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Research Article

OPTIMIZATION OF MEDIUM COMPOSITION FOR ANTIBACTERIAL METABOLITE PRODUCTION FROM *STREPTOMYCES SP.*

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ABSTRACT

Objectives: This paper aimed to optimize some essential nutritional components (carbon, nitrogen, and phosphate) of fermentation medium necessary for the production of antibacterial metabolites from *Streptomyces* sp.

Materials and Methods: *Streptomyces* sp. LH9 previously isolated from desert soil in Karbala Province, Iraq. This strain produced antibiotic against 4 pathogenic bacteria, including *Escherichia coli, Staphylococcus aureus, Streptococcus agalagtiae,* and *Pseudomonas aeruginosa*. For optimizing, the essential nutritional requirements such as carbon, nitrogen, and phosphate in fermentation media different concentrations of these sources were used to improve the antibacterial metabolite production.

Results: All the studied nutritional parameters were had impacts on the antibacterial metabolite production from *Streptomyces* sp. LH9. The actinobacterial strain produced a highest antibiotic metabolites when was grown in the fermentation medium supplemented with 2% dextrose (as a sole carbon source), 0.05% peptone (as a sole nitrogen source), and 0.05% K_2HPO_4 at pH 7 and incubated under optimal conditions; at 30°C with 250 rpm (revolutions/min) agitation for 7 days.

Conclusion: *Streptomyces* sp. LH9 was a good producer for antibacterial against Gram-positive and Gram-negative bacteria, which required simple nutritional supplements in the fermentation medium. Furthermore, could be utilized the industrial waste for improving the production in the most economic manner.

Keywords: *Streptomycetes* sp., Carbon, Nitrogen, Phosphate source.

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INTRODUCTION

The presence of multidrug-resistant pathogenic bacteria creates the need to search for new potential antibiotics against it, which became an important area of antibiotic research in the contemporary scenario. Natural bioactive substances having unique structures have been observed to perform natural biological activities. The soil is a natural source for microorganisms and their antimicrobial products [1]. Organisms from extreme habitats have gained considerable attention in recent years because of their diversity and biological activities which come mainly from their ability to produce novel chemical compounds of high commercial value [2,3].

Actinomycetes have identified as one of the major groups of the soil population, which may vary with the environment of ground type [4]. *Streptomyces* are filamentous bacteria with a complex life cycle. Aerial growth coincides with the production of many secondary metabolites; *Streptomyces*is widely recognized as industrially relevant microorganisms for their ability to produce different types of novel secondary metabolites including many clinically important antibiotics [5].

Streptomyces species produces about 75% of commercially and medically useful antibiotics [6]. They have provided more than half of the naturally occurring antibiotics discovered to date and continue to screen for useful compounds [7]. Still, microbial natural products remain the most promising source of novel antibiotics although new approaches are required to improve the efficiency of the discovery process [8].

It is well known that designing an appropriate fermentation medium is of critical importance in the production of secondary metabolites [9]. Prior knowledge and experience in developing a suitable basal medium may play a significant role in the further media optimization [10]. Production of secondary metabolites through fermentation is influenced by various environmental factors, including nutrients nitrogen, phosphorus, and carbon sources [11-13]. Furthermore, production of valuable metabolites by *Streptomyces* differs qualitatively and quantitatively depending on the strains used in fermentation as one of the most significantroles as sources of precursors and energies for secondary metabolite production [14]. Therefore, influences of medium components and environmental conditions are an initial and important step to improve metabolite production of the genus *Streptomyces*.

The primary objective of this research was studying the effects of nutritional factors (carbon, nitrogen, and phosphorous sources) on antimicrobial metabolite production under optimum fermentation conditions to formulate excellent medium for improving the antibiotic production.

MATERIALS AND METHODS

Microorganisms used in the study

Streptomyces sp. LH9 previously isolated from desert soil at Karbala Province, Iraq. This isolate was identified according to microbial, cultural, biochemical and physiological characteristics [15,16]. The strain produced antibiotic against Gram-positive and Gram-negative bacteria.

Four test pathogenic bacteria, including Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) and Gram-positive (*Staphylococcus aureus* and *Streptococcus agalagtiae*), were used as test microorganisms for evaluation the antibacterial activity of *Streptomyces* sp. All the

tested pathogenic bacteria were obtained from laboratory of general health in Karbala province. All bacterial isolates were preserved in slant media at 4°C until used.

Antibiotic production from *Streptomyces* sp.

The antibacterial metabolite was produced from *Streptomyces* sp. LH9 using the sterile fermentation broth as a basal medium, which composed from (g/100 ml distilled water): 2.5% glucose, 0.5% soybean, 0.5% NaCl, 2% K_2HPO_4, 0.05% $MgSO_4$, and 0.01% $CaCO_3$ at pH 7 [17].

The inoculum was separately prepared by cultivated the actinobacterial isolate on starch casein nitrate agar at 30°C for 7 days. Then, the liquid media (50 ml media/250 ml Erlenmeyer flask) were incubated with 2 disks (6 mm in diameter) of actinobacterial inoculum and incubated at 30°C in a rotary shaker at 250 rpm (revolution per minutes) for 7 days. After that, the culture broth was centrifuged at 8000 rpm at 4°C for 20 minutes. The aliquot supernatant was filtered using 0.45 µm membrane filter (millipore). The filtrate of actinobacterial isolate was tested as antibacterial metabolite [18].

The antibacterial activity was determined, pathogenic bacteria were grown overnight in nutrient broth at 37°C for 24 hrs (O.D = 0.5; McFarland 1×10^8 CFU/ml). The cultures of test organisms were streaked on Mueller-Hinton agar medium separately. Wells (6 mm in diameter) were prepared in each seeded agar plate, and each well was filled with 60 µl of the aliquot supernatant containing the active metabolites [19]. The plates were kept at 4°C for 24 hrs for the diffusion of the metabolites. Then, the plates were incubated at 37°C for 24 hrs. After the incubation period, the diameter of inhibition zones was measured. Each test was in triplicate, and the activities were shown as the mean of the diameter of the inhibition zone [20,21].

Optimization of culture conditions for antibiotic production

The optimization of cultural conditions was done for improving the production of antimicrobial metabolites from selected isolate using batch culture fermentation.

Carbon source

Streptomyces sp. LH9 was grown under optimum conditions (in triplicate), including the basal medium, incubation temperature 30°C with shaking 250 rpm for 7 days. The basal medium composed from 2.5% carbon source, 0.5% soybean, 0.5% NaCl, 0.2% K_2HPO_4, 0.05% $MgSO_4$, and 0.01% $CaCO_3$ at pH 7. Various carbon sources (2.5%) were separately added to the production media such as sucrose, sorbitol, dextrose, galactose, glucose, glycerol, and maltose. The basal fermentation medium without any carbon source was used as a control. All carbon sources were sterilized separately with 0.45 µm millipore filter and then, added separately to the sterile basal fermentation medium under aseptic conditions [22].

The best carbon sourcequantity was determined using different concentrations of sugar ranged from 1% to 3.5%, in triplicate. The antibiotic production was performed under optimal conditions.

Nitrogen source

The nitrogen source was optimized using different sources (0.5%) were added to basal fermentation media separately, such as malt extract, soybean, peptone, beef extract, and casein. The basal medium composed of the optimum carbon source and the same nutrition components as mentioned above, except the nitrogen source was changed. The control represents the basal medium without any nitrogen sources [23]. The antibiotic production was performed under optimal conditions, incubation temperature 30°C, and shaking 250 rpm for 7 days, in triplicate.

The best nitrogen concentration was studied using different levels of the best nitrogen source ranged from 0.0005 to 10/100 ml medium, in triplicate. The antibiotic production was performed under optimal conditions.

Phosphate source

The *Streptomyces* sp. LH9 isolate was grown under optimal culture conditions, the basal medium containing the optimum carbon and nitrogen sources and the other nutrition components as mentioned above, except the phosphate which was used from different sources. The phosphate sources were included Na_2HPO_4, $(NH_4)\ H_2PO_4$, NaH_2PO_4, K_2HPO_4, KH_2PO_4, $(NH_4)\ _2HPO_4$ at concentration 0.2% and the control represents the basal medium without any phosphate sources, in triplicate.

The phosphate concentration was optimized for the highest level of antibiotic production; different levels of the best phosphate source were supplemented to the media (0.125, 0.25, 0.50, 1.00, 2.00, 4.00, and 8.00) g/100 ml, in triplicate. The antibiotic production was performed under optimal conditions.

Statistical analyses

Data were analyzed using the SPSS version 22 software Fisher's exact with a significant value of <0.05.

RESULTS AND DISCCUSION

In previous research, the *Streptomyces* sp. LH9 strain was recovered from desert soil and preserved in starch casein nitrate agar slants at 4°C. This strain produced antibacterial metabolites against pathogenic Gram-positive and Gram-negative bacteria, including *S. aureus*, *S. agalagtiae*, *E. coli*, and *P. aeruginosa*. The optimal conditions for antibiotic production were 30°C in shaker incubator (250 rpm) for 7 days using basal fermentation medium composed of (g/100 ml distilled water): 2.5% glucose, 0.5% soybean, 0.5% NaCl, 2% K_2HPO_4, 0.05% $MgSO_4$, and 0.01% $CaCO_3$ at pH 7. In the present study, the effect of nutrition media components such as carbon, nitrogen, and phosphate sources were investigated to formulate the excellent medium for highest antibiotic production.

Carbon source

The efficiency of antibiotic production was studied from *Streptomyces* sp. LH9 by growing it in media supplemented with different carbon sources (sucrose, sorbitol, dextrose, galactose, glucose, glycerol, and maltose). The results revealed that *Streptomyces* sp. LH9 produced variable levels of antibiotic metabolites with the variance of carbon sources of media (Fig. 1). The best carbon source was dextrose, which exhibited high ability to stimulate the antibiotic productivity.

It is possible that the carbon sources (sucrose, sorbitol, galactose, glucose, glycerol, and maltose) are utilized rapidly for the synthesis of cellular material; hence, that little would be available as carbon and energy source for antibiotic synthesis [24, 25]. While dextrose may be a good stimulator for growth and antibiotic production in addition to other media components. It is interesting, however, to note that antibiotic formation is not solely dependent on cellular growth. This result was in agreement with that obtained by Pandey *et al.* [26] when some carbohydrates were investigated for their effect on the growth of *Streptomyces kanamyceticus* M27 and antibiotic production. Dextrose proved to be an excellent carbon source for antibiotic biosynthesis [26]. Our results also were in agreement with the result revealed by Sharon *et al.* [27], when they studied the effect of different carbon sources for the production of antibacterial compounds from *Streptomyces* sp. KOD10 and the optimal carbon source was dextrose sugar.

In our study, the dextrose was an excellent carbon source for antibiotic production from *Streptomyces* sp. LH9, different levels of dextrose were tested to determine the optimal concentration for antibiotic production. The results in Fig. 2 showed the dextrose at a concentration of 2 g/100 ml induced the maximal antibiotic level, whereas the gradual increasing in dextrose concentration up to 3.5 g/100 caused decreasing the yield.

These results were in agreement with Sharon *et al.* [27], who found the different dextrose concentration impact on *Streptomyces* sp.

KOD10 growth and antibiotic production, but the maximum dextrose concentration for excellent antibiotic production was 1.5%.

Nitrogen source

For investigating the influence of nitrogen source on the antimicrobial metabolite production from *Streptomyces* sp. LH9, different nitrogen sources (malt extract, yeast extract, peptone, beef extract, and casein) were detected. The results showed the excellent nitrogen source was peptone, whereas the other sources (malt extract, yeast extract, beef extract, and casein) appeared less efficiency in supporting the production of antibacterial metabolites as shown in Fig. 3.

These results were consistent with the results of Oskay [28], who found the excellent nitrogen source for antimicrobial production from *Streptomyces* sp. KGG32 was peptone. Furthermore, these results were in agreement with results obtained by Ababutain *et al*. [29], among several nitrogen sources were tested, the peptone was preferable to produce the antibiotic from *Streptomyces* sp.

The nature and the availability of nitrogen from the organic and inorganic nitrogen compounds in fermentation media showed variable influences on the antibiotic levels. High yields of antibacterial compounds are favored by a tight temporal coupling between biomass accumulation and antibacterial compounds synthesis.

As peptone was the best nitrogen source for antibiotic production by *Streptomyces* sp., in our results, the effects of different peptone concentration the productivity of antimicrobial agent were studied. The results showed the maximum peptone concentration was required to reach maximum antibacterial metabolite production from *Streptomyces* sp. LH9 was 0.05 g/100 ml as shown in Fig. 4.

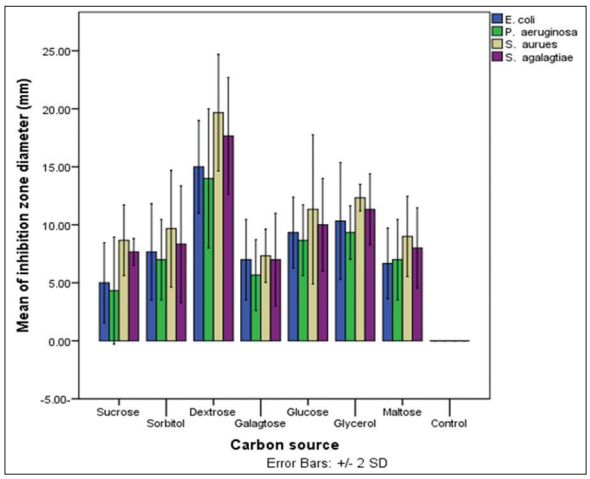

Fig. 1: Effect of carbon sources on the antibacterial metabolite production from *Streptomyces* sp. LH9

The actinobacterial strain was grown in liquid media containing 2.5% carbon source at pH 7, 30°C and 250 rpm for 7 days. The antibiotic activities were measured according to the agar diffusion method using Mueller-Hinton agar; each well (6 mm in diameter) was filled with 60 µl of the aliquot supernatant containing actinobacterial metabolites and the plates were incubated at 37°C for 24 hrs.

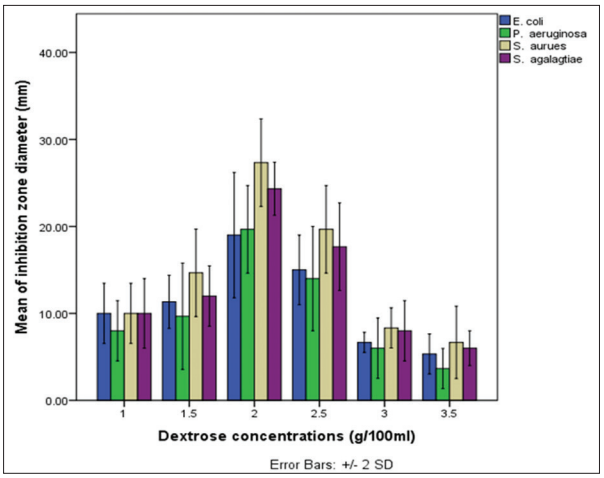

Fig. 2: Effect of dextrose concentrations on the antibacterial metabolite production from *Streptomyces* sp. LH9

The strain was grown in liquid media (pH 7) at 30°C and 250 rpm for 7 days containing different concentrations of dextrose. The antibiotic activities were measured according to the agar diffusion method using Mueller-Hinton agar; each well (6 mm in diameter) was filled with 60 µl of the aliquot supernatant containing actinobacterial metabolites and the plates were incubated at 37°C for 24 hrs.

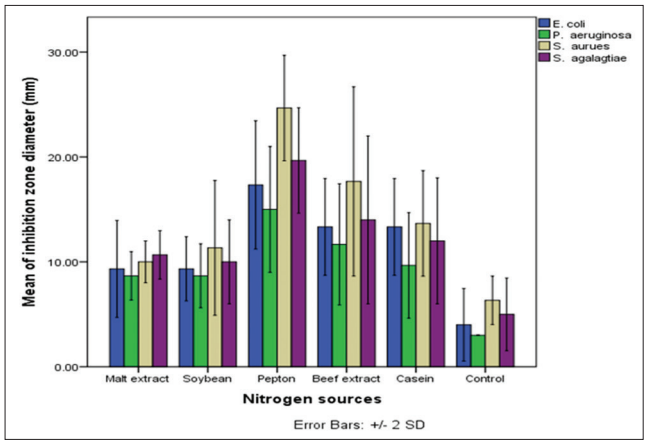

Fig. 3: Effect of nitrogen sources on the antibacterial metabolite production from *Streptomyces* sp. LH9

The strain was grown in liquid media (pH 7) at 30°C and 250 rpm for 7 days containing different nitrogen sources (0.5%). The antibiotic activities were measured according to the agar diffusion method using Mueller-Hinton agar; each well (6 mm in diameter) was filled with 60 µl of the aliquot supernatant containing actinobacterial metabolites and the plates were incubated at 37°C for 24 hrs.

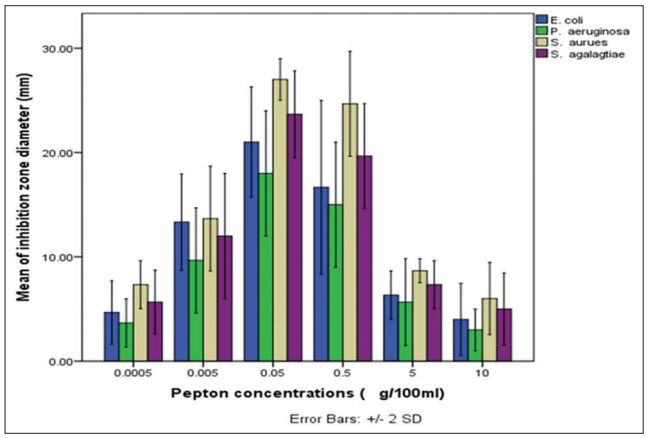

Fig. 4: Effect of peptone concentrations on the antibacterial metabolite production from *Streptomyces* sp. LH9

The strain was grown in liquid media (pH 7) at 30°C and 250 rpm for 7 days containing different concentrations of peptone. The antibiotic activities were measured according to the agar diffusion method using Mueller-Hinton agar; each well (6 mm in diameter) was filled with 60 µl of the aliquot supernatant containing actinobacterial metabolites and the plates were incubated at 37°C for 24 hrs.

These results were consistent with results obtained by Ababutain *et al*. [29] how found among several peptone concentrations were tested, 0.04 g/100 ml peptone was preferable for antibiotic production by *Streptomyces* sp.

Phosphate source

Phosphate is the crucial growth-limiting nutrient in many antibiotic fermentations; hence, the effect of different phosphate source (Na_2HPo_4, $(NH_4)H_2po_4$, NaH_2PO_4, K_2HP_{04}, KH_2PO_4, $(NH_4)_2HPO_4$) was evaluated for their influence on antibiotic production from *Streptomyces* sp. LH9. The results showed the efficiency of different phosphate compounds in supporting the antibacterial metabolite production from actinobacterial strain. The K_2HPO_4 salt was the optimum phosphate compound for antibacterial metabolite production. While the other phosphate sources exhibited low efficiency for antibiotic production, which were evident by weak activities against tested pathogenic bacteria, as shown in Fig. 5.

The results were in agreement with the results of Majumdar and Majumdar [30], who reported that K_2HPO_4 is required for maximum yield of neomycin which produced from *Streptomyces fradiae*. Furthermore, the results were consistent with the results obtained by Narayana and Vijayalakshmi [31] how found among different minerals tested, only K_2HPO_4 showed the positive effect on antibiotic production from *Streptomyces albidoflavus.*

As K_2HPO_4 was the best phosphate source for antibiotic production from *Streptomyces* sp. LH9, the consequences of supplying different concentrations (0.125, 0.25, 0.50, 1.00, 2.00, 4.00, and 8.00) g/100 ml on the productivity of antimicrobial agent were studied. The results showed the different levels of inhibition zones of testing pathogenic bacteria included Gram-negative bacteria (*E. coli* and *P. aeruginosa*) and Gram-positive bacteria (*S. agalagtiae* and *S. aureus*). Whereas the highest level was obtained by the K_2HPO_4 concentration in 0.05 g/100 ml, but the upper or lower K_2HPO_4 concentrations caused decreases in antibiotic production from *Streptomyces* sp. LH9, as shown in Fig. 6.

These results were in agreement with the results of Raytapadar and Paul [32] who reported the optimum concentration of K_2HPO_4 for growth, and antibiotic production was 0.135 and 0.675 g/l.

Phosphate regulates the syntheses of antibiotics belonging to different biosynthetic groups; these include peptide antibiotics, polyene macrolides, tetracyclines, and biosynthetically sophisticated antibiotics. Industrial production of these medicines is carried out at growth-limiting concentrations of inorganic phosphate. Phosphate in concentrations ranging from 0.3 to 300 m supports huge cell growth, but the concentrations of 10 mm and above suppress the biosynthesis of many antibiotics [33]. Because of the various antimicrobial metabolites regulated by phosphate are synthesized through different pathways. Many different mechanisms may exist, or else a standard regulatory effector may act on the biosynthetic pathways [34]. Phosphate addition not only interferes with antibiotic synthesis but also after several hours causes a reversal of non-growing, antibiotic-producing cells back to a growing, non-producing state [35].

CONCLUSION

Streptomyces sp. LH9 was a good producer for antibacterial against Gram-positive and negative bacteria, which required essential nutritional supplements in the fermentation medium. Furthermore, could be utilized the industrial waste for improving the production in the most economical manner.

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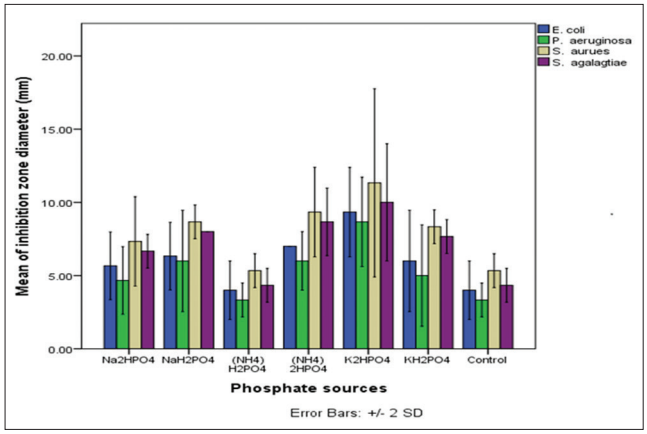

Fig. 5: Effect of phosphate sources on the antibacterial metabolite production from *Streptomyces* sp. LH9

The strain was grown in liquid media (pH 7) at 30°C and 250 rpm for 7 days containing different sources of phosphate. The antibiotic activities were measured according to the agar diffusion method using Mueller-Hinton agar; each well (6 mm in diameter) was filled with 60 µl of the aliquot supernatant containing actinobacterial metabolites and the plates were incubated at 37°C for 24 hrs.

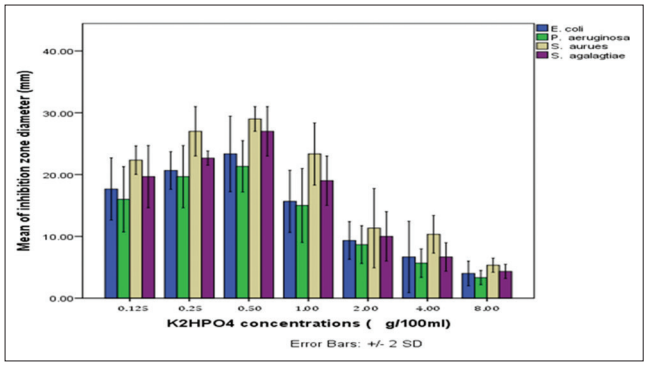

Fig. 6: Effect of K_2HPO_4 concentrations on the antibacterial metabolite production from *Streptomyces* sp. LH9

The strain was grown in liquid media (pH 7) at 30°C and 250 rpm for 7 days containing different concentrations of K_2HPO_4. The antibiotic activities were measured according to the agar diffusion method using Mueller-Hinton agar; each well (6 mm in diameter) was filled with 60 µl of the aliquot supernatant containing actinobacterial metabolites and the plates were incubated at 37°C for 24 hrs.

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