figure (3). As observed in Figure (2), the antibiotic production by Streptomyces sp. Z1 increased with the addition of dextrose in production media although starch, maltose and glycerol were a good carbon sources for antibiotic production, the effect of different dextrose levels (1%, 2%, 3%, 4%, 5%, 6%) on antibiotic production was studied. The diameter of inhibition zone was measured after incubation of Streptomyces sp. Z1 cultures on plates of K. pneumoniae for 24 hr at 37ºC. Optimum dextrose concentration for antibiotic production was 4%, the diameter of inhibition zone reached to 30.1 mm, where low antimicrobial activity observed with dextrose at 1%, 2%, 3%, 5%, 6%, the zone of inhibition were 10.5, 18.0, 27.0, 10.0, 10.0 mm, respectively, as shown in figure (4). Also the effect of number of nitrogen sources (Yeast extract, L-valine, sodium glutamate, NH4SO4,
NaNO₃, NH₄NO₃) on antibiotic production by Streptomyces sp.Z1 was studied. It has been observed that the optimum nitrogen source for antibiotic production by Streptomyces sp. Z1 was yeast extract, and the diameter of inhibition zone against K. pneumonia was reached to 28.5 mm, followed by sodium glutamate, L-valine, NH₄NO₃, NaNO₃, NH₄SO₄, that the zone of inhibition were 24.1, 18.6, 12.5, 11.0, 9.0 mm, respectively, as shown in Figure (5). Results in present study showed that organic nitrogen source were exceeded inorganic nitrogen sources in production of antibiotic. The optimum nitrogen sources in this study was yeast extract, the effect of different Yeast extract concentrations (0.6%, 0.9%, 1.2%, 1.5%, 1.8%, 2.1%) on the biosynthesis of antimicrobial antibiotic by Streptomyces sp. Z1 was studied. Yeast extract at 1.5% was found to be the optimum concentration for antibiotic production although production of antibiotic at other concentrations but at less quantities, the diameter of inhibition zone against K. pneumoniae was 28.7 mm, where the zone of inhibition at 0.6, 0.9, 1.2, 1.8, 2.1%, was 14.2, 18.0, 26.5, 28.7, 17.2, 12.0 mm, respectively, as shown in Figure (6).

Figure (2): Effect of carbon source on antibiotic production by *Streptomyces sp. Z1*.

Figure (3): Effect of carbon source (1. Dextrose, 2. Starch, 3. Glucose, 4. Maltose) on Antibiotic production by *Streptomyces sp. Z1*. 

<table>
<thead>
<tr>
<th>Carbon Source</th>
<th>Zone of Inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextrose</td>
<td>30</td>
</tr>
<tr>
<td>Starch</td>
<td>26.1</td>
</tr>
<tr>
<td>Lactose</td>
<td>14</td>
</tr>
<tr>
<td>Manitol</td>
<td>18</td>
</tr>
<tr>
<td>Glucose</td>
<td>10.5</td>
</tr>
<tr>
<td>Galactose</td>
<td>0</td>
</tr>
<tr>
<td>Glycerol</td>
<td>20.1</td>
</tr>
<tr>
<td>Maltose</td>
<td>25</td>
</tr>
</tbody>
</table>
Figure (4): Effect of dextrose concentration on antibiotic production by Streptomyces sp. Z1.

Figure (5): Effect of nitrogen source on antibiotic production by Streptomyces sp. Z1.
Discussion:-
Actinomycetes produce approximately two-thirds of all known antibiotics of microbial origin, including over 6,000 different chemical structures, and they continue to be an excellent source of novel compounds. Many of these natural products are commercially important medicinal compounds with a variety of therapeutic uses \(^{(3,18,19)}\). Results in present study indicated that local isolate of *Streptomyces* sp. Z1 have ability to produce antibiotic against isolates of pathogenic bacteria included: *Escherichia coli*, *Shigella dysenteria*, *Pseudomonas aeruginosa*, *K. pneumoniae*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Bacillus subtilis*. These results are comparable with some *Streptomyces* species recorded to secrete antibiotics against bacteria, fungi and yeast, such as production of actinorhodin by *S. coelicolor* A3 \(^{(2, 20)}\), pristinamycin by *S. pristinaespiralis*\(^{(21)}\), leucomycin by *S. kitasatoensis*, cephalosporin by *S. clavuligerus* \(^{(22, 23)}\), also *S. rimosus* is a known industrial producer of oxytetracycline and was originally isolated from soil \(^{(24)}\). Indeed, most known microbial producers of the different tetracyclines are bacteria native to soil.

Actinomycetes are usually present in large numbers in soil, and they constitute about 10% of the cultivable microbial population, exceeding 1 million CFU/g of soil \(^{(25, 26)}\). Antibiotic production is greatly influenced by cultural conditions and media components, which vary from organism to organism \(^{(27, 28)}\), in present study attempted to optimize such nutritional constituents as carbon and nitrogen sources to improve antimicrobial agent production by *Streptomyces* sp. Z1, results in this study indicate that dextrose was the best carbon source for antibiotic production where less antibiotic production found with glucose, and lactose. Dextrose may be utilized less rapidly, and thus it is available during the phase of antibiotic production, in a study with *Streptomyces kanamyceticus* M27, dextrose proved to be an excellent carbon source for antibiotic production \(^{(29)}\) where glucose, usually utilized rapidly for the synthesis of cellular material so that little would be available as carbon and energy source for antibiotic synthesis, also glucose interferes with the biosynthesis of many antibiotics such as bacitracin \(^{(30)}\) and actinomycin \(^{(31)}\). During studies on fermentation medium development, polysaccharides or oligosaccharides are often found to be better than glucose as carbon.

Figure (6): Effect of Yeast extract concentration on antibiotic production by *Streptomyces* sp. Z1.
sources for antibiotic production (32). Other studies indicated that *S. hygroscopicus* D1 and *S. venezuelae* produced antibiotic optimally with glycerol as the carbon source (20,33). As dextrose was an excellent carbon source for antibiotic production by *Streptomyces* sp. Z1, different levels of dextrose were tested to determine the optimal concentration for antibiotic production. Dextrose at 4% was the best concentration for antimicrobial agent production Figure (3), where higher dextrose levels decreased the activity of antibiotic. At higher concentration of carbon source with a slow uptake rate, the production of the antibiotic decreased. This is probably because of accumulation of carbon source in the fermentation broth leading to the fragmentation and autolysis of the mold (34). Antibiotic production is in general subjected to the suppressive effects caused by an excess of nutrients such as carbon, nitrogen, and phosphate sources (2, 20), also it found that the optimal dextrose concentration for antibiotic production by *S. Kanamyceticus*, was 2%, where at higher dose of dextrose decreased of antibiotic production (29).

The nature and the amount of the nitrogen source are both critical in determining the onset of antibiotic production. In this study different nitrogen sources were added to the production medium included organic nitrogen source (Yeast extract, sodium glutamate, L-valine) and inorganic nitrogen sources (NH4SO4, NH4NO3, NaNO3), it has been found that organic nitrogen sources were exceeded inorganic nitrogen sources in production of antibiotic by *Streptomyces* sp. Z1 (Figure 5), these results agreed with other studies which indicated that organic nitrogen sources were better in antibiotic production rather than inorganic nitrogen sources (35,36,37), where inorganic nitrogen source, ammonium sulphate was favored for cephalosporin production by *C. acremonium*, it was observed to be the best nitrogen source for the higher production of cephalosporin (6,21), also maximum antibiotic production by *S. kanamyceticus* was obtained in a synthetic medium containing (NH4)H2PO4 as the nitrogen source (29). The absence of production or the delay observed with some nitrogen sources could result either from a shortage of precursors or from a negative regulation of the enzymes involved in antibiotic biosynthesis (21).

High content of nitrogen is found to decrease the production of antibiotic. In this study Yeast extract at 1.5% was optimum concentration for antibiotic production by *Streptomyces* sp. Z1, and the high concentrations prevented antibiotic production. It might be due to the reason that it interferes to the process of differentiation of mycelium to swollen hyphal fragments and arthrospores during the production stage. Production of CPC by many strains of *C. acremonium* is known to be stimulated by the addition of the amino acid methionine, it was observed that at higher concentration of methionine, the percentage of unicellular arthrospores increased from 55% to 80% and CPC production is decreased to 85% of that obtained with normal methionine level. This decrease in production of antibiotic might be due to its toxic effect on the mold (38,39). In addition, the same delay in pristinamycin production by *S. pristinaespiralis* that was observed with high levels of glutamate and ammonium (21,23).

Further studies are recommended to study the effect of other nutritional factors on antibiotic production by *Streptomyces* sp. Z1, and to purify, characterize the antibiotic.
References:


