Original Article

Antibacterial Compound Productivity by *Streptomyces* spp. Isolated from Clinical Samples in Iraq: Optimization of Their Production

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Abstract

Background: *Streptomyces* can manufacture a variety of biologically active secondary metabolites (antibacterial compounds). **Objectives:** The study involved optimizing the conditions for antibacterial productivity of two selected *Streptomyces* isolates recovered from the clinical samples of patients. **Materials and Methods:** Two isolates of *Streptomyces*, *Streptomyces* isolate 04 (SI-04) and *Streptomyces* isolate 10 (SI-10), were selected and found to be antibiotic producers. Four different parameters were used to achieve maximum antibacterial productivity for both isolates. The parameters include the use of four different broth media for antibacterial productivity, the use of five various pH values (pH: 5, 6, 7, 8, 9), the use of a variety of incubation temperatures (26, 28, 30, 32, and 34°C), and the use of various incubation times (1, 2, 3, 4, 5, 6, and 7 days) and its relation to antibacterial productivity. The antibacterial ability of each *Streptomyces* isolate for productivity related to all parameters was determined by measuring the inhibition zone diameters against test bacterial isolates. Analysis of variance test was used for data analysis using SPSS V-25. **Results:** Results revealed that the optimization of antibacterial productivity was applied using the following parameters: soya bean broth 1 medium, pH adjusted at 7.0, and the incubation time for 4 days at 30°C for *Streptomyces* isolates SI-04 and soya bean broth 1 medium, pH adjusted at 7.0, and incubation for 5 days at 30°C for *Streptomyces* isolate SI-10. **Conclusion:** The optimization of antibacterial production conditions was an important and a crucial step for improving and enhancing the antibacterial productivity of *Streptomyces* spp.

Keywords: Antibiotic production, clinical samples, improvement, *Streptomyces*

Introduction

Actinobacteria are the phylum to which the genus of *Streptomyces* belongs and constitutes the family, Streptomycetaceae. Streptomycetes are the most predominant group among the actinomycetes.[1] *Streptomyces* spp. are Gram-positive bacteria characterized by a sophisticated multicellular progression, in which their germinating spores comprise hyphae with multinuclear aerial mycelium, which comprises septa at arranged interval forming a chain of single-nucleated spores.[2]

Generally, the primary metabolites are those involved directly in development, normal growth, and reproduction. It usually generates a physiological function in the organism. Typically, they are present in various cells or organisms. On the other hand, the secondary metabolites are organic compounds that are not directly related to normal growth; their synthesis is highly related to many growth conditions (as types of culture media) and they are frequently produced as groups of closely related molecules. Searching for novel bioactive compounds (especially antibiotics) whose effectual toward multiple drug-resistant pathogenic

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bacteria (that are associated with infectious diseases) is considered an important field of antibiotic research.[3] Actinomycetes (*Streptomyces* genus) are considered industrially significant as a reason for their ability to produce multiple secondary metabolites, particularly antibiotics.[4-6] Bacteria related to the *Streptomyces* genus are extremely recorded as an important origin of antibiotics and other significant new bioactive metabolites such as antifungal, antitumor, antihelminthic, and even herbicides.[7] The production of secondary metabolites usually occurs at the stationary phase. The nature of secondary metabolites is genetically determined; nevertheless, the gene expression can be affected by the environmental fluctuation. Manipulation in nutritional and physical parameters of the culturing conditions can be related to improvement in the growth and antibacterial production. Designing a convenient fermentation medium is of great importance in the production of secondary metabolites. Types of nitrogen and carbon sources have been recorded to affect antibiotic productivity in *Streptomyces*.[8] Many cultivation parameters including pH, incubation period, and temperature play a crucial role in the biosynthesis of secondary metabolites.[9]

The present study was aimed for optimizing the circumstances required to achieve maximum productivity of antibacterial compounds by *Streptomyces* spp. isolated from various clinical samples of patients in Babylon Province, Iraq, by adjusting various parameters including time, pH, incubation temperature, and various kinds of culture media.

Materials and Methods

Isolates of *Streptomyces*

In the current study, various isolates of *Streptomyces* were recovered from clinical samples (blood) of patients with cancer and pneumonia from different hospitals at Babylon Province, Iraq during the period from April 2021 to May 2022. A screening test was performed on these isolates to select which produced more antibiotics.[10]

Test microorganisms

The present study involved the use of two indicator bacteria (test bacterial isolates) that were isolated from patients with severe otitis media (the indicator bacteria used are definitely pathogenic bacteria). The test bacterial isolates used were *Escherichia coli* and *Staphylococcus aureus* that were placed and stored aseptically in deep freezer for a long time. The test bacterial isolates (mentioned above) were activated prior to any test by subculturing on nutrient agar medium.

Following are the culture media for the determination of antibacterial productivity (Shirling and Gottlieb)[11]:

1. **ISP2 Broth Medium** (Modified from Pridham et al.[12]:

The components of this medium are Bacto-Malt Extract, 10 g; Bacto-Yeast Extract, 4.0 g; Bacto-Dextrose, 4.0 g; and distilled water, 1.0 L. These components were mixed together and solubilized, and then the pH was adjusted to be 7.0. After that, the broth medium was autoclaved and was ready for inoculation with plugs of *Streptomyces* obtained from soya bean agar medium. Following the inoculation, the inoculated flasks were incubated in shaking incubator at 30°C for 4 days.

2. **Soya Bean Broth 1:**

The components of this medium are soya bean meal, 10.0 g; glucose, 10.0 g; NaCl, 10.0 g; and distilled water, 1.0 L. The pH was adjusted to be 7.0 by adding drops of 1N NaOH, and then the medium was solubilized and autoclaved. After autoclaving, the medium was left to cool and can be used when required. The same conditions were applied for the inoculation and subsequent incubation as with the two media mentioned above.[13]

3. **Soya Bean Broth 2:**

The components of this medium are soya bean meal, 20.0 g; mannitol, 20.0 g; and distilled water, 1.0 L. These two components were mixed together and solubilized well, without adding agar and then, the pH was adjusted to be 7.0. This broth medium was also autoclaved and was left to cool. Then, it was inoculated with plugs as with the above broth medium, followed by incubating the inoculated flasks in shaking incubator at 30°C for 4 days.[14]

4. **Starch-Casein Broth Medium:**

The components of this medium are K_2HPO_4, 0.5 g; casein, 3.0 g; starch, 10 g; peptone, 1.0 g; yeast extract, 1.0 g; malt extract, 1.0 g; and distilled water, 1.0 L. The pH was adjusted by adding few drops of 1N NaOH until it reaches 7.0. The medium was mixed, homogenized, solubilized properly, and then autoclaved. After autoclaving, it was left to cool at room temperature. The same conditions were followed as with the three media mentioned above concerning the inoculation and subsequent incubation.

Preparation of cell-free filtrate

Each productivity broth culture was incubated and then centrifuged in cooler centrifuge at 4000 revolution per minute (rpm) and 5°C for 20 min. After that, the cell-free filtrate was collected from each broth culture of *Streptomyces* and examined for their antibacterial activities using the agar-well diffusion technique.[15] Following the incubation of plates (at 37°C for 18–24 h), zones of inhibition (in mm) were recorded (if present).

Optimization of antibacterial productivity

In this study, four different parameters were used to optimize the antibacterial productivity of both *Streptomyces isolates* (SI-04 and SI-10). The use of four different broth media for antibacterial productivity (as mentioned above) was

the first parameter involving the use of two test bacterial isolates (*Escherichia coli* and *Staphylococcus aureus*). The study involved the use of five various pH values (pH: 5, 6, 7, 8, and 9) as a second parameter. The relationship between various pH values and the antibacterial productivity for each isolate of *Streptomyces* (SMI-04 and SMI-10) was determined individually, and this productivity was performed against the test bacterial isolates (*S. aureus* and *E. coli*).

The third parameter involves studying the effects of several incubation temperatures (26, 28, 30, 32, and 34°C) to evaluate which temperature is convenient, optimum, and accompanied with the highest antibacterial potentials. In this experiment, soya bean broth 1 medium and pH 7 were fixed, and only the incubation temperature was changed. The fourth parameter studied is the effect of using various incubation times (days) and its relation to antibacterial productivity. It involved using different incubation times (1, 2, 3, 4, 5, 6, and 7 days) and evaluated daily for 7 days to determine which time is accompanied with higher antibacterial activity using the soya bean broth 1 medium with pH 7 and incubated at 30°C. The antibacterial ability of each *Streptomyces* isolate for productivity in relation to each incubation time was determined through inhibition zone diameter measurement.

Statistical analysis

In the present study, regression and correlation were used for analysis of data by applying analysis of variance test utilizing statistical package for the social sciences version 25 (SPSS, IBM Company, Chicago, Illinois).

Ethical approval

The study proposal was in agreement with the ethical standard of Helsinki and was approved by the local ethics committee according to the reference number B521 on March 3, 2021.

Results

Many isolates of *Streptomyces* were obtained from clinical samples; the screening test was performed on these isolates to select the potent isolates regarding antibacterial productivity. Two isolates of *Streptomyces*, which are *Streptomyces* isolate 4 (SI-04) and *Streptomyces* isolate 10 (SI-10), were found to be potent producers and so selected for further investigations.

Effect of different types of production media

In this study, the relationships between the use of various types of broth media (that encourage the antibacterial productivity of *Streptomyces* isolates, SI-04 and SI-10) were studied as best shown in Figures 1 and 2. The results obtained revealed that among the four antibacterial production media used, soya bean broth 1 medium gave the highest antibacterial productivity (maximum inhibition zones). This result was identical for both *Streptomyces* isolates and found that *S. aureus* was more susceptible than *E. coli* regarding all media. According to the statistical analysis, the correlation coefficient (*R*) values of the isolate SI-04 were 0.979 for *S. aureus* and 0.968 for *E. coli*, whereas the values of isolate SI-10 were 0.957 and 0.988 for *S. aureus* and *E. coli*, respectively. These *R* values represent a higher relationship (correlation) between the fermentation medium type and inhibition zones.

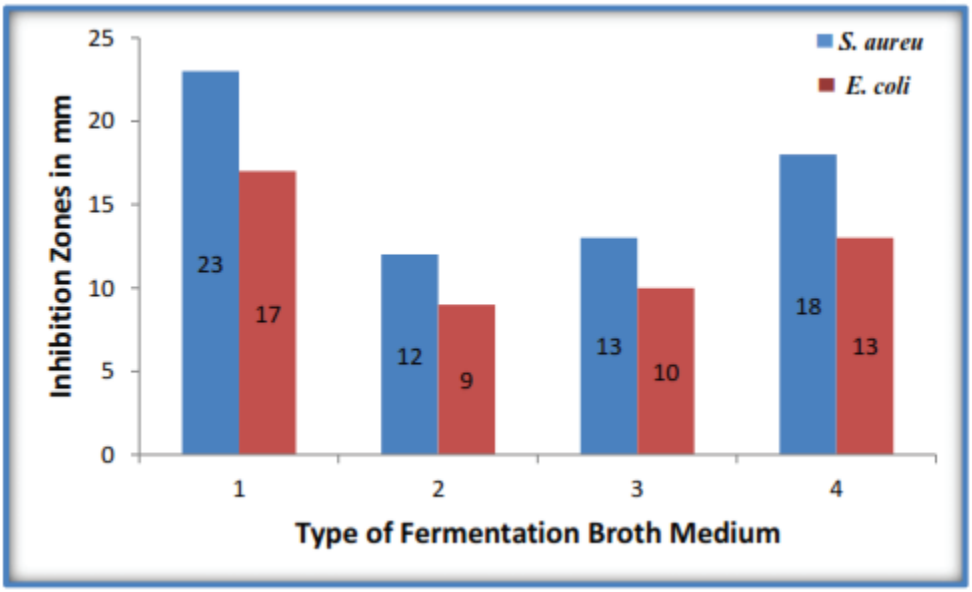

Figure 1: Effect of different production media on antibacterial productivity of *Streptomyces* isolate SI-04. ** 1: Soya bean broth 1 medium; 2: Soya bean broth 2 medium; 3: Starch-casein broth medium; 4: ISP2 broth medium

Effect of pH

The effect of various pH degrees on antibacterial metabolite productivity by *Streptomyces* isolates SI-04 and SI-10 is best clarified in Figures 3 and 4, respectively. The maximum bioactive metabolite production of both SI-04 and SI-10 was obtained at pH 7 followed by pH 8. This explained that both isolates were favored a neutral pH for antibacterial productivity. Statistical analysis demonstrated that *R* values were 0.967 and 0.964 for *S. aureus* and *E. coli* subsequently regarding isolate SI-04, while isolate SI-10 recorded 0.930 for *S. aureus* and 0.951 for *E. coli*.

Effect of incubation temperatures

In this study, the incubation temperature of 30°C was associated with maximum antibacterial activity for both isolates followed by the temperature of 28°C as summarized in Figures 5 and 6. These results give an indication that *Streptomyces* isolates SI-04 and SI-10

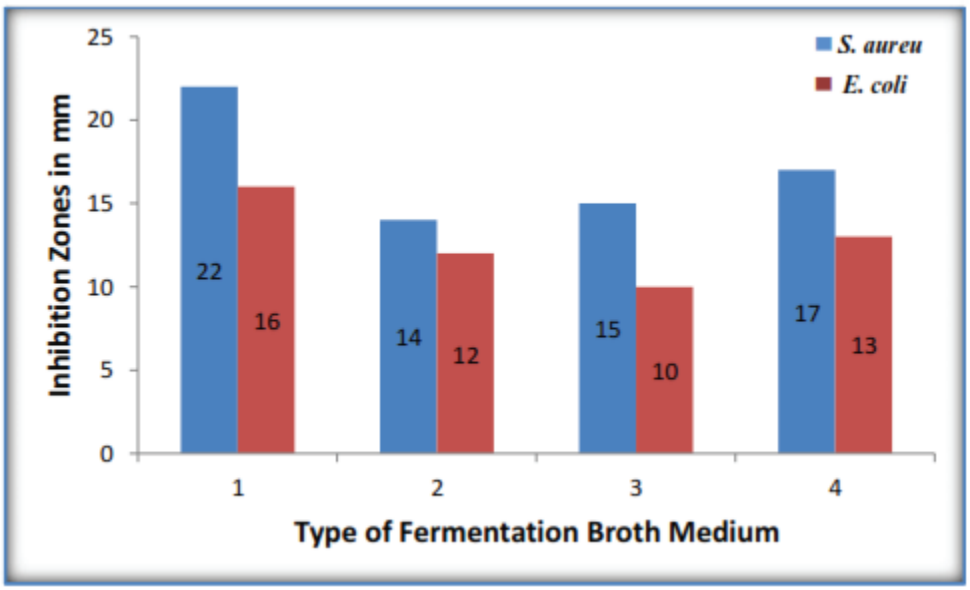

Figure 2: Effect of different antibacterial production media on antibacterial productivity of *Streptomyces* isolate SI-10. ** 1: Soya bean broth 1 medium; 2: Soya bean broth 2 medium; 3: Starch-casein broth medium; 4: ISP2 broth medium

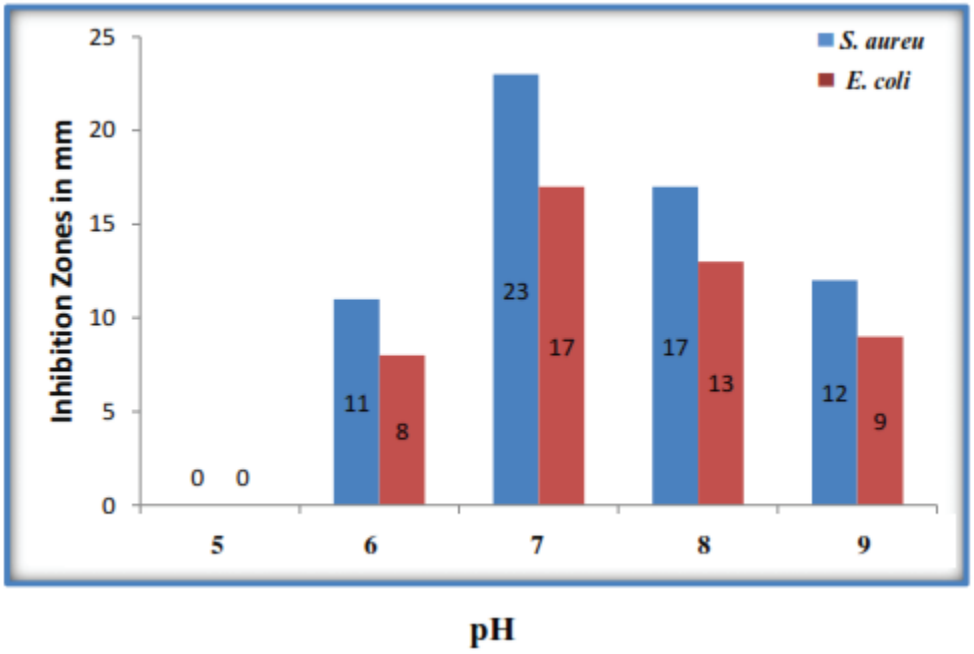

Figure 3: Effect of different pH on antibacterial productivity of *Streptomyces* isolate SI-04

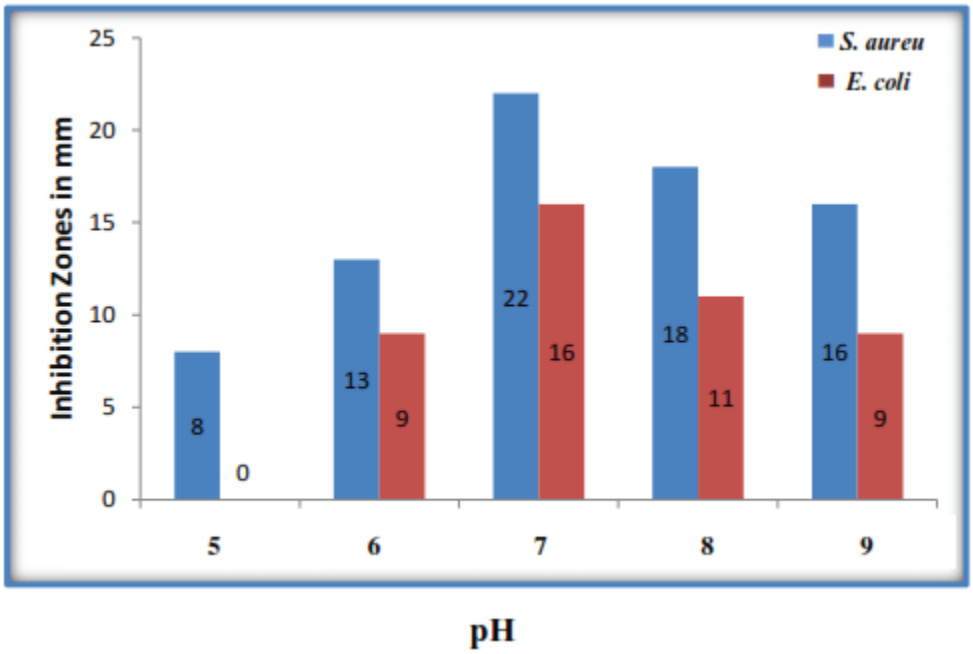

Figure 4: Effect of different pH on antibacterial productivity of *Streptomyces* isolate SI-10

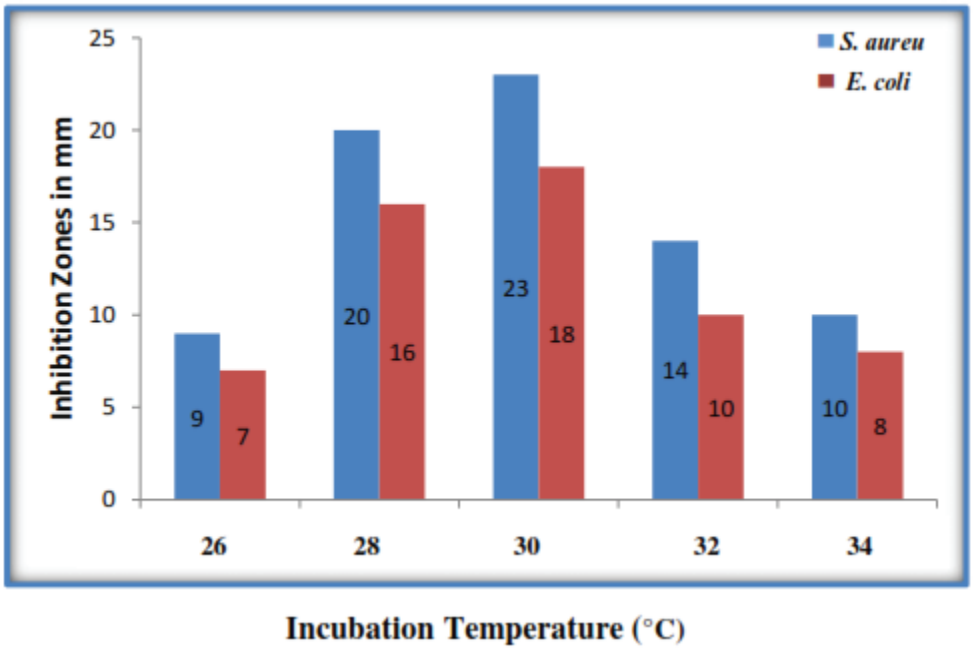

Figure 5: Effect of different incubation temperatures on antibacterial productivity of *Streptomyces* isolate SI-04

have a mesophilic nature. Statistical analysis indicated that *R* values were 0.920 and 0.879 for *S. aureus* and *E. coli* subsequently regarding the isolate SI-04, while isolate SI-10 included *R* values of 0.957 for *S. aureus* and 0.988 for *E. coli*.

Effect of incubation time

This study revealed that *Streptomyces* isolate SI-04 exhibited the highest antibacterial activity at the fourth day of incubation (the activity began from the third day, maximized at the fourth day and then declined at the fifth, sixth days reaching the seven day where no activity seen) as shown in Figure 7. Concerning the *Streptomyces* isolate SI-10 [Figure 8], this study revealed that maximum antibacterial activity was obtained at the fifth day of incubation period; the activity began from the second day, increased gradually till it maximized at the fifth day followed by declination at the sixth day and more

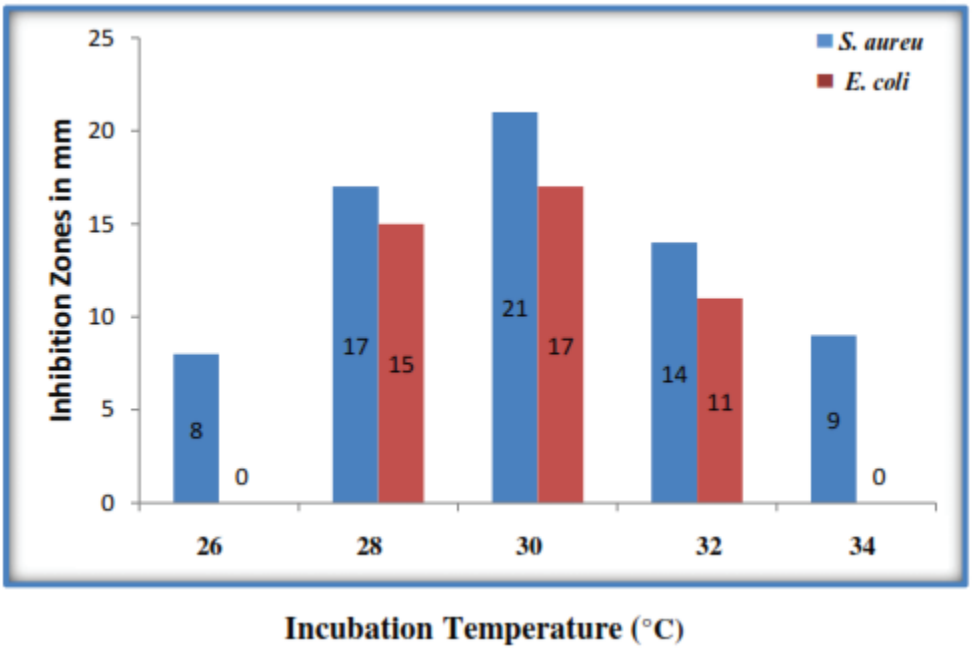

Figure 6: Effect of different incubation temperatures on antibacterial productivity of *Streptomyces* isolate SI-10

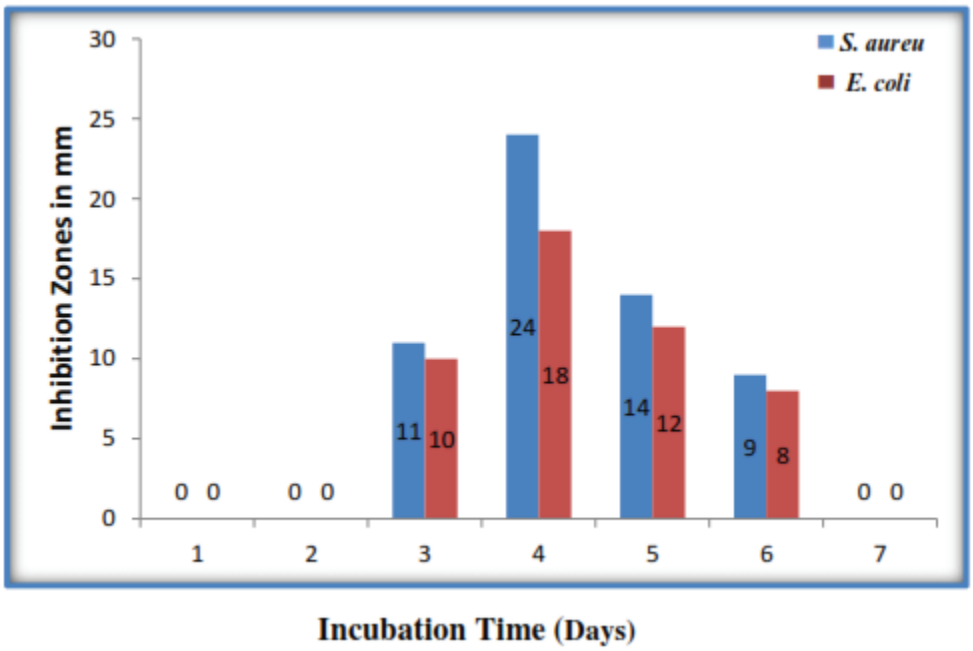

Figure 7: Effect of different incubation periods on antibacterial productivity of *Streptomyces* isolate SI-04

decrement obtained at the seventh day. Concerning the statistical analysis, the *R* values related to the isolate SI-04 were 0.859 and 0.887 for *S. aureus* and *E. coli* subsequently, while regarding the isolate SI-10, the *R* value was 0.990 for both *S. aureus* and *E. coli*.

Discussion

The type of fermentation media is very useful for the antibacterial compound productivity. Bhavana et al.[16] tested different glucose concentrations and its relation with antibiotic productivity, and they found that optimum glucose concentration for maximum antibiotic productivity was 10 g/L. Soya bean broth 1 medium used in the present study contained glucose at a concentration of 10 g/L compatible with that recorded by Bhavana and his colleagues. Narayana and Vijayalakshmi[17] found that soya bean meal enhanced the production of antibiotics by *Streptomyces albidoflavus.* Analogous outcomes were reported by Elbadawy et al.,[18] who suggested that

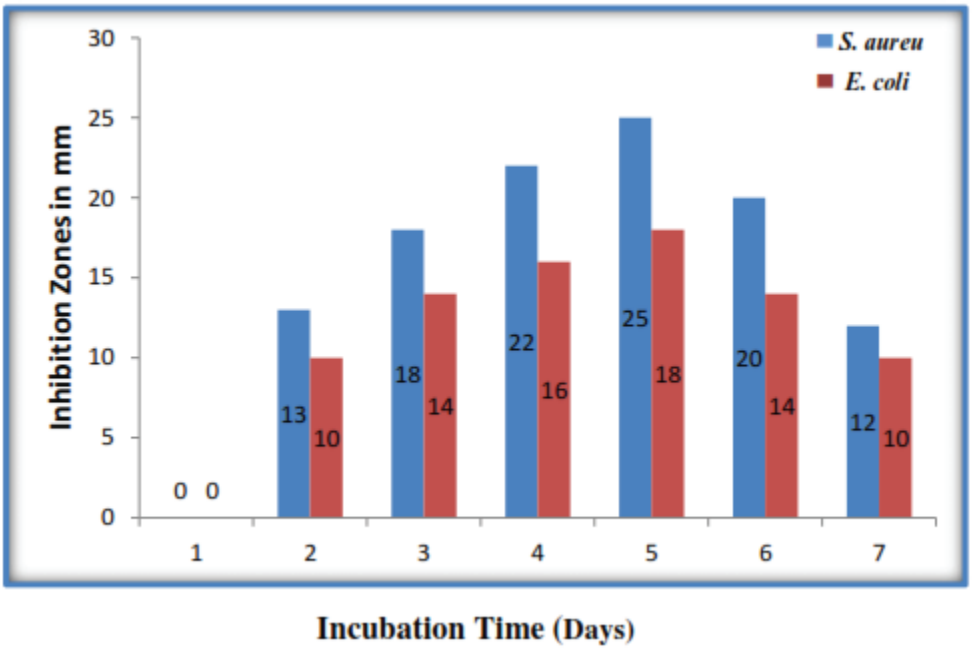

Figure 8: Effect of different incubation times on antibacterial productivity of *Streptomyces* isolate SI-10

chitinase synthesized by *Amycolatopsis orientalis* A13 sp. can be optimized to reach the highest yield and used as a biocontrol agent.

The value of pH of the culture medium (fermentation medium) is a critical point that determines the outcome of antibacterial metabolite productivity. The correct choice of pH will enhance and permit maximum productivity. The outcomes of this study agreed with that of Mangamuri et al.[19] who stated that maximum pH of 7.5 represented the preferred pH for both growth and bioactive metabolite productivity. On the other hand, Nwankwo et al.[20] reported that the isolated *Streptomyces* spp. preferred pH of 10 for optimum growth as well as for efficient antibiotic productivity.

Incubation temperature plays a crucial role in detecting the productivity volume of secondary metabolites since each microorganism may favor a temperature different from others. Therefore, the selection of suitable temperature will enhance the productivity. A study by Kavitha and Vijayalakshmi[21] recorded that the incubation temperature of 30°C was preferred for both the growth of cell and secondary metabolite productivity. In a similar study, Vijayakumar et al.[22] stated that *Streptomyces* sp. VPTS3-1 culture filtrate exhibited maximum antimicrobial activity at 30°C, while the activity was disappeared at 5, 10, and 20°C. The results of this study slightly disagreed with the results of Ripa et al.;[23] they recorded that the optimum antibiotic activity of isolated *Streptomyces* spp. was obtained when they applied incubation temperature of 39°C for the culture medium.

The duration of incubation of secondary metabolite producers (*Streptomyces*) is a critical step that associated with higher degree of productivity when properly selected. Bioactive metabolites collected from 4-days-old cultures of *Streptomyces psammoticus* exhibited good antimicrobial activity against methicillin-resistant *Staphylococcus aureus*.[24] Thakur et al.[25] recorded that 6-day-old culture of *Streptomyces* spp. 201 expressed good antimicrobial activity. At the end, the resulting optimum conditions for antibacterial productivity of *Streptomyces* isolate SI-04 were applied using these parameters: soya bean broth 1 medium, pH adjusted at 7.0, and incubation for 4 days at 30°C. On the other hand, the resulting optimum conditions for antibacterial productivity of *Streptomyces* isolate SI-10 were also applied using these parameters: soya bean broth 1 medium, pH adjusted at 7.0, and incubation for 5 days at 30°C.

It is important to increase the number of studies that involve the productivity of antibiotics from microorganisms (enhancing and improving the conditions and adding certain adjuvants that potentiate the productivity) since it may lead to the discovery of novel antibiotics to overcome untreatable infection caused by the growth of highly resistant bacteria (the use of antimicrobial susceptibility testing is regarded as an important way to minimize the antibiotic resistant).[26,27]

Conclusion

The optimization of production conditions was an important and crucial step for improving and enhancing

the antibacterial productivity of *Streptomyces* spp. isolated from clinical samples in Iraq.

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Nil.

Conflicts of interest
There are no conflict of interest.

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