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Research Article

OPTIMIZATION PRODUCTION CONDITIONS OF ANTIBACTERIAL METABOLITE FROM *STREPTOMYCES* SP.

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ABSTRACT

Objectives: The paper aimed to isolate *Streptomyces* strain having the ability to produce antibacterial metabolites and optimize some environmental parameters for excellent antibiotic production.

Methods: Different soil samples were collected from extreme environments of desert regions at Karbala Province, Iraq. Actinomycetes were isolated using different media. The primary screening for antibacterial production was accomplished, and the antibacterial activities were tested against pathogenic bacteria, including *Escherichia coli, Staphylococcus aureus, Streptococcus agalactiae,* and *Pseudomonas aeruginosa*. The most potent strain was chosen for optimizing some of environmental parameters to increase the bioactive metabolite production. Different parameters were studied such as culture media, temperature, pH, and agitation rate.

Results: About eight *Streptomyces* strains were isolated from soil samples. All isolates appeared variable levels of antibiotic productions against Gram-positive and negative pathogenic bacteria, and the best one was *Streptomyces* sp. LHR 9. The antibacterial metabolite production from *Streptomyces* sp. LHR 9 was affected by various cultural parameters. Glucose soybean meal broth as a fermentation medium at pH 7 yielded the highest antibiotic production under the optimal fermentation conditions, including the temperature at 35°C with 200 rpm (revolution/min) agitation rate and 7 days incubation period.

Conclusion: The *Streptomyces* sp. LHR 9 showed antibacterial activity against both Gram-positive and negative pathogenic bacteria. It may consider as a potential source of drug production. Further study needs to purification and characterization of antibiotic and analyzes the mechanism for the antimicrobial activity of this bioactive compound.

Keywords: *Streptomyces*, Antibacterial metabolite, Optimization, Environmental condition.

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INTRODUCTION

Actinomycetes comprise an extensive and diverse group of Gram-positive aerobic mycelial bacteria. They are widely distributed group in nature, particularly the soil inhabit [1]. They have produced many important bioactive substances with high pharmaceutical values and approximately two-thirds of antibiotics [2]. Almost 80% of the world's antibiotics are known to produce from actinomycetes, mostly from the genera *Streptomyces* and *Micromonospora* [3].

According to the World Health Organization, over prescription and the continuous use of antibiotics have led to evolved the antibiotic resistance in many bacterial pathogens. Recently, the drug-resistant pathogenic bacteria have emerged more quickly than the rate of discovery of new medicines and antibiotics. Therefore, many researchers and the pharmaceutical industries have actively worked to find new sources of antibiotic production. The isolation of actinomycetes from different extreme habitats and screening for novel antibiotic productions are continually [1]. Severe infections caused by multi-resistant bacteria to commonly used antibiotics and become a major global health-care problem in the 21st century [4]. *Staphylococcus aureus* and other nosocomial infection bacteria, that are responsible for different types of infections, have become resistant to most classes of antibiotics [5]. Clinicians and public health officials have faced hospital-acquired drug-resistant *S. aureus* and *Pseudomonas aeruginosa* which resist wide spectrum of antibiotics. Hence, there is a need to rediscover new drugs active against these drug-resistant pathogens.

The majority of the actinomycetes inhabit in soil that are essential drug sources remain uncultivable, as a result, inaccessible for novel antibiotic discovery. Goodfellow and Haynes reviewed the literature on isolation of actinomycetes and suggested that only 10% of the actinomycetes are isolated from nature [6]. Most of the antibiotics in use today are derivatives of secondary metabolites produced by actinomycetes and fungi [7,8]. Actinomycetes can be isolated from soil and marine sediments. Although the pharmaceutical industry has screened soils for about 50 years, only a small fraction of the surface of the globe has sampled, and only a small fraction of actinomycetes taxa has been discovered [9,10]. Hence, we need to screen more and more actinomycetes from different habitats for antimicrobial sources in the hope of getting some actinomycetes strains producing new antibiotics that have not been discovered yet and active against multiple drug-resistant pathogens.

Filamentous soil bacteria belonging to the genus *Streptomyces* widely recognized as industrially relevant microorganisms because of their ability to produce many kinds of novel secondary metabolites including antibiotics and enzymes [11]. Indeed, different *Streptomyces* species produces about 75% of commercially and medically useful antibiotics [12].

Under different conditions, antibiotics production by *Streptomyces*, it is possible to improve or lose altogether, so promoting the growth and production of secondary metabolites from *Streptomyces* can be carried out by manipulating the nutritional, chemical, and physical parameters of the culturing conditions. The cultural factor optimization

plays a remarkable role in the productivity and economics of the essential process [13-19]. Hence, our study was aimed to the isolation of *Streptomyces sp.* having the ability to produce antibacterial metabolites and optimize some environmental parameters for excellent antimicrobial metabolite production.

METHODS

Actinomycetes isolation

Soil samples (50) were collected from 10 to 15 cm depth of the upper surface of the ground in the desert region away from about 3 km from Al-Razzaza lake at Karbala Province, Iraq. The samples were placed in sterile plastic containers separately, tightly sealed and transported to the laboratory.

The collected soil samples were dried in a hot air oven at 60-65°C for about three hours for reducing the vegetative bacterial. Subsequently, the soil samples which containing spores of actinomycetes were transferred to sterile tubes separately and store at 4°C until the screening performed.

The starch-casein-nitrate-agar medium was used to isolate of actinomycetes. The medium was prepared according to the manufacturer company instructions. The pH value of the medium was adjusted to 7-7.2 and then sterilized in an autoclave at 121°C for 15 minutes. The medium was then allowed to cool to around 45-50°C, and 1 ml of cycloheximide (50 µg/ml) was added before pouring into plates. Then, the medium was poured into the plates with different thick to prevent drying during the incubation period.

One gram of dried soil sample was suspended in 9 ml sterile normal saline to prepare the spore stock solution. Serial dilutions (10^{-1}-10^{-4}) were made from the stock. Four Petri dishes containing isolation medium were cultured by transferring 0.1 ml of the spore suspension from each dilution and spreading on the surface of agar medium using a sterile glass spreader. Then, the plates were incubated at 30°C for 5 days. After the incubation period, the plates were examined for typical actinomycetes colonies, which had regular round, small, opaque, compact, frequently pigmented with white, brown, gray-pink, or other colors, the colonies were examined under a light microscope to observe their colonial morphology and distinguished from fungi colonies. The isolated actinomycetes were recultured in nutrient broth and nutrient agar slants and stored at 4°C for further study [20,22].

Test organisms used in the study

Four pathogenic bacteria, including Gram-negative (*Escherichia coli* and *P. aeruginosa*) and Gram-positive (*S. aureus* and *Streptococcus agalactiae*), were used as test microorganisms for evaluation the antibacterial activity of actinomycetes. All the tested pathogenic bacteria were obtained from Laboratory of General Health in Karbala Province.

Primary screening for antibacterial activity

Primary screening of antagonism was performed on Muller Hinton agar using the perpendicular streak plate method against four test organisms, including Gram-negative (*E. coli* and *P. aeruginosa*) and Gram-positive pathogenic bacteria (*S. aureus* and *S. agalactiae*). The medium was prepared according to manufacturer company instructions. The actinobacterial isolate was streaked across the surface of the agar medium at the middle position of the plate and incubated at 30°C for 7 days, in triplicate. After that, the test organisms were streaked perpendicularly with actinobacterial growth and the space of 2-3 mm between two streaks. Then, the plates were incubated at 37°C for 2 days for the test organism growing. After that, the plates were then examined, and the presences of the clear zone between the actinobacterial growth and test microorganism indicate growth inhibition of test organisms.

Secondary screening for antibacterial activity

Actinomycetes isolates were screened for their ability to produce bioactive compounds against test microorganisms using the agar diffusion methods.

The antibacterial metabolites were produced from actinomycetes isolates using the sterile fermentation broth, 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 [22].

The inoculum was separately prepared by cultivated the actinobacterial isolates 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 two disks (6 mm in diameter) of actinobacterial inoculum and incubated at 30°C in a rotary shaker at 150 rpm (revolution per min) 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 each actinobacterial isolate was tested as antibacterial metabolite [23].

For antibacterial activity determination, pathogenic bacteria were grown overnight in nutrient broth at 37°C for 24 h (O.D=0.5; McFarland 1×10^8 CFU/ml). The cultures of test organisms were streaked on Moller Hinton agar medium separately. Wells (6 mm in diameter) were made in each seeded agar plate, and each well was filled with 60 µl of the aliquot supernatant containing the active metabolites [24]. The plates were kept at 4°C for 2-4 h for the diffusion of the metabolites. Then, the plates were incubated at 37°C for 24 h. After the incubation period, the diameter of inhibition zones was measured. Each test was in triplicate, and the activities were estimated as the mean of the diameter of the inhibition zone [25,26].

The isolates which exhibited higher production of bioactive compounds were rescreened for antimicrobial activity against four pathogenic bacteria using the agar well diffusion method. The best active actinobacterial isolate was chosen for identification and antimicrobial metabolite production.

Optimization of antibiotic production conditions

The optimization of cultural conditions was determined for improving the production of antimicrobial metabolites from selected isolate. The experiments were performed as batch fermentation.

Fermentation media

The selected isolate was cultured in different types of culture media at pH 7 (50 ml media/250-ml Erlenmeyer flask), which composed of the following constituents (g/l):

- Glucose-soybean (GS) medium: Glucose 25, soybean 5, NaCl 5, K_2HPO_4 2, $MgSO_4$ 0.5, and $CaCO_3$ 1 [22].
- Glucose-yeast-meat-peptone (GYMP) in medium: Glucose 10, yeast extract 1, meat extract 4, peptone 4, and NaCl 2 [27].
- Glucose-glycerol-soybean (GGS) medium: Glucose 15, glycerol 2.5, soybean meal 25, NaCl 2.5, $NaNO_3$ 4, K_2HPO_4 5, $ZnSO_4$ 0.04, and $CaCO_3$ 0.4 [28].
- Starch-yeast-malt-casein-peptone medium (SYMCP): Starch 10, malt extract 10, casein 3, peptone 1, yeast extract 1, and K_2HPO_4 0.5 [29].
- Dextrose-yeast-malt medium: Malt extract 10, dextrose 4, and yeast extract 4 [29].

All media were sterilized by autoclave at 121°C for 15 minutes; then, they were inoculated with the actinobacterial inoculum (2 disk/50 ml medium with 6 mm in diameter) and incubated with shaking incubator at 150 rpm (revolutions/minute) and 30°C for 7 days, in triplicate for each medium.

Optimization incubation temperature

The optimum temperature for antibiotic production was determined using different incubation temperatures ranged from 15°C to 45°C with 5°C intervals. The optimum media at pH 7 were inoculated with the actinobacterial isolate and incubated with shaking incubator at 150 rpm for 7 days, in triplicate for each temperature [30].

The initial pH value of production medium

The effect of the initial pH values of production medium was studied, each 150 ml of optimum liquid medium was adjusted to initial pH values ranged

from 5 to 10 using 1N HCl or NaOH, and then it distributed into three flasks (50 ml medium/250 ml flask). The media were inoculated with selected isolate and incubated at the optimum temperature with shaking incubator at 150 rpm for 7 days, in triplicate for each pH value [30].

Agitation rate of production medium

The optimum medium was inoculated with the selected isolate and incubated at the optimum temperature with different agitation rates ranged from 100 to 350 rpm, in addition to the static condition (0 rpm) for 7 days, in triplicate for each agitation rate [31].

Statistical analyses

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

RESULTS AND DISCUSSION

Actinomycetes isolation and antibiotic screening

From the desert soil samples, 8 actinomycetes isolates were recovered and identified depending on microbial, cultural, biochemical, and molecular characteristics (unpublished data). These isolates were preserved in starch-casein-nitrate-agar slants at 4°C for further analysis.

The antibiotic screening (primary and secondary) was done against four pathogenic bacterial strains, including Gram-negative (*E. coli* and *P. aeruginosa*) and Gram-positive pathogenic bacteria (*S. aureus* and *S. agalactiae*). The results revealed that all actinobacterial isolates appeared the ability to produce antibiotic substances against the test microorganisms (Table 1).

All actinobacterial isolates exhibited a broad spectrum of antibacterial activity against Gram-positive and negative bacteria. The isolate LHR 9 followed by the isolates LH 12 and LH 3 which showed the highest activity among the others. The LHR 9 isolate showed the highest antibacterial activity, according to Fisher's least significant difference (LSD) (LSD=3.565) against tested Gram-negative bacteria. It formed inhibition zones were 9.333 1.527 mm and 8.667±1.527 mm for *E. coli* and *P. aeruginosa*, respectively, whereas the isolate LH 12 gave an inhibition zone of 7.667±1.527 mm for *E. coli* and 6.000±1.000 for *P. aeruginosa*. Furthermore, it had high antibacterial activity against tested Gram-positive bacteria. These results were consistent with the previous studies [24,32,33] which reported the Gram-positive bacteria were more susceptible than Gram-negative bacteria to antibacterial substances that produced from actinomycetes. The moderate antibacterial activity of actinomycetes secondary metabolites against the Gram-negative bacteria could be ascribed to the presence of an outer membrane that possesses hydrophilic lipopolysaccharide chains and forms an additional barrier for the entry of antibiotic extract into the cells [16,24,34,35].

Due to the highest antibacterial activity and the excellent growth properties, the isolate LHR 9 a yellowish white-colored actinomycetes was chosen for production of an antibacterial metabolite. This strain was identified according to morphological, physiological, and molecular properties and found it related to *Streptomyces* sp. [21] (unpublished data).

Optimization fermentation parameters of antibiotic production from *Streptomyces sp.*

The antimicrobial metabolite from microorganisms influences by different environmental parameters such as fermentation media composition, temperature, pH, and agitation rate. The optimizing production conditions of any microbial product are a crucial step to stimulate overproduction from microorganisms such as actinobacterial species. Due to the most secondary metabolites such as antimicrobial substances are yielded from different pathways in microbial cells, the easiest way to improve the production is optimizing fermentation parameters. Logically, if we want to determine the effect of any parameter, must be non-changed the others, so all the experiments were designed at this trend.

Fermentation media

Preliminary, the best fermentation medium was selected depending on the results of antibiotic production from *Streptomyces sp.,* when it was grown in five different media (Fig. 1). According to the statistical analysis (LSD=3.751), the best production medium was GS medium,

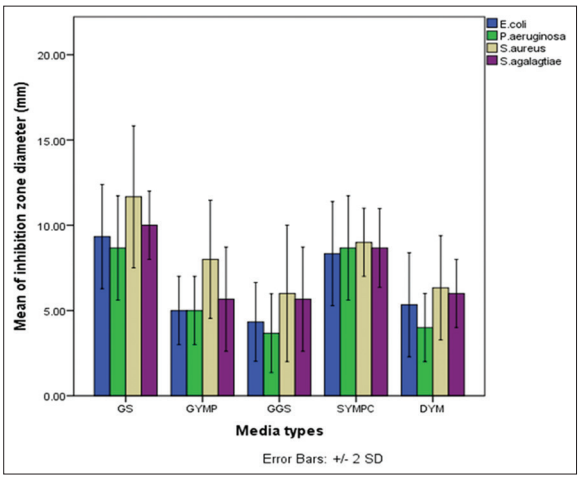

Fig. 1: Effect of different fermentation media on antimicrobial production from *Streptomyces* sp. LHR 9. *Least significant difference=3.751 at a level 0.05; The actinobacterial strain was grown in liquid media (pH 7) at 30°C and 150 rpm for 7 days. The antibiotic activities were measured according to the agar diffusion method using Moller Hinton agar; each well (6 mm in diameter) was filled with 60 µl of the aliquot supernatant containing actinobacterial metabolites produced from *Streptomyces* sp. LHR 9, and the plates were incubated at 37°C for 24 hr

Table 1: Secondary screening of antibiotic production from actinomycetes isolates against pathogenic bacteria

Actinomycetes	Inhibition zone diameter (mm)±SD			
	E. coli	*P. aeruginosa*	*S. aureus*	*S. agalactiae*
LH 3	5.333±1.527	5.666±1.154	12.667±2.516	9.000±1.000
LH 6	5.667±1.154	6.000±1.000	10.333±1.527	6.667±1.154
LH 7	3.667±0.577	4.333±1.527	6.667±2.081	4.000±1.732
LHR 9	9.333±1.527	8.667±1.527	11.333±2.309	10.000±2.000
LH 10	4.000±1.732	4.333±1.527	6.000±1.732	5.333±0.577
LH 11	4.333±1.154	4.000±1.000	6.667±1.527	6.000±2.645
LH 12	7.667±1.527	6.000±1.000	11.000±1.000	8.000±1.000
LH 18	5.000±1.732	4.333±2.309	8.667±1.527	5.667±1.527

*Fisher's LSD (LSD=3.565) at a level 0.05. All isolates were grown in liquid media (pH 7) at 30°C and 150 rpm for 7 days. The antibiotic activities were measured according to the agar diffusion method using Moller Hinton agar; each well (6 mm in diameter) was filled with 60 µl of the aliquot supernatant containing actinobacterial metabolites produced from actinomycetes and the plates were incubated at 37°C for 24 hr. *E. coli: Escherichia coli, S. aureus: Staphylococcus aureus, S. agalactiae: Streptococcus agalactiae, P. aeruginosa: Pseudomonas aeruginosa*, SD: Standard deviation, LSD: Least significant difference

which resulted highest inhibition zones of the test microorganisms, *E. coli* (9.333±1.527 mm), *P aeruginosa* (8.667±1.527 mm), *S. aureus* (11.667±2.081 mm), and *S. agalactiae* (10.000±1.000 mm). The medium was containing the highest glucose concentration (2.5%) and the suitable concentration of soybean meal (0.5%), in addition to essential mineral salts. Followed by SYMCP medium which contains more complex nutrients such as carbon and nitrogen sources (starch, yeast extract, malt, casein, and peptone), but at least concentrations ranged between 1% and 0.1%. While the GGS medium appeared little antibiotic production, in spite of it contains organic and inorganic carbon and nitrogen sources. It contains glucose (1.5%), glycerol (0.25%), soybean meal (2.5%), and $NaNO_3$ (0.4%), in addition to other essential mineral salts. These results indicate that the *Streptomyces* sp. LHR 9 was preferred high concentration of glucose and low concentration of nitrogen source to stimulate its growth and antimicrobial metabolites production, due to it adapted to grow in a weak nutrient environment (desert, the local of isolation). On the other hand, the composition of production medium effects on the level of antimicrobial metabolites production depending on the structural components of the antibiotics [24].

In spite of all tested media were supplemented with the necessary components such as carbon, energy sources, minerals, and growth factors for the microorganism growing. However, it appeared variable quantities of antibiotic production; this may be due to the nature of the organism and its genetic content [21,34]. Glucose has been found to increase the growth of *Streptomyces* sp. LHR 9 and antibiotic production; this result was in agreement with other investigations of the researchers who stated that glucose was the excellent carbon source for antibiotic production [35,36]. The presence of NaCl in producing medium was enhanced microbial growth because it was isolated from extreme environments. Furthermore, it induced the release of bound antibiotic from the mycelia; these results were in agreement with the result of previous studies [24,37] which reported the production of the antimicrobial metabolite was increased with the presence of high concentration of glucose and sodium chloride. Soybean meal is a complex nitrogen source and contains some amino acids such as lysine, methionine, threonine, tryptophan, aspartic acid, glutamine, proline, alanine, valine, and isoleucine. The presence of tryptophan in soybean meal increases antibiotic production up to a certain level [38]. Due to the GS medium was the best fermentation medium which stimulates highest antibiotic production levels from *Streptomyces* sp. LHR 9, this medium was used to optimize other fermentation conditions.

These results were disagreed with studying of Mukhtar *et al.* [39] which concerned with the production of antitumor antibiotic from *Streptomyces capoamus* in batch fermentation and concluded the GYMP medium as the best medium for the production of secondary metabolites.

Incubation temperature

Any microorganism has an optimum temperature for growing but not always it is optimized for secondary metabolite production. Hence, the actinobacterial strain (*Streptomyces* sp. LHR 9) was grown in different incubation temperature to determine the optimum for antibiotic production using the optimum medium (GS). The preliminary experiments showed that the optimum growth temperature was 30°C. The present results revealed that the optimum temperature for the maximum antibiotic production was 35°C, which indicated by the increasing of inhibition zone of tested pathogenic microorganisms (LSD=5.003). The inhibition zones reached to 15.667±2.886 mm, 12.000±3.000 mm, 19.333±2.081 mm, and 17.000±1.000 mm for *E. coli, P. aeruginosa, S. aureus,* and *S. agalactiae*, respectively (Fig. 2). Whereas, at 30°C, the inhibition zones were 10.000±2.000 mm, 8.000±2.000 mm, 17.333±1.527, and 11.000±2.000 mm for *E. coli, P. aeruginosa, S. aureus,* and *S. agalactiae,* respectively. The isolate produced the antibiotic at a range of temperature from 20 to 45 in GS medium at pH 7, agitation 160 rpm for 7 days.

From these results, we can conclude that the optimum temperature for the antibacterial metabolite production was 35°C and used this

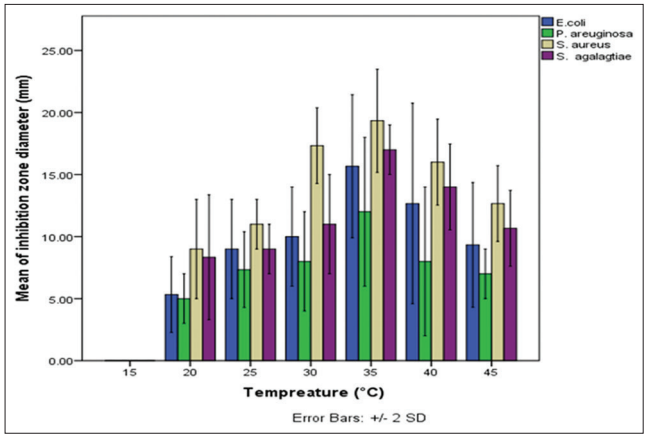

Fig. 2: Effect of incubation temperature on antimicrobial production from *Streptomyces* sp. LHR 9. *Least significant difference=5.003 at a level 0.05; the actinobacterial strain was grown in optimum medium GS at pH 7 and 150 rpm for 7 days and incubated at different temperatures. The antibiotic activities were measured according to the agar diffusion method using Moller Hinton agar; each well (6 mm in diameter) was filled with 60 µl of the aliquot supernatant containing actinobacterial metabolites produced from actinobacterial strain and the plates were incubated at 37°C for 24 hr

degree for antibiotic production in the next experiments. Therefore, the strain was strictly mesophilic for secondary metabolite production. These results were in agreement with the results of Saha *et al*. [40] who studied the optimum conditions of antimicrobial metabolite production from *Streptomyces* sp. MNK7 and they obtained that the optimum temperature of antimicrobial fermentation was 35°C. Furthermore, our results are consistent with previous studies of Sekhar *et al*. [41] and Narayana and Vijayalakshmi [42].

Initial pH of production medium

To determine the optimum initial pH value of the fermentation medium for maximum antibiotic production; the *Streptomyces* sp. LHR 9 was grown in GS medium at different pH values ranging from 5 to 10 and optimum temperature 35°C with agitation rate 150 rpm for 7 days. The results revealed that the actinobacterial strain produced the antibacterial metabolite in GS medium in the different range of pH values (5-10). The maximum production at neutral environment pH7 (LSD=4.667); The inhibition zones were 18.333±2.516 mm, 19.333±3.214 mm, 24.000±1.732 mm, and 20.000±2.000 for *E. coli, P. aeruginosa, S. aureus,* and *S. agalactiae*, respectively (Fig. 3).

The hydronium ion concentration (pH) of the media affects the activity of cellular enzymes, nutrient ionization, and transporting through cell membranes, consequently its influence on the bacterial growth rate and secondary metabolites biosynthesis. The present results were in agreement with previous studies [43,44] which reported the optimum for antibiotic production from *Streptomyces* species; *Streptomyces hygroscopic* was pH 7 or near neutral environments. Another study said that the optimum pH for the fermentation of antibiotic, granaticin by *Streptomyces thermoviolaceus* was 6.5-7.0 and the *Streptomyces* isolate failed to grow at acidic pH but grow well at pH 7-8 [45].

Agitation rate

The intensity of the agitation applied during fermentation may have an impact on growth rate, antibiotic biosynthesis, metabolism through transport of nutrients and enzyme activities, cell damage, morphology, and broth viscosity in the case of non-Newtonian rheology. Hence that, the actinobacterial strain was grown in optimum GS medium at pH 7 and different agitation speeds were applied at 35°C for 7 days (Fig. 4). The results appeared *Streptomyces sp.* LHR 9 had the ability to produce antibiotic with various agitation rates (100-350 rpm), and

the maximum production (LSD = 4.567) occurred at 200 rpm but not in static conditions (0 rpm). That may attribute to the decrease in the agitation rate that might reduce the dissolved oxygen level in the fermentation broth that could effect on the antimicrobial metabolite production [46].

Furthermore, the agitation speed increased consequences gradually, increasing the production of antimicrobial metabolites, reaching its maximum level. The fermentation media filtrate of *Streptomyces* sp. LHR 9 gave inhibition zones 20.000±2.000 mm, 18.667±2.309 mm, 26.557±1.527 mm, and 23.333±1.527 mm for *E. coli, P. aeruginosa, S. aureus,* and *S. agalactiae,* respectively, at agitation speed 200 rpm. That may be a result of the aeration level in the culture medium when increased lead to sufficient supplies of dissolved oxygen in the culture media and the nutrient uptake by bacteria also will be increased. On the other hand, as the agitation speed rose to 350 rpm, the activity of antimicrobial metabolites produced from *Streptomyces sp.* LHR 9 in fermentation media filtrate decreased gradually and reached its minimum levels as showed as inhibition zones in Fig. 4. Due to increasing the agitation speed tends to increase the amount of dissolved oxygen available to the culture that makes depletion for the cell growth and antimicrobial compound production [14].

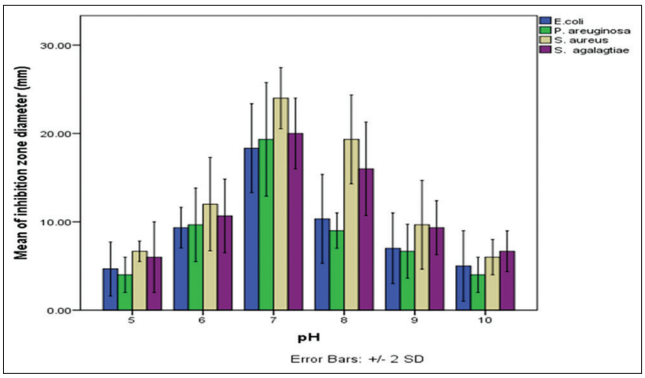

Fig. 3: Effect of different pH values of fermentation media on antimicrobial production from *Streptomyces* sp. LHR 9. *Least significant difference=4.667 at a level 0.05; The actinobacterial strain was grown in optimum medium GS in various pH values, at 35°C and 150 rpm for 7 days. The antibiotic activities were measured according to the agar diffusion method using Moller Hinton agar; each well (6 mm in diameter) was filled with 60 µl of the aliquot supernatant containing actinobacterial metabolites produced from actinobacterial strain, and the plates were incubated at 37°C for 24 hr

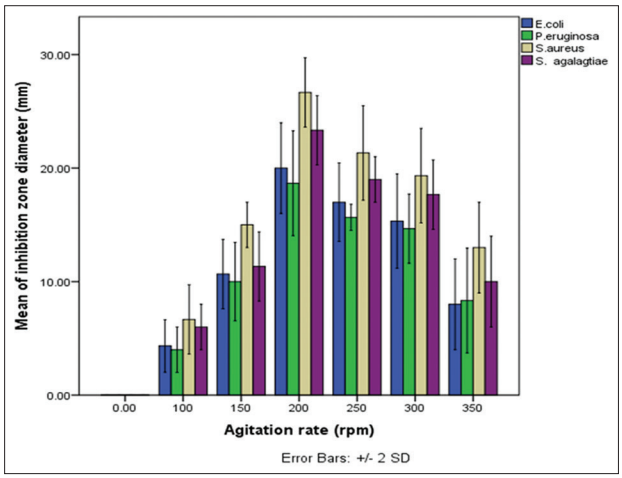

Fig. 4: Effect of agitation rates on antimicrobial production from *Streptomyces sp.* LHR 9. *Least significant difference=4.567 at a level 0.05; the actinobacterial strain was grown in optimum medium GS (pH 7) at 35°C for 7 days and in different agitation rates. The antibiotic activities were measured according to the agar diffusion method using Moller Hinton agar; each well (6 mm in diameter) was filled with 60 µl of the aliquot supernatant containing actinobacterial metabolites produced from actinobacterial strain and the plates were incubated at 37°C for 24 hr

Our results were in agreement with the findings of many researchers [16,20,32] how found that the productivity increased by culture shaking in comparison with the static culture shaking in comparison with the static culture. Due to *Streptomycetes* are obligate aerobic organisms [21], while AL-Zahrani [34] found that the shaking culture reduced the productivity of *Streptomyces* J12. Whereas, other species of actinomycetes produced antibiotics under static conditions [18,24].

CONCLUSION

Streptomyces sp. LHR 9 produced vital antimicrobial metabolites against Gram-positive and negative bacteria. It may act as a potential source of drug production. Further study needs to purification and characterization of antibiotic and analyze the mechanism for the antimicrobial activity of this bioactive compound.

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