Production of Hygromycin-B antibiotic from *Streptomyces crystallinus*, AZ-A151: II. Parameters Controlling of Antibiotic Production

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Abstract: Growth pattern and antimicrobial profile of *Streptomyces crystallinus,* AZ-A151 were studied on Starch Nitrate (SN) broth medium. An attempt has been made to evaluate the optimal cultural conditions for obtaining high yields of bioactive metabolites. The optimum temperature and pH for bioactive metabolite production of the strain were recorded as 35 °C and 8.0 respectively. Production of bioactive metabolites by the strain was high in Starch nitrate (SN) broth medium as compared to other tested media tested. The strain utilized starch, sodium nitrate and 200 ppm of vitamin H, as good carbon, nitrogen and vitamin sources for the elaboration of bioactive metabolites. The secondary metabolites exhibited high antimicrobial activity against *Staphylococcus aureus*, NCTC 7447; *Escherichia coli*, NCTC 10416; *Klebsiella pneumonia*, NCIMB 9111; *Salmonella typhi*; *Saccharomyces cerevisiae,* ATCC 9763; *Aspergillus flavus*, IMI 111023; *Alternaria alternate* and *Fusarium verticillioides*. This is the first report on the optimization studies of bioactive metabolites by *Streptomyces crystallinus,* AZ-A151.
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Keywords: Effect of Environmental Conditions and Nutritional Requirements

1. Introduction

Most of the antibiotics in use today are derivatives of natural products of actinomycetes and fungi [Newman and Cragg, 2007]. Antibiotics produced by actinomycetes and other microbes have been evolving for one billion years [Baltz, 2005] and their activity has been tested against microbes based on their ability to inhibit target enzymes and macromolecules. Actinomycetes can be isolated from soil and marine environments. Since collecting soil is relatively inexpensive, much is known about the distribution and abundance of terrestrial actinomycetes. Although soils have been screened by the pharmaceutical industry for the past five decades, only a small fraction of actinomycetes have been discovered [Baltz, 2005]. Actinomycetes from unexplored habitats have gained considerable attention in recent years for the production of bioactive metabolites. The list of novel actinomycetes and products derived from poorly explored areas of the world stresses the importance of investigating new habitats [Nolan and Cross, 1988].

The search for novel natural products with useful pharmacological activities often includes the isolation of actinomycetes, such as *Streptomyces* species, from soil samples [Ritacco *et al.,* 2003; Sembiring and Goodfellow, 2008]. Actinomycetes have been especially useful to the pharmaceutical industry for their seemingly unlimited capacity to produce secondary metabolites with diverse chemical structures and biological activities. Searching for novel actinomycetes constitutes an essential component in natural product-based drug discovery [Valan Arasu *et al.,* 2008]. Actinomycetes are also the focus of attention due to their production of secondary metabolites that may have a range of pharmaceutical and biotechnological applications. Microbial natural products still appear to be the most promising source of the future antibiotics that society expects to be developed and they are the origin of most of the antibiotics on the market today [Kaltenpoth, 2009; Thumar *et al*., 2010]. The search for new antibiotics or new microorganism strains producing antibiotics continues to be of utmost importance in research programs around the world, because of the increase of resistant pathogens and toxicity of some used chemical antibiotics. Therefore, there is an alarming scarcity of new antibiotics currently under development in the pharmaceutical industry. 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 (Thumar *et al.,* 2010). In the past two decades however, there has been a decline in the discovery of new lead compounds from common soil-derived actinomycetes [Valan Arasu *et al.,* 2008]. It is well known that designing an appropriate fermentation medium is of critical importance in the production of secondary metabolites [Gao *et al.,* 2009]. Prior knowledge and

experience in developing a suitable basal medium may play an important role in further medium optimization [Jia *et al.,* 2008]. Production of secondary metabolites through fermentation is influenced by various environmental factors including nutrients (nitrogen, phosphorous and carbon source), growth rate, feedback control, enzyme inactivation and variable conditions (oxygen supply, temperature, light & pH) [Lin *et al.,* 2010; Ruiz *et al.,* 2010; Sánchez *et al.,* 2010]. In addition, production of valuable metabolites by actinomycetes differs qualitatively and quantitatively depending on the strains used in fermentation. As one of the most significant components in the medium, carbon source plays a critical role as sources of precursors and energies for synthesis of biomass building blocks and secondary metabolite production [Wang *et al.,* 2008 & 2010; Jia *et al.,* 2009]. Therefore, influences of medium components and environmental conditions are an initial and important step to improve metabolite production of the genus *Streptomyces*.

In the present study, optimal conditions for the production of bioactive metabolites by *Streptomyces crystallinus,* AZ-A151 were determined and the metabolites thus extracted showed good antimicrobial activity against Gram positive, Gram negative bacteria and unicellular and filamentous fungi.

2. Material and Methods

2.2. Test organisms

2.2.1. Gram Positive: *Staphylococcus aureus*, NCTC 7447.

2.2.2. Gram Negative: *Escherichia coli*, NCTC 10416; *Klebsiella pneumonia*, NCIMB 9111; *Salmonella typhi*.

2.2.3. Unicellular fungi: *Saccharomyces cerevisiae,* ATCC 9763.

2.2.4. Filamentous fungi: *Aspergillus flavus*, IMI 111023 and *Alternaria alternata*.

2.3. Effect of environmental conditions

2.3. 1. Incubation period:

For such a purpose the spores of cultures were allowed to grow on a Basal Starch Nitrate Medium (BSNM) as mentioned before.

Fifty mls of the medium were dispensed among conical flasks of 250 ml. three flasks were used for each particular incubation period. The flasks were then sterilized, cooled, inoculated and incubated on a rotary shaker of 120 rpm. at 30°C. Cultures were removed after 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 days of incubation and tested for antimicrobial biosynthesis.

2.3.2. Initial pH values:

The initial pH values of the basal starch nitrate broth media were adjusted to cover the range from 4, 5, 6, 7, 8, 9, 10 and 11 before sterilization.

Three flasks were always used for each particular pH value. The experiment was terminated after maximum biosynthesis of metabolite was attained and the broth filtrate was used for assessing the antimicrobial potency.

2.3.3. Incubation temperatures:

Most antibiotic producing microorganisms are mesophilic that is the optimum temperature for their growth in the range of 23-37°C. This experiment was constructed to determine the optimum growth temperature at which maximum biosynthesis of the active agent occurred. The liquid nutrient media were inoculated as previously mentioned and incubated at temperatures range from 15, 20, 25, 30, 35, 40, 45, 50 and 55°C. The antimicrobial agent biosynthesis was assessed at each temperature.

2.3.4. Shaking at different speed (rpm):

The inoculated flasks of the previous medium were incubated in incubator shaker at 40, 80, 120, 160 and 200 (rpm). At the end of incubation period, the antibiotic biosynthesis was assessed.

2.3.5. Inoculum age:

Liquid medium of Starch Nitrate (BSNM) used for each particular inoculum age, each flask contained 50 mls of the liquid medium, inoculated with 1.0 ml from suspension of organism using different inocula ages of: 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30 days. At the end of incubation period, antibiotic biosynthesis was assessed.

2.3.6. Inoculum size:

The investigated strains were grown on starch nitrate agar medium plate for 10 days at 28°C. Spores were harvested and re-suspended in water. Spore suspension was inoculated in 250 ml Erlenmeyer flasks containing 50 ml of the basal fermentation medium at 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20% (v/v), and incubated on a rotatory shaker. Then calculate the total colonies forming units (C.F.U./ml) for each studied strain. At the end of incubation period the obtained clear filtrates were tested for their antimicrobial activities.

2.4. Effect of nutritional conditions

2.4.1. Growth media:

For such a purpose the investigated strains were grown on six different growth media media such as starch nitrate, Starch casein, Yeast extract

malt extract, Glycerol asparagines, Inorganic salt starch and tryptone yeast extract (the composition of these media are as mentioned before). Fifty mls of each medium was dispensed among conical flasks of 250 ml. three flasks were used for each medium, sterilized, cooled, inoculated and incubated on a rotary shaker of 120 rpm at 30°C. Cultures were removed after 10 days of incubation and tested for antimicrobial biosynthesis.

2.4.2. Carbon source:

Eleven carbon sources (Starch, Galactose, Arabinose, Trehalose, Glycerol, Glucose, Mannose, Mannitol, Cellulose, *meso*-inositol and Raffinose) were applied. The liquid basal medium (without carbon source) was supplemented with each individual carbon source at an equivalent amount of carbon to that located in starch (2 %). The tested sugars were separately sterilized by diethyl ether and left to dry and then were added to the basal nutrient medium. The initial pH of the various media was adjusted at 7.0 before sterilization. Flasks after incubation filtrated for assayed antibiotic biosynthesis.

2.4.3. Nitrogen source:

The following nitrogenous compounds such as (Ammonium nitrate, Casein, Sodium nitrate, Potassium nitrate, Magnesium nitrate, Ammonium sulphate, Ammonium carbonate) and amino acids such as (L-Phenylalanine, DL-Cystine, DL Methonine, Lysine, L-Leucine, Glycine, Treptophane, Asparagine, Proline, L-Asparatic acid, L-Serine) were supplemented to the basal medium. The liquid basal medium was supplemented with each individual nitrogen source at an equivalent amount to that nitrogen located in 0.2 % ($NaNO_3$).The appropriate level of each amino acid tested was soaking in diethyl ether for overnight and then added to the basal medium. Flasks were inoculated and incubated, and antibiotic biosynthesis was assessed at the end of incubation period.

2.4.4. Vitamins:

The previously mentioned production broth medium (BSNM) was used for studying the effect of different vitamins on antibiotics biosynthesis. Vitamins used were, riboflavin, pantathonic acid, folic acid, vitamin H, thiamin, B12 and D2. Each vitamin was added at three concentrations viz, 50, 100 and 200 ppm. Appropriate weights of these vitamins were sterilized by soaking in 96 % ethyl alcohol for 24 hours, flasks were inoculated, incubated, filtrated and then the antibiotic biosynthesis was assessed at the end of incubation period.

2.4.5. Potassium monohydrogen phosphate Concentration:

Various concentrations (g/L) of Potassium monohydrogen phosphate: 0.2, 0.4, 0.6, 0.8, 1.0, 2.0 and 3.0 were supplemented to basal liquid medium. The flasks were sterilized, inoculated, and incubated at 30 °C. At the end of incubation period, the antibiotic biosynthesis was assessed.

2.4.6. Magnesium sulphate Concentration:

Various concentrations (g/L) of Magnesium sulphate: 0.1, 0.3, 0.5, 0.7, 1.0 and 2.0 were supplemented to basal liquid medium. The flasks were sterilized, inoculated, and assessed as mentioned before.

3. Results

3.1. Effect of environmental conditions

3.1.1. Effect of incubation periods:

The Effect of incubation periods on biomass and bioactive metabolite production of *Streptomyces crystallinus*, AZ-A151 was recorded (Fig. 1). Cell growth and the yield of bioactive metabolites were found to be optimum when the strain was cultured at incubation period (day) of 10 and given highly potency against all test organisms in series *Staphylococcus aureus* (35), gram-negative bacteria *Klebsiella pnumoniae* (24.5), *Alternaria alternata* (20.5), and *Saccharomyces cerevisiae* (16.8) respectively.

3.1.2. Effect of different pH values:

Data recorded in Fig. (2), contained that maximum biosynthesis level of antimicrobial agents by *Streptomyces crystallinus*, AZ-A151 could be recorded within an pH value 8 and given highly potency against bacterial test organism*s* *Staphylococcus aureus* (34.5mm), *Klebsiella pnumoniae* (24mm), followed by pH 7 in *Alternaria alternata* (21mm) and *Saccharomyces cerevisiae* (16.5mm).

3.1.3. Effect of incubation temperatures:

The Effect of incubation temperatures on biomass and bioactive metabolite production of *Streptomyces crystallinus*, AZ-A151 was recorded (Fig. 3). Cell growth and the yield of bioactive metabolites were found to be optimum when the strain was cultured at 35°C indicating its mesophilic nature. This was declared by the higher activity in relation to *Staphylococcus aureus* (37.5), *Klebsiella pnumoniae* (28.0), *Alternaria alternata* (23.5), and *Saccharomyces cerevisiae* (19.5) in AZ-A151.

3.1.4. Effect of shaking at different speeds (rpm):

Effect of shaking at different speeds (rpm) on biomass and bioactive metabolite production of *Streptomyces crystallinus*, AZ-A151 was recorded (Fig. 4). The maximum biosynthesis level of antimicrobial agents could be recorded within an shaking speed (rpm) at 160, and given the highest potency against all test organisms *Staphylococcus aureus* (36.5), *Klebsiella pnumoniae* (25.5), *Alternaria alternata* (21.5) and *Saccharomyces cerevisiae* (18.8).

3.1.5. Effect of inoculum age:

Effect of inoculum age on biomass and bioactive metabolite production of *Streptomyces crystallinus*, AZ-A151 was recorded (Fig. 5). The maximum biosynthesis level of antimicrobial agents was recorded within an inoculums age (days) at 12 and 15, as it was detected by the highest potency against *Staphylococcus aureus* (36.5), *Klebsiella pnumoniae* (26.0), *Alternaria alternata* (22.5) and *Saccharomyces cerevisiae* (18.5) tested in AZ-A151.

3.1.6. Effect of inoculum size:

Data recorded in Fig. (6), determined that the maximum biosynthesis level of antimicrobial agents by *Streptomyces crystallinus*, AZ-A151 could be represented within 10 % (v/v) inoculum size concentration and given highest potencies (mm) against *Staphylococcus aureus* (44) and *Klebsiella pnumoniae* (32) compared to (25.5) of *Alternaria alternata* and (22.5) of *Saccharomyces cerevisiae* at 8 % (v/v) inoculum size concentration.

3.2. Effect of nutritional requirements

3.2.1. Effect of different growth media:

Data recorded in Fig. (7), the maximum biosynthesis level of antimicrobial agent(s), could be recorded on Starch nitrate (SN) medium which given a highly potency against all test organisms as inhibition zone (mm) such as *Staphylococcus aureus* (35.8 and 33.5), *Klebsiella pnumoniae* (24.5 and 24.0), *Alternaria alternata* (20.3 and 21.0) and *Saccharomyces cerevisiae* (16.8 and 16.5) in case of *Streptomyces crystallinus*, AZ-A151. This was followed by Inorganic salts nitrate medium. On the other hand, a decrease of antimicrobial agent(s) productivity was detected in the presence of other tested growth media, while antimicrobial production was not produced in tryptone yeast extract (TYE).

3.2.2. Effect of various carbon sources:

Impact of several carbon sources on biomass and bioactive metabolite yield was shown in Fig. (8). Among the carbon sources tested, starch and glycerol are the best carbon source for productivity of antimicrobial agent(s) which exhibited the highest potencies (i.e. inhibition zone in mm) as follows: against *Staphylococcus aureus* (38), *Klebsiella pnumoniae* (26.5), *Alternaria alternata* (23) and *Saccharomyces cerevisiae* (20.5) in case of AZ-A151. On the other hand, a decrease of biosynthesis antimicrobial agent(s) was detected in the presence of other tested carbon sources.

3.2.3. Effect of various nitrogen sources:

The effect of different nitrogen sources on the production of biomass and bioactive metabolites of the strain was studied (Fig. 9). Among the nitrogen sources tested, sodium nitrate is the best nitrogen source for productivity of antimicrobial agent(s) by AZ-A151 given high potency inhibition zone (mm) against all test organisms as *Staphylococcus aureus* (34.5), *Klebsiella pnumoniae* (24.8), *Alternaria alternata* (21.0), and *Saccharomyces cerevisiae* (16.5) in case of AZ-A151. This was followed by Ammonium sulphate. On the other hand, a decrease of antimicrobial agent(s) productivity was detected in the presence of other tested nitrogen sources.

3.2.4. Effect of different vitamins:

The effect of different vitamins sources on the production of biomass and bioactive metabolites of the strain was studied (Fig. 10). The maximum activity of antimicrobial agent(s) produced by AZ-A151 was detected in the presence of 200 ppm of vitamin H and highest potencies against *Staphylococcus aureus* (42.5) *Klebsiella pnumoniae* (30.5), followed by Riboflavine which declared (25) in *Alternaria alternata* and (19) in *Saccharomyces cerevisiae* at the same concentration, comparable to control (which contained no vitamins).

3.2.5. Effect of various amino acids:

The effect of various amino acids on the production of biomass and bioactive metabolites of the strain was studied (Fig. 11). Among the nitrogen sources tested, Asparagine are given highly potency i.e., inhibition zone (mm) against *Staphylococcus aureus* (30), *Klebsiella pnumoniae* (22.3), *Alternaria alternata* (18.5) and *Saccharomyces cerevisiae* (14). This was followed by Trptophane, DL-Cystine, Lysine and Proline respectively.

3.2.6. Effect of various K_2HPO_4 concentrations:

Data recorded in Fig. (12), the highest yield of the antimicrobial agent biosynthesis produced by *Streptomyces crystallinus*, AZ-A151 was achieved in a medium fortified with 0.08 % (w/v) concentrations of K_2HPO_4, that recorded a high potency against *Staphylococcus aureus* (37.0 mm) and *Klebsiella pnumoniae* (26.0 mm) and found maximum

inhibition zone at 0.06 % (w/v) against *Alternaria alternata* (23.0 mm) and *Saccharomyces cerevisiae* (19.5 mm).

3.2.7. Effect of $MgSO_4$. $7H_2O$ concentrations:

Data recorded in Fig. (13), the highest yield of the antimicrobial agent biosynthesis was attained in a medium fortified with 0.7 $MgSO_4.7H_2O$ and given highly potency against all test organisms as follows *Staphylococcus aureus* (36.0), *Klebsiella pnumoniae* (25.5), *Alternaria alternata* (21.5) and *Saccharomyces cerevisiae* (18.0) in the case of AZ-A151.

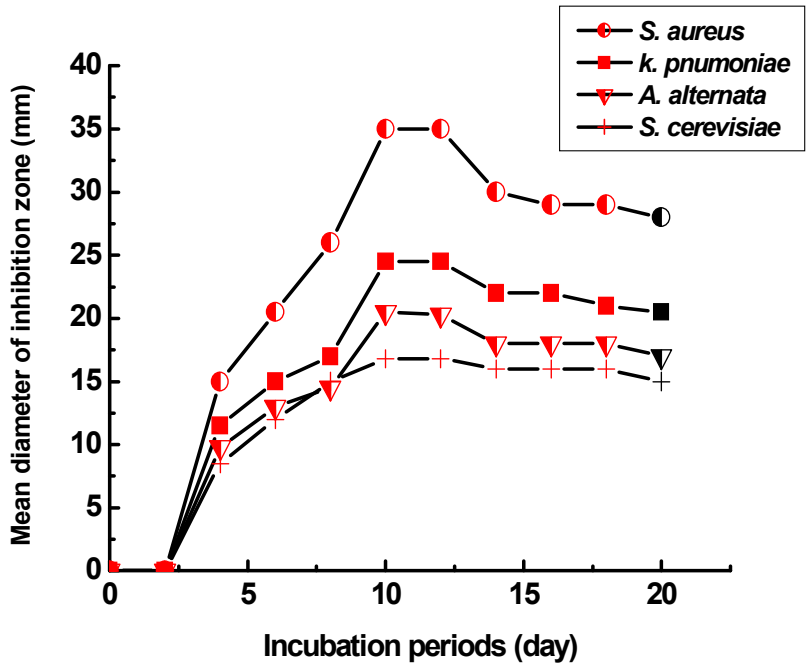


Figure 1. Effect of different incubation periods on antimicrobial agent biosynthesized by *Streptomyces crystallinus*, AZ-A151.

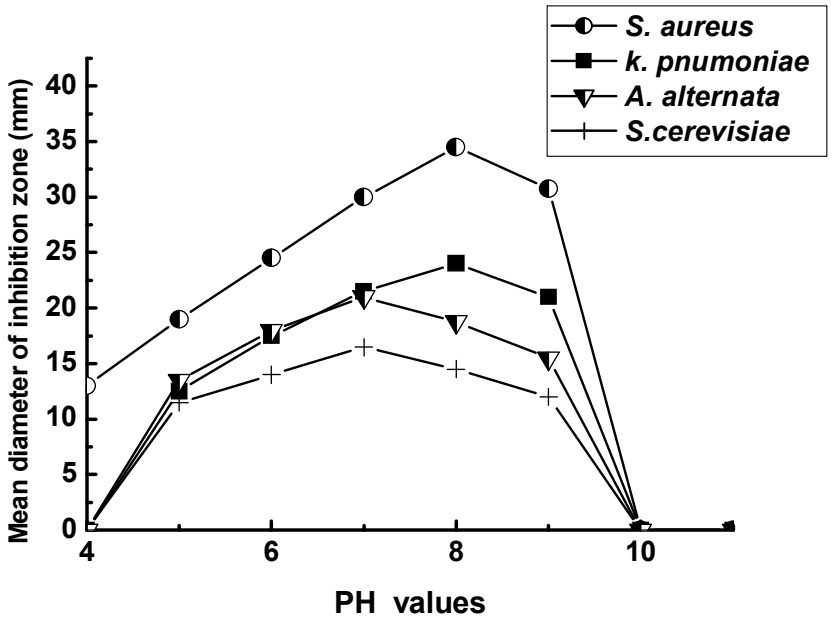


Figure 2. Effect of pH values on the biosynthesis of antimicrobial agent produced by *Streptomyces crystallinus*, AZ-A151.

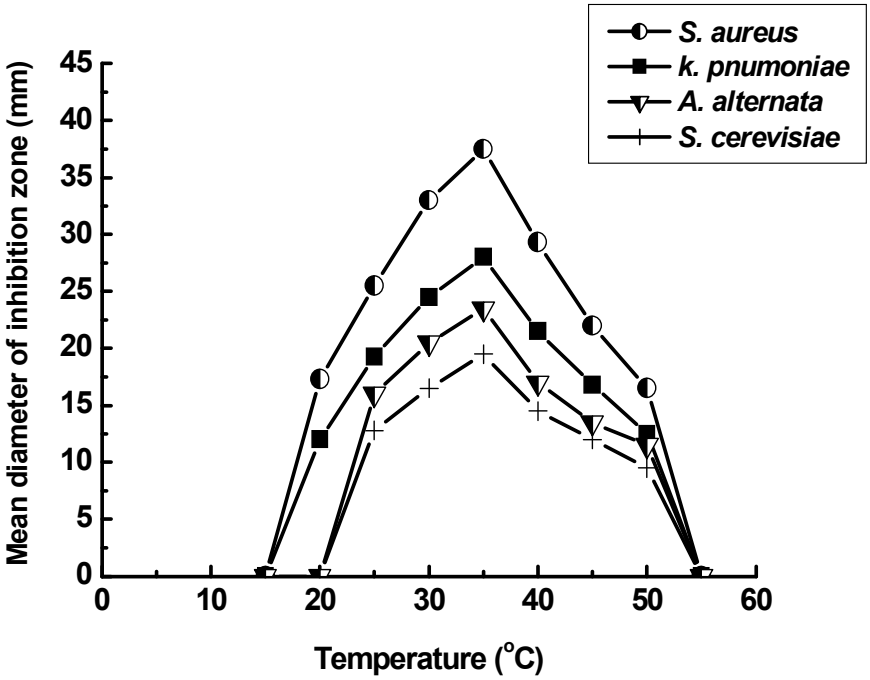


Figure 3. Effect of incubation temperatures (°C) on biosynthesis of antimicrobial agent produced by *Streptomyces crystallinus*, AZ-A151.

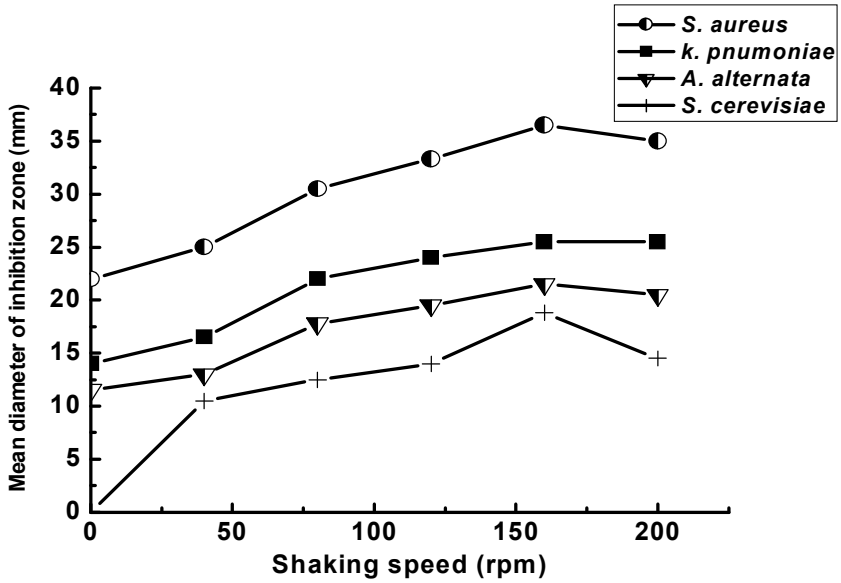


Figure 4. Effect of various shaking speed (rpm) on biosynthesis of antimicrobial agent produced by *Streptomyces crystallinus*, AZ-A151.

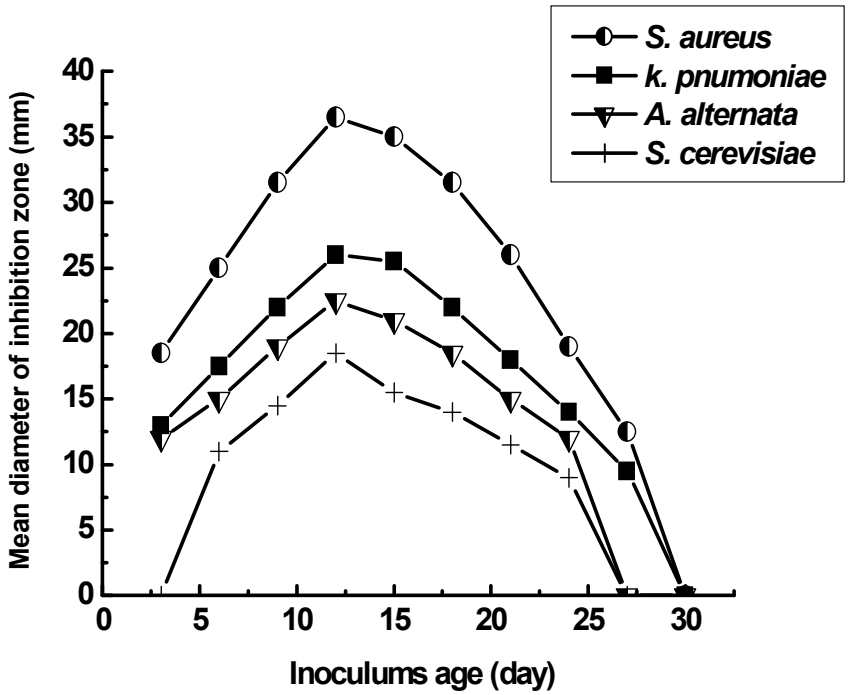


Figure 5. Effect of inocula ages on biosynthesis level of antimicrobial agent produced by *Streptomyces crystallinus*, AZ-A151.

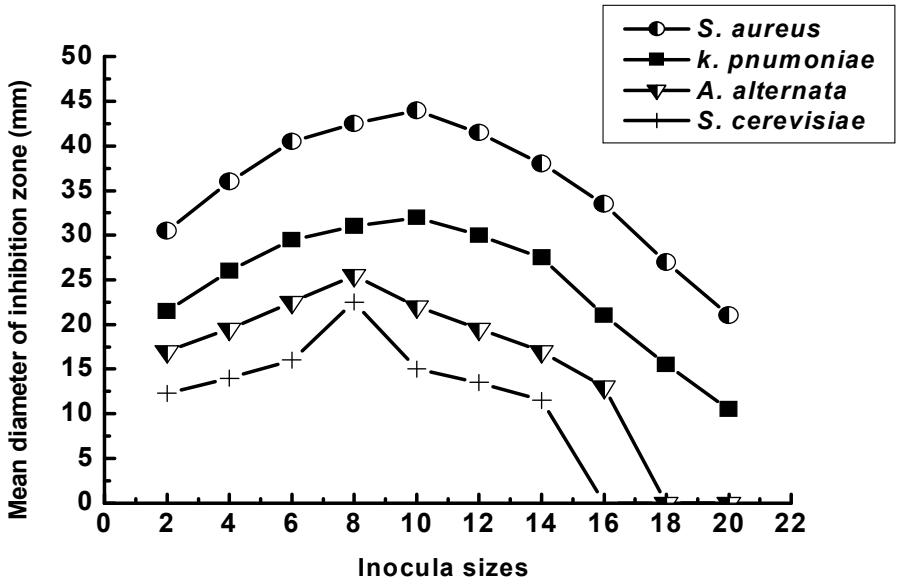


Figure 6. Effect of inocula sizes on biosynthesis of antimicrobial agent produced by *Streptomyces crystallinus*, AZ- A151.

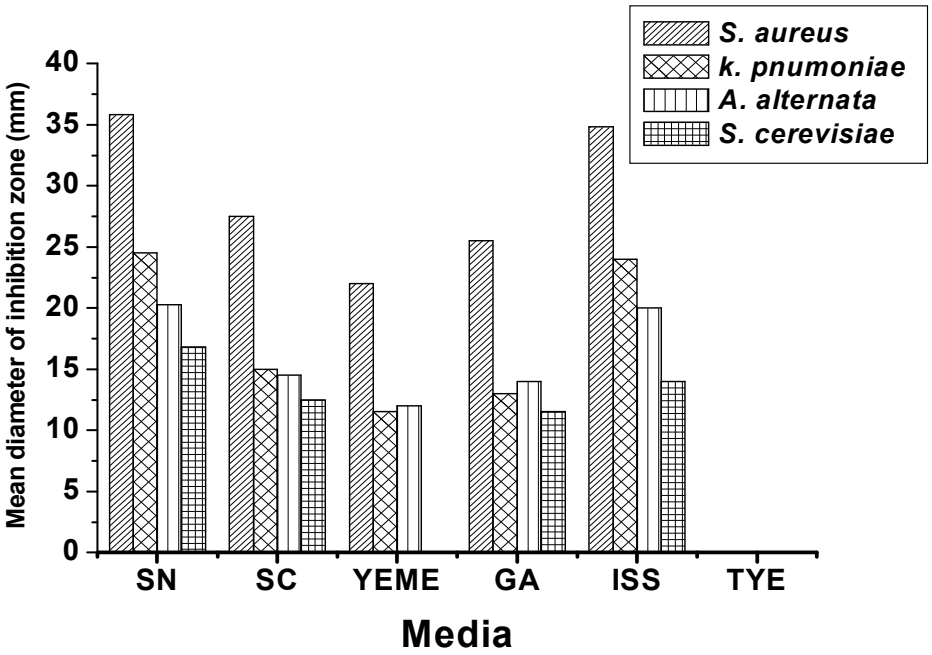


Figure 7. Effect of different media on the biosynthesis of antimicrobial agent produced by *Streptomyces crystallinus*, AZ- A151.

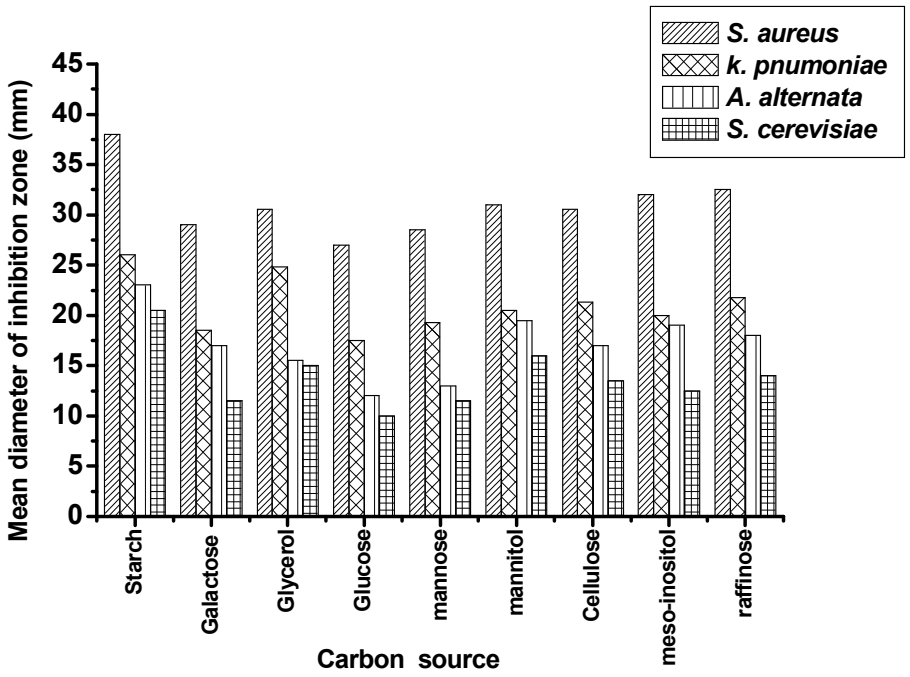


Figure 8. Effect of Carbon sources on biosynthesis of antimicrobial agent by *Streptomyces crystallinus*, AZ-A151.

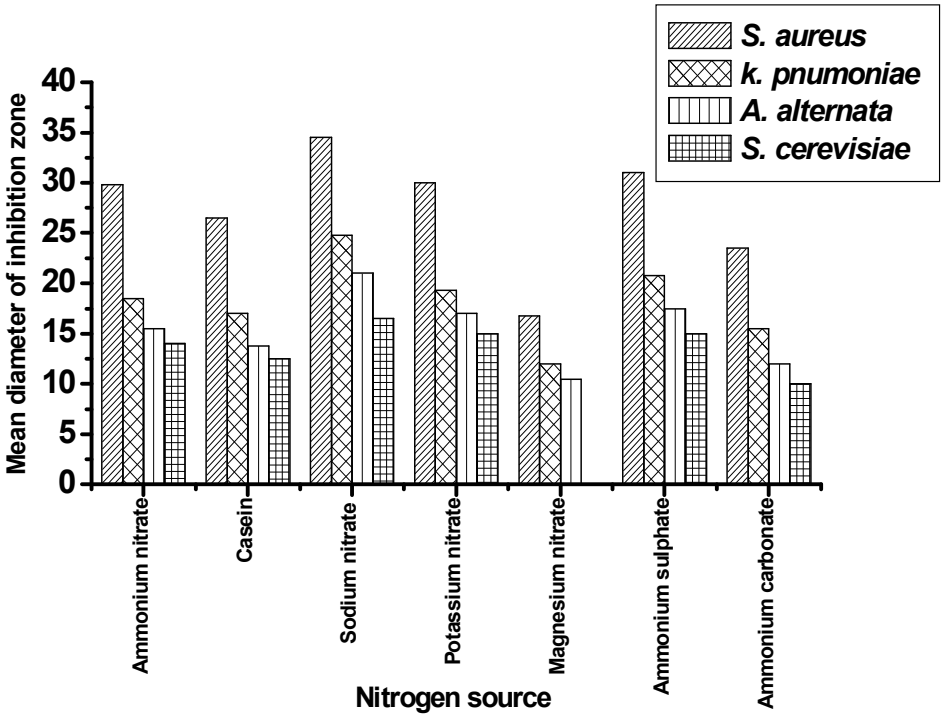
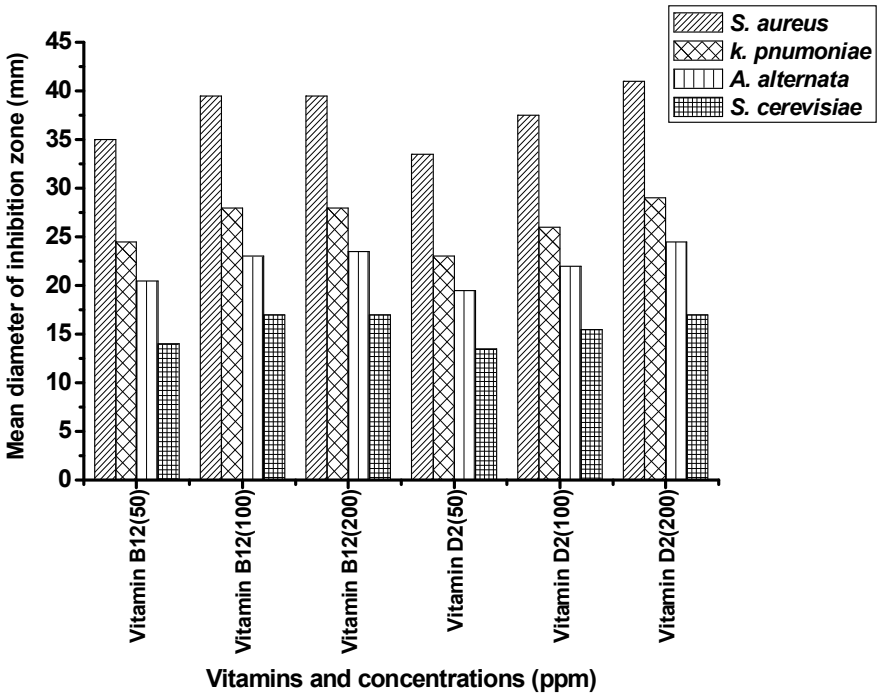
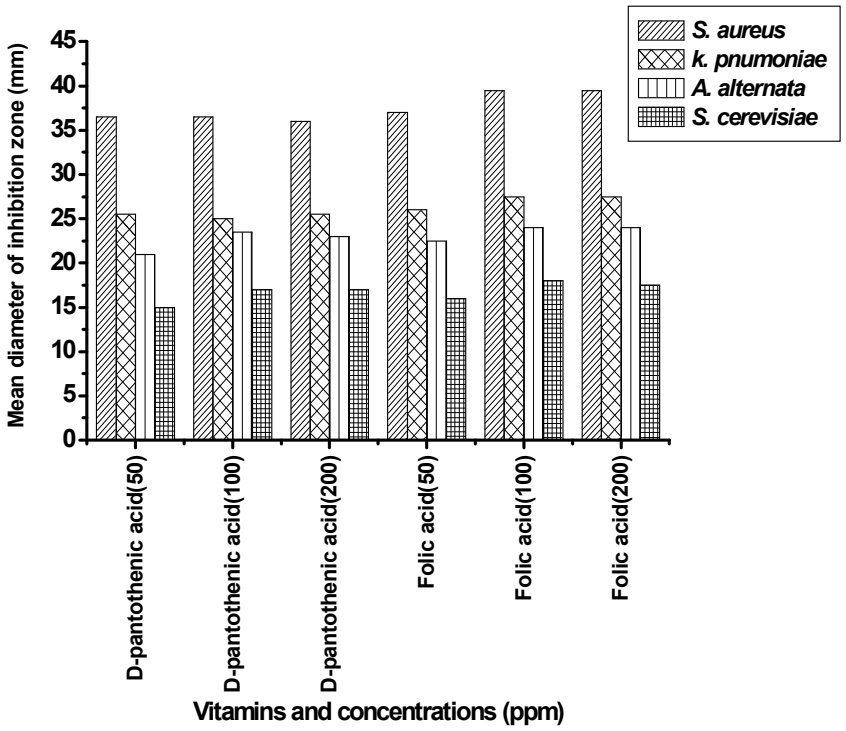


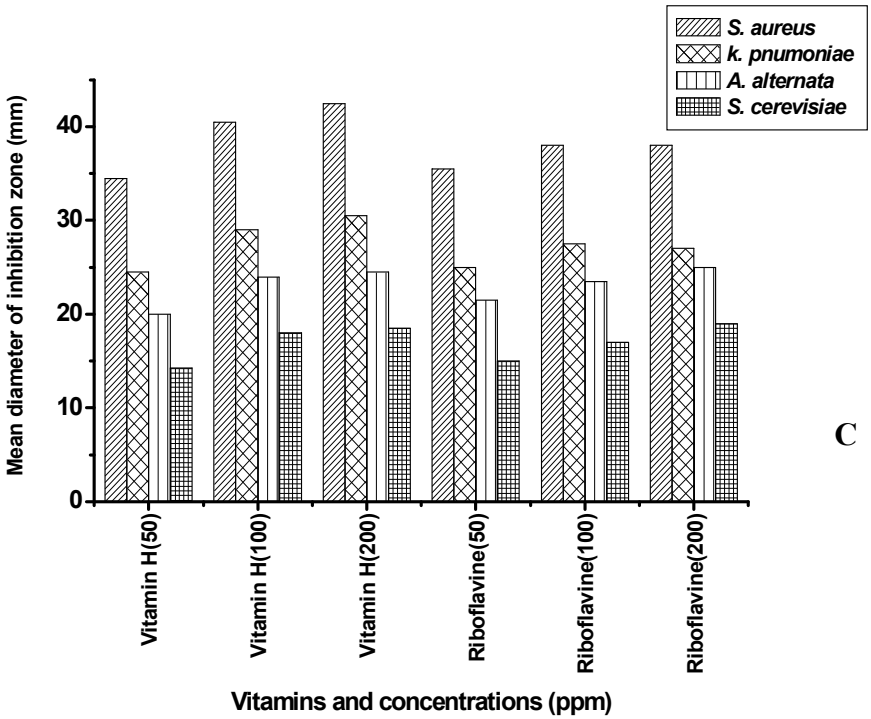
Figure 9. Effect of different nitrogen sources on the biosynthesis of antimicrobial agent produced by *Streptomyces crystallinus*, AZ-A151.



A


S. aureus
k. pnumoniae
A. alternata
S. cerevisiae
Mean diameter of inhibition zone (mm)
0
5
10
15
20
25
30
35
40
45
D-pantothenic acid(50)
D-pantothenic acid(100)
D-pantothenic acid(200)
Folic acid(50)
Folic acid(100)
Folic acid(200)
Vitamins and concentrations (ppm)

B


S. aureus
k. pnumoniae
A. alternata
S. cerevisiae
Mean diameter of inhibition zone (mm)
0
10
20
30
40
Vitamin H(50)
Vitamin H(100)
Vitamin H(200)
Riboflavine(50)
Riboflavine(100)
Riboflavine(200)
Vitamins and concentrations (ppm)

C

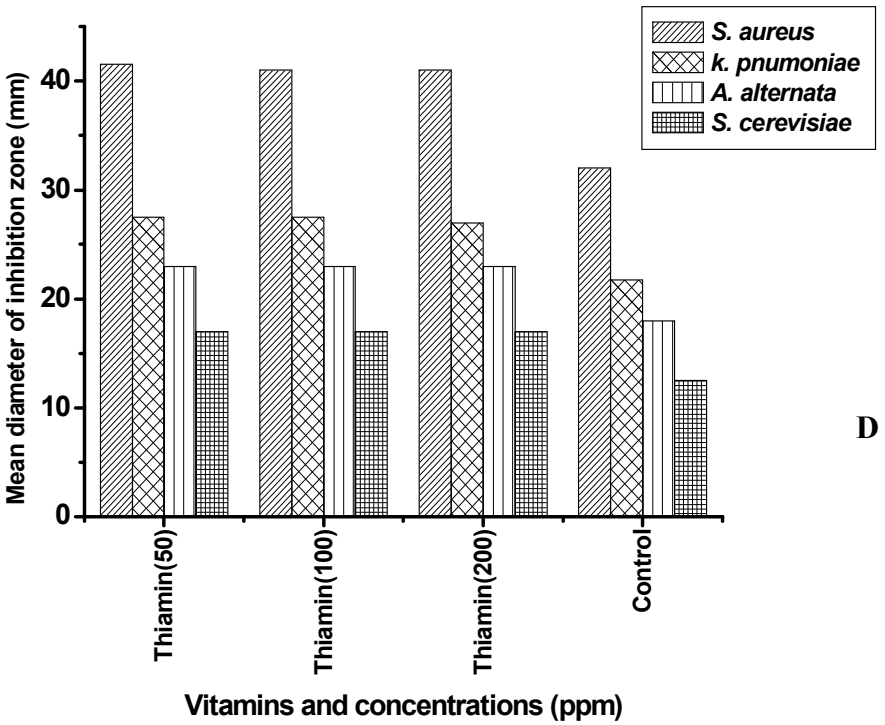


Figure 10. Effect of different vitamins and their concentrations on biosynthesis of antimicrobial agent produced by *Streptomyces crystallinus*, AZ-A151.

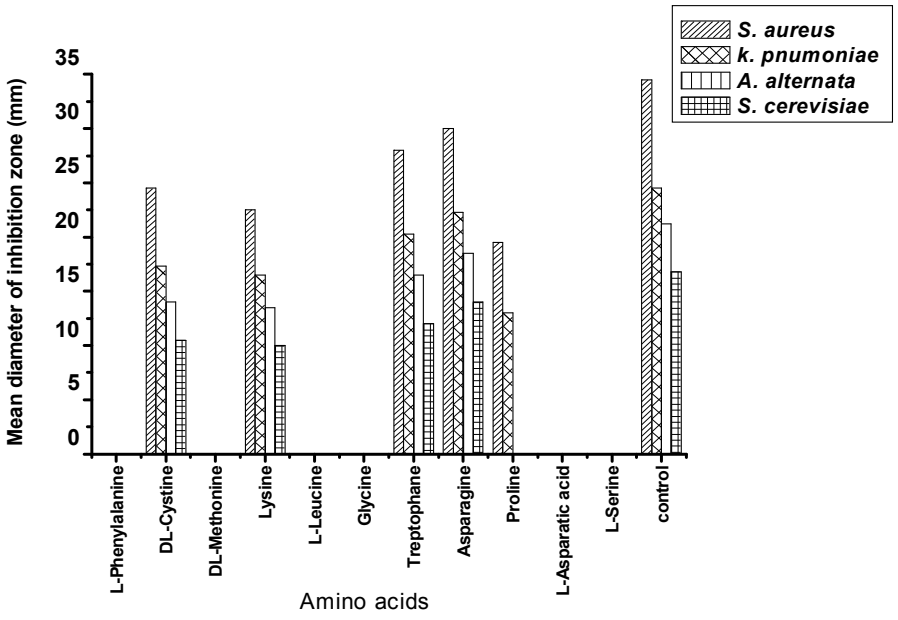


Figure 11. Effect of amino acids on the biosynthesis of antimicrobial agent produced by *Streptomyces crystallinus*, AZ-A151.

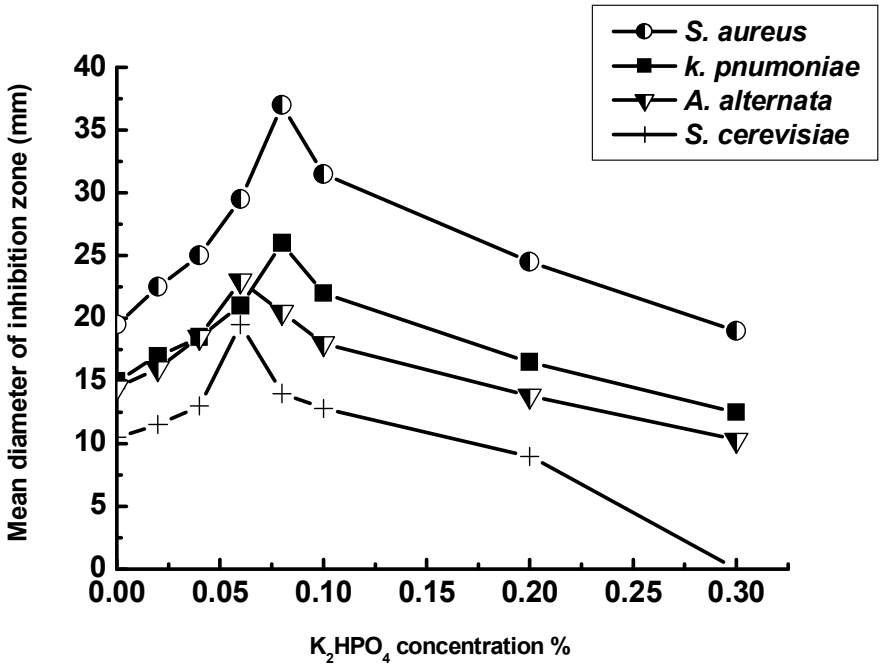


Figure 12. Effect of various K_2HPO_4 concentrations on the biosynthesis of antimicrobial agent produced by *Streptomyces crystallinus*, AZ- A151.

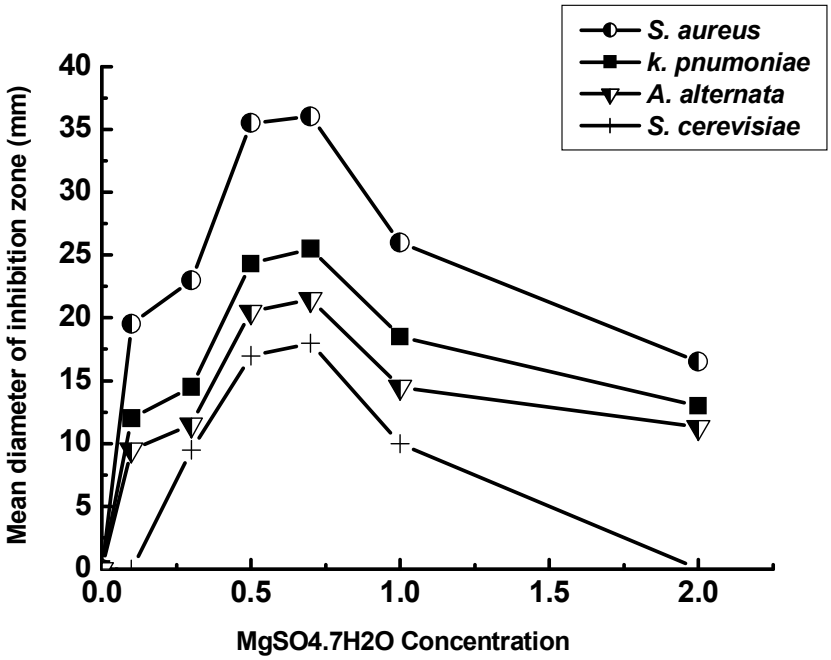


Figure 13. Effect of various concentrations of $MgSO_4.7H_2O$ on the biosynthesis of antimicrobial agent produced by *Streptomyces crystallinus*, AZ-A151.

4. Discussions

The effect of different environmental and nutritional factors on the antimicrobial activity was studied for optimizing the cultural conditions to obtain the highest quantities of antimicrobial compounds produced by *Streptomyces crystallinus*, AZ-A151. The maximum biosynthesis of the antimicrobial activities produced by *Streptomyces crystallinus,* AZ-A151 were obtained after incubation 10 days. Similar result was obtained by [Martin *et al.,* 1979]. Our results agreed with [El-Naggar *et al.,* 2006] who stated that the maximum Meroparamycin production by the three isolated *Streptomyces* sp. strain MAR01 were obtained after days 5, 6 and 7. Also [Bruheim *et al.,* 2002] stated that high-yield actinorhodin production was occurred during 5 days of cultivation. [Barun *et al.,* 1997] reported that the best antibiotic yield was recorded when fermentation was carried out for 5 days. [Sousa *et al.,* 2002] showed that the production of actinomycin-D by *S. parvulus* reached their maximum values at around 144 h. Maximum antibiotic production by *Streptomyces albidoflavus* was observed on the 8th day of incubation [Augustine *et al.,* 2005] found that peak antibiotic production by *Streptomyces sp.* occurred at 72 h in a batch culture.

The maximum antibiotic production was obtained at incubation temperature 35ºC for *Streptomyces crystallinus,* AZ-A151. These results are in agreement with [Hassan *et al.,* 2001] that showed that maximum antibiotic production was obtained by *S. violatus* at 30ºC. Also [Adinarayana *et al.,* 2003] stated that maximum neomycin production by *Streptomyces marinensis* was obtained at 30ºC. [Suetsuna and Osajima, 1990] showed that 28ºC is the optimum temperature for griseorhodin production by *S. californicus* JCM6910. [Augustine *et al.,* 2004] reported that deviation from optimum temperature for antifungal metabolite production severely affected the yield of antifungal metabolite.

The antagonistic activities were found to be influenced by the pH of the medium. The optimum pH value for the antimicrobial metabolite biosynthesis varied from acidic, neutral and alkaline environments were reported to have a great effect in this respect [Egorov, 1985]. The initial pH value of the present data showed a significant influence on the maximum productivity of the antibiotic as well as on the growth of the test organism. The isolate *Streptomyces crystallinus,* AZ-A151 showed the maximum antibiotic activity was obtained at an initial pH of 8.0. Our results were comparable with some *Streptomyces* species recorded to secrete antibiotics against Gram posistive and gram negative bacteria and unicellular and filamentous fungi; actinorhodin, a blue pigment-antibiotic, was produced extracellularly in *S. coelicolor* cultures at pH values around 8 [Bystrykh *et al.,* 1996]. [Sathi *et al.,* 2001] found that pH 7- 8 was the most suitable for large scale production of antibiotics *from Streptomyces* Species.

The ability of Streptomycete to form antibiotic was not a fixed property but can be greatly increased or completely lost under different conditions of nutrition and cultivation [Waksman, 1961]. Therefore, the medium constitution together with the metabolic capacity of the producing organism greatly affected antibiotic biosynthesis [Barratt and Oliver, 1994; Abbanat *et al.,* 1999].

The choice of carbon source greatly influenced secondary metabolism and therefore antibiotic production [Spizek and Tichy, 1995]. A quickly metabolized substrate such as glucose may often achieved maximum cell growth rates, but it was known to inhibit the production of many secondary metabolites. This “catabolite repression” was thought to be due to intermediates generated from the rapid catabolism of glucose interfering with enzymes in the secondary metabolism process.

We explored the effect of carbon sources on antibiotic production by inoculating the selected isolates on the basal medium having different sugars like Sucrose, Cellulose, Mannitol, Galactose, Lactose, Maltose, Glucose, starch, and Fructose. The maximal cell efficiency for antibiotic production was in the following manner (starch > cellulose> raffinose> meso-inositol> mannitol>glycerol> galactose> glucose >) by *Streptomyces crystallinus.* AZ- A151. These results indicated that starch was the excellent carbon source for antibiotic production by *Streptomyces crystallinus.*

The present study agreed with [Atta *et al.*, 2011] who stated that glycerol, starch, and maltose were excellent carbon sources for neomycin production by *Streptomyces fradiae*. Also [Sousa *et al.,* 2002] stated that when glucose was substituted by fructose in 30 g/L concentrations, all the actinomycins antibiotic concentrations produced by the three tested species of *Streptomyces*, *S. parvulus*, *S. felleus* and *S. regensis*, were higher than with glucose. [Bandi *et al.,* 2003] found that effective neomycin production by *Streptomyces marinensis* (6920 mg/L) was achieved with maltose. [Sultan *et al.,* 2002] reported that glycerol as a carbon source was the most suitable for large scale production of antibiotic by *Streptomyces* Species. [Ramadan, 2000] showed that glucose was the most adequate carbon source followed by starch for antibiotic production. [Mellouli *et al.,* 2003] reported that Antibiotic production was only observed when starch was used as carbon source.

The use of unsuitable amino acids as a nitrogen source can inhibit the biosynthesis of

secondary metabolites [Ahronowitz, 1980 and Martain and Demain, 1980]. Conversely, specific amino acids can, in some cases, enhanced antibiotic production.

The best nitrogen source for the biosynthesis of antimicrobial agent(s) produced by Streptomyces *crystallinus,* AZ-A151 were obtained at $NaNO_3$. Similar results were obtained by other workers on other metabolites produced by other microorganisms, e.g.: [Atta *et al.,* 2011].

Sources of nitrogen were important for the production of antibiotic by microorganisms. [Hobbs *et al.,* 1990] reported that the carbon and nitrogen sources affected actinorhodin production by *S. coelicolor*. Similarly, growth and pristinamycin production in *Streptomyces pristinaespiralis* had been recorded to be governed by nitrogen sources [Francois and Stephane, 2001]. In *Streptomyces clavuligerus*, amino nitrogen as well as urea support cephalosporin production [Aharonowitz and Demain, 1979]. Optimization of cultural conditions for antibiotic production had also been attempted [Haque *et al.,* 1995] in *Streptomyces antibioticus* Sr15.4 and *S. californicus* JCM6910. However, [Lee and Hwang, 2002] reported that inoganic nitrogen sources played an important role in determining the production profile of rifamycin B with KNO_3 showing a positive influence on antibiotic production. [Vasavada *et al.,* 2006] reported that *S. kananmyceticus* M27 yielded maximum antibiotic production with sodium nitrate. [Farid *et al.,* 2000] ammonium sulphate, sodium nitrate was the suitable nitrogen sources in supporting the antibiotic production.

In the present data the favorable level of K_2HPO_4 for antimicrobial agent(s) production was 0.8g/l in case of *Streptomyces crystallinus*. AZ-A151. The antimicrobial agent(s) production was decreased by increasing concentration of K_2HPO_4. These results agree with [El-Tayeb *et al.,* 2004b] who stated that, increasing the concentration of KH_2PO_4 above 0.1% caused a marked decrease in rifamycin B production (36- 45%), while total elimination of KH_2PO_4 caused only 12% decreased. [Sujatha *et al.,* 2004] also reported that K_2HPO_4 at a concentration of 1.2 g/l gave maximum yield of antibiotic.

The final consideration in terms of basic media composition for secondary metabolic production was which trace elements to add [Weinberg, 1970]. The present results indicated that the optimal level of $MgSO_4.7H_2O$ for antibiotic production was 0.7 g/l in case of *Streptomyces crystallinus,* AZ-A151. The optimal level of vitamins for antimicrobial agent(s) biosynthesis was 200 ppm from vitamin H at shaking speed 160 r.p.m in case of *Streptomyces crystallinus,* AZ- A151. These results agree with [Hassan *et al.,* 2001] who showed that addition of 0.5g/l magnesium sulphate to the culture medium was optimal for the production of a maximum yield of antibiotic by *S. violatus*. [Sujatha *et al.,* 2004] also showed that the addition of 0.5 g/l of magnesium sulfate to the culture medium was optimal for antibiotic production.

5. Conclusion

Growth pattern and antimicrobial profile of *Streptomyces crystallinus,* AZ-A151 were studied on Starch Nitrate (SN) broth medium. An attempt has been made to evaluate the optimal cultural conditions for obtaining high yields of bioactive metabolites against *Staphylococcus aureus*, NCTC 7447; *Escherichia coli*, NCTC 10416; *Klebsiella pneumonia*, NCIMB 9111; *Salmonella typhi*; *Saccharomyces cerevisiae,* ATCC 9763; *Aspergillus flavus*, IMI 111023; *Alternaria alternate* and *Fusarium verticillioides*.

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7. References

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