ISSN 2229 – 6824

IJPI's Journal of Biotechnology and Biotherapeutics

Visit

www.ijpijournals.com

Optimization of a Cultural Medium for Antifungal Antibiotic Production by Strain no.10/2 (*S. albovinaceus*)

Thaer Abdelghani*[1], Ellaiah Poluri[2]

[1] Department of Medical Lab Sciences, Faculty of Allied Medical Sciences, Arab American University, P.O. Box 240, Jenin, PALESTINE.

[2] College of Pharmaceutical Sciences, Andhra University, Visakhapatnam-530003 A.P., INDIA

***Corresponding Author:** Thaer Abdelghani

ABSTRACT:

A broad antifungal and antibacterial activity of a new actinomycete (*S. albovinaceus*) strain no.10/2 was isolated from soil sample of Palestine and characterized. The medium composition for antifungal production for strain no.10/2 (*S. albovinaceus*) was optimized using shake-flask methodology. 1.25 % mannitol and 1 % malt extract were found to be best carbon and nitrogen sources respectively for growth and antifungal production. Similarly initial pH of 7.2, 10 % level of inoculum, incubation period of 96 h, and agitation in 250 ml bottom indented flask and medium to flask ratio of 1:10 (aeration) were found to be optimal. Optimization of medium and cultural conditions resulted in good increase in antifungal yield.

Key words*:* Optimization, *Streptomyces, S. albovinaceus*, antifungal.

1. INTRODUCTION

The new drug discovery processes have proved that novel skeletons of drugs come from natural sources in majority of cases[1]. This involves the screening of microorganism and plant extracts[2]. Microbial production of antibiotics is one of the rapidly expanding branches of industrial microbiology. The exploration of new habitats plays a pivotal role in search of new microbes possessing potentials to produce novel metabolites, and is urgent to counter the threats posed by the fast emerging phenomenon of antibiotic resistance[3]. Antibiotic production is predominantly a feature of soil fungi and bacteria. Filamentous soil bacteria belonging to the genus Streptomyces are widely recognized as industrially important microorganisms because of their ability to produce many kinds of novel secondary metabolites including antibiotics[4]. Different Streptomyces species produce about 75% of commercially and medically useful antibiotics. In the course of screening for new antibiotics, several studies are oriented towards isolation of new Streptomyces species from different habitats[5]. The genus Streptomyces has received considerable attention especially in view of its importance as a source of several secondary metabolites of industrial interest, in particular antibiotics. The continuing success of microbiologists in the search among microbial metabolites for use as antibiotics in combating human, animal and plant diseases has stimulated the belief that microorganisms constitute an inexhaustible reservoir of interesting compounds[6]. Several researchers have already reported antimicrobial activity of *Streptomyces* against fungal pathogens[7,8]. Waksman[9] has recognized some natural substrates as ideal sources for the isolation of actinomycetes, of which soil was found to be the richest source. Different soils all over the world had been exploited in search of bioactive actinomycetes. Influence of particular nutrients on the antibiotic biosynthesis is determined by the chemical structures of antibiotic substances. The choice of medium is virtually as important to the success of an industrial fermentation as the selection of organism to carry out the fermentation[10]. The search for new drugs against fungal infections is a major challenge to current research in mycotic diseases. In recent years the literature on antifungal antibiotics has been adequately reviewed[11,12]. The current efforts to find new antibiotics are faced with the difficulty that the probability of discovering them is declining as more and more natural substrates have been exploited, especially in the last 30 years when antibiotics research and actinomycetes screening was at the peak.

There is a need for the development of new antibiotics to overcome the problems associated with the existing antibiotics. The screening programs for new actinomycetes and for their antibiotics are still proceeding at a very rapid pace. To discover the new antibiotics it will be necessary to continue the use of conventional screening programs. So far, few reports appeared in literature about production of antifungal antibiotic from microorganisms isolated from soil samples of Palestine. As such, it was proposed to carry out the investigation of different soil samples of Palestine and to optimize fermentation conditions for antifungal production. This paper deals with the effect of medium composition on antifungal production by strain of *S. albovinaceus* (10/2) in batch cultures. The aim of the present work is to increase volumetric production and yield of the antifungal by selecting the suitable medium components.

2. MATERIAL AND METHOD

2.1 Microorganism and growth conditions:

Producer strain was isolated from a soil sample of Palestine. The strain was characterized at the College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, India. The strain was identified as a strain no.10/2 (*S. albovinaceus*) with broad antifungal and antibacterial activities[13]. Stock cultures were maintained on yeast extract malt extract (YEME) agar medium and incubating at 28 ºC and stored at 4 ºC. Glycerol was added to YEME broth at a final concentration of 15% (v/v) and stored at –20 ºC for long storage.

2.2 Media and fermentation conditions:

The inoculation as well as the production media was they are the same and contained % (w/v): dextrose 0.1,

potato starch 2.4, yeast extract 0.5, meat extract 0.3, tryptone 0.5, calcium carbonate 0.2, pH was adjusted to 7.0 before sterilization[24]. The inoculums culture was prepared by inoculating one full slant culture into 50 ml of inoculum medium and incubated on rotary shaker (220 rpm) at 28 ºC for 48 h. The fermentation medium was inoculated with 10 % level of 48 h inoculum and incubated on rotary shaker (220 rpm) at 28 ºC for 96 h.

2.3 Media optimization:

The literature indicated that yield is not only dependent on the nature of the strain and composition of medium but also on the cultural conditions[14]. Nutritional manipulation enhances the positive regulatory mechanisms of the production strains during cultivation[15]. To determine the optimal nutritional and cultural conditions for growth and antibiotic production, our production medium was used as the base. Also different parameters like selection of a suitable inoculum medium, selection of a suitable production medium, and level of inoculums were used to study their effect on growth and antibiotic production. It was supplemented with different carbon and nitrogen sources with different concentrations to study their effect on growth and antibiotic production. The effect of cultural conditions like different initial pH, incubation period, aeration, agitation, on growth and antibiotic production was studied. Finally production of antifungal with the optimum conditions (using modified production medium and cultural conditions formulated based on the above observations) was studied.

2.4 Antifungal activity:

Antifungal activity from culture broth was assayed against *Candida albicans*. It was performed by withdrawing samples at the end of the fermentation cycle (96 h), centrifuged, and the supernatant was assayed for extracellular antifungal activity by standard cup plate method

3. RESULTS

Effect of inoculum medium on antifungal production

In order to minimize the time lag in fermentation process, inocula are raised in media with a composition similar to that of fermentation medium. Three different inoculum media IM1, IM2 and IM3 which are similar to the selected production medium were tested. The results are presented in Fig. 1. Inoculum medium (IM3) gave maximum antifungal yield; so it was used in further studies as the inoculum medium.

Effect of production medium on antifungal production

Antifungal production of the isolate was carried out in 8 different production media PM1 to PM8. This was done in order to select the best production medium for maximum antifungal. The results are shown in Fig. 2. Production medium (PM3) gave maximum antifungal production. Hence it was selected for further optimization studies for antifungal production. It was shown that the inoculation as well as the production media used was the same and the composition is as mentioned before in materials and methods.

Suitability of different carbon sources for antifungal production

For maximum metabolite production different carbon sources were supplemented to the production medium to study their effect on antifungal production. This includes glucose, lactose, sucrose, fructose, maltose, mannitol, Jowar starch, soluble starch, potato starch, and control (without carbon source). Each of the carbon sources was incorporated at 1% level into the basal medium PM_3 in place of potato starch. The results were shown in Fig. 3. Mannitol was found to be the best carbon source for maximum antifungal production followed by maltose. So, mannitol was selected for further optimization studies for antifungal production. The cultivation medium supplemented with mannitol was thus employed in all further experiments.

Antifungal production with different concentrations of mannitol

Earlier experiment has shown that mannitol is the best carbon source for antifungal production. It was thought, therefore, to test whether antifungal production could be improved by varying mannitol concentration in the cultivation medium. For this purpose mannitol was applied in different concentrations varied from 0.25 to 3 % (w/v).The results are shown in Fig. 4. Mannitol at a concentration of 1.25 % (w/v) gave maximum antifungal yield. Therefore, mannitol at a concentration of 1.25 % (w/v) was used in the subsequent experiments.

Suitability of different nitrogen sources for antifungal production

Different nitorgen sources at a concentration of 1 %, organic (peptone, yeast extract, malt extract, meat extract, casein, tryptone, corn steep liquor, and urea), inorganic (sodium nitrate and ammonium sulphate), and control (without nitrogen source) were tested to study their effect on antibiotic production. The results are presented in Fig. 5. Nitrogen source exhibited a significant effect on the antifungal production. The best nitrogen source for supporting antifungal production was malt extract.

Antifungal production with different concentrations of malt extract

Earlier experiment has shown that malt extract is the best nitrogen source for antifungal production. It was thought, therefore, to test whether antifungal production could be improved by varying malt extract concentration in the cultivation medium. For this purpose malt extract was applied in different concentrations varied from 0.25 to 2 % (w/v).The results are shown in Fig. 6. Malt extract at a concentration of 1.0 % (w/v) gave maximum antifungal yield. Therefore, malt extract at a concentration of 1.0 % (w/v) was used in the subsequent experiments.

Influence of initial pH on antifungal production

In order to study the effect of pH on antifungal production, production media with different initial pH varied from 5.5 to 8.5 were studied. The results are shown in Fig. 7. The yield of antifungal was maximum when medium with an initial pH 7.2 used.

Influence of inoculum level on antifungal production

The effect of inoculum level on the antifungal production was studied. Different inocula levels varied from 2.5 to 15 % (v/v) were studied. The results are presented in Table 1. Inoculum level of 10 % yielded maximum antifungal production.

Influence of incubation period on antifungal production

The effect of incubation period on antifungal production was studied. Different incubation periods varied from 12 to 132 h were studied. The results are presented in Fig. 8. The yield of antifungal was maximum when an incubation period of 96 h was used.

Effect of aeration on antifungal production

The effect of aeration on antifungal production was studied. Different volumes of production medium varied from 25 to 100 ml in 250 ml EM flasks (v/v) were studied. The results are presented in Table 2. The yield of antifungal was maximum when 25 ml production medium in 250 ml EM flask was used.

Effect of agitation on antifungal production

In order to estimate the effect of agitation on antifungal production, different types of flasks with different capacities were tried. The results are presented in Table 3. The yield of antifungal was maximum when fermentation was carried out in 250 ml bottom indented flask.

Production of antifungal with optimum conditions

Finally, the antibiotic production was tested employing the modified medium and optimized cultural conditions. The results are presented in Fig. 9. Significant improvement in the antifungal titer was observed with the modified production medium for antifungal activity.

Figure 1: Effect of inoculum medium on antifungal production

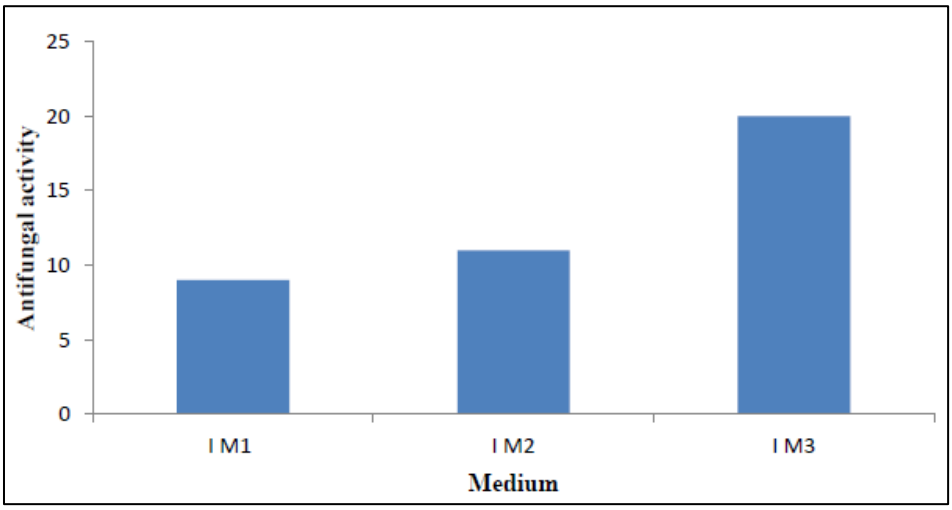


Figure 2: Effect of production medium on antifungal production

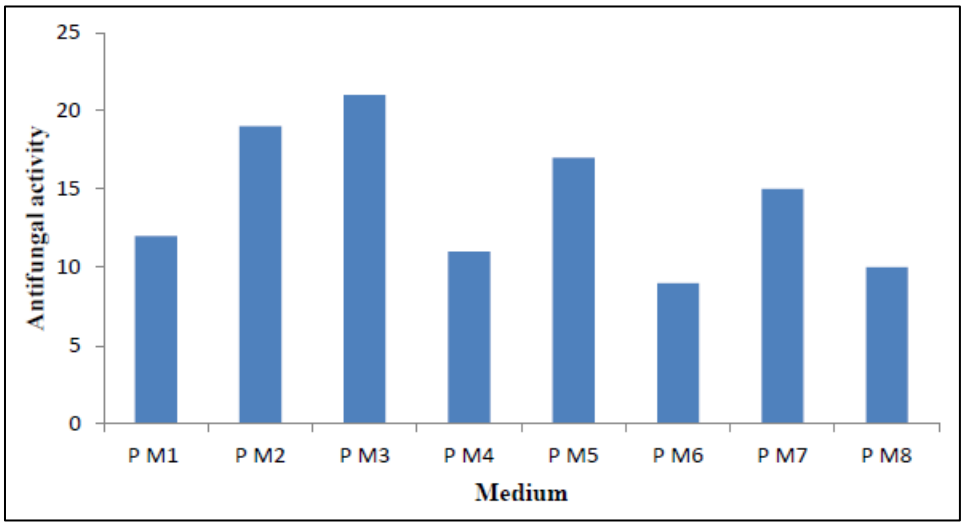


Figure 3: Suitability of different carbon sources for antifungal production

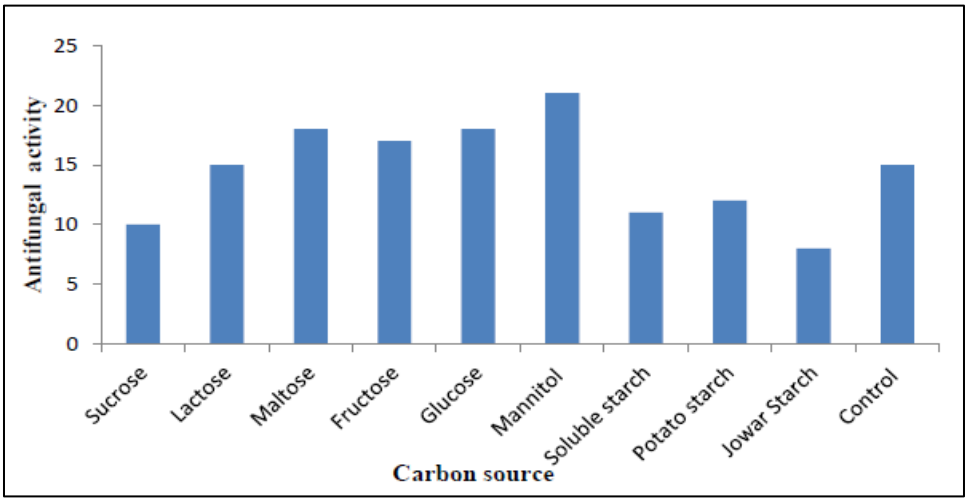


Figure 4: Antifungal production with different concentrations of mannitol

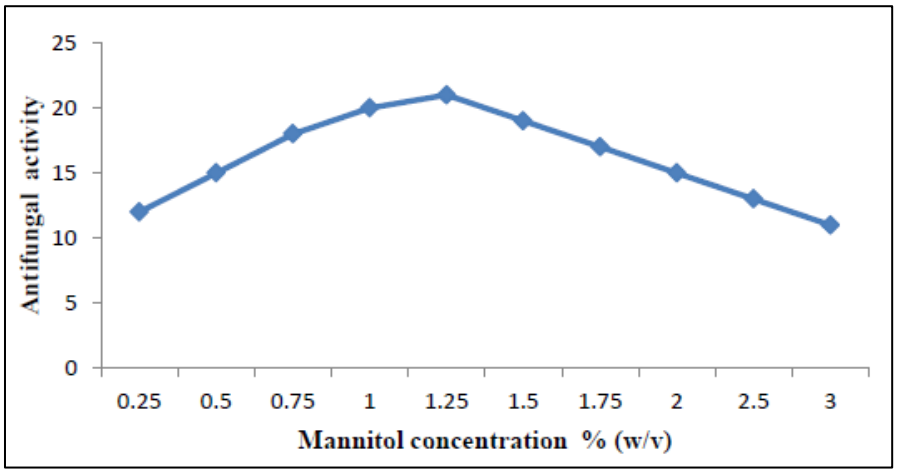


Figure 5: Suitability of different nitrogen sources for antifungal production

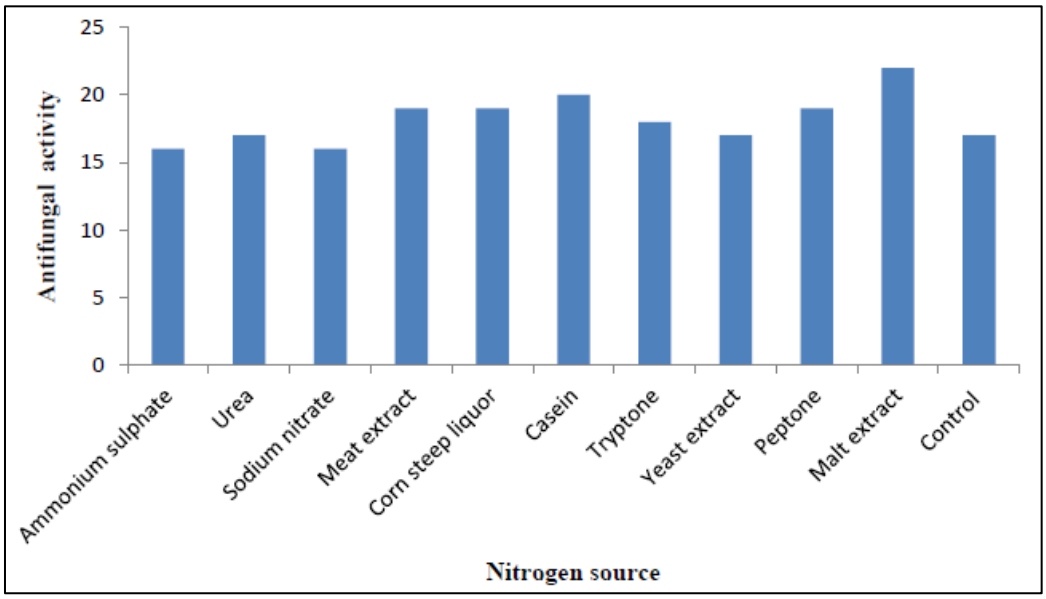


Figure 6: Antifungal production with different concentrations of malt extract

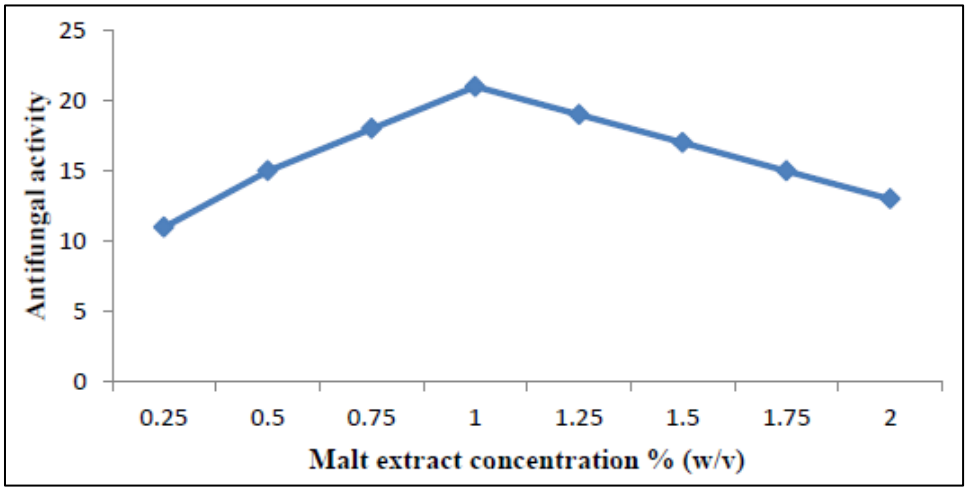


Figure 7: Influence of initial pH on antifungal production

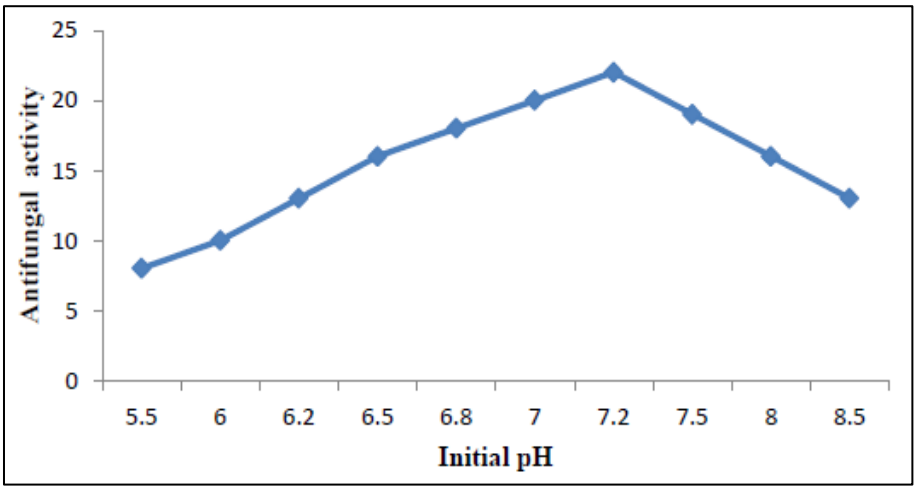


Table 1: Influence of inoculum level on antifungal production

Level of inoculum in % (v/v)	Antifungal activity
	Inhibition zone diameter (mm)
2.5%	13
5.0%	16
10.0%	**22**
15.0%	17

Figure 8: Influence of incubation period on antifungal production

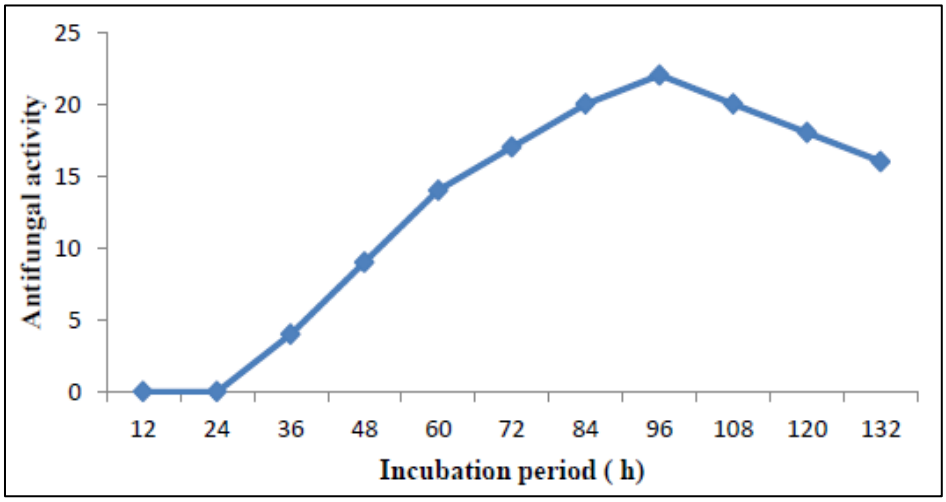


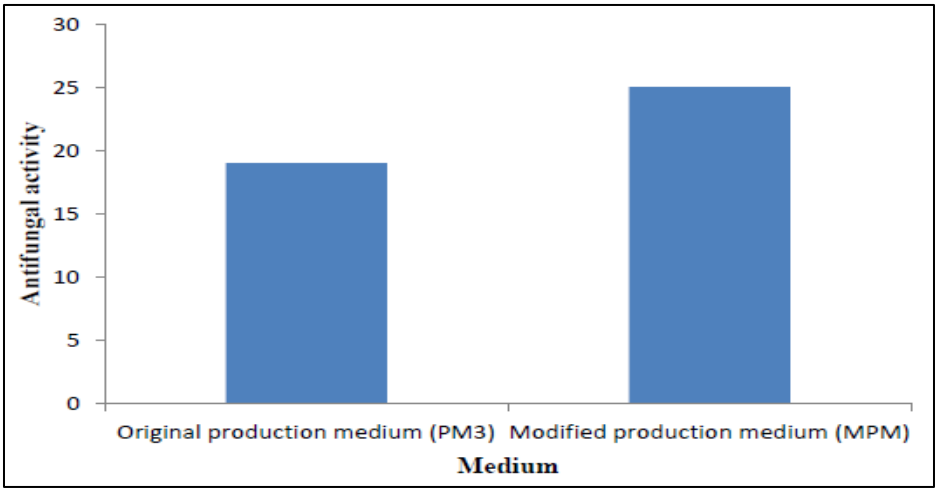
Table 2: Effect of aeration on antifungal production

Volume of medium (ml)	Antifungal activity
	Inhibition zone diameter (mm)
25	**22**
50	19
75	16
100	13

Table 3: Effect of agitation on antifungal production

Type of flask	Antifungal activity
	Inhibition zone diameter (mm)
Un-indented	
250 ml	19
500 ml	13
Side indented	
250 ml	20
500 ml	15
Bottom indented	
250 ml	**23**
500 ml	16

Figure 9: Production of antifungal with optimum conditions



4. DISCUSION

The choice of medium is virtually as important to the success of an industrial fermentation as the selection of organism to carry out the fermentation[16]. The medium requires the selection of carbon, nitrogen and inorganic salts, as well as energy sources that will support not only good microbial growth but also maximize the product yield, minimize the synthesis of compounds closely related to the product, and enhance product recovery[15,16,17,18,19].

Actinomycetes perform significant biogeochemical roles in nature and are highly valued for their unparallel ability to produce wide variety of biologically active secondary metabolites[20,21]. Actinomycetes are able to utilize a great variety of organic compounds as sources of energy[22,23]. Influence of particular nutrients on the antibiotic biosynthesis is determined by the chemical structures of antibiotic substances. Medium composition plays a critical role in both volumetric and specific antibiotic production which is reflected directly on the process economics[17].

It was indicated that antibiotic synthesis is dependence on the medium constituents[5]. Variation in fermentation environment like cultural characteristic and media composition often resulted in change in antibiotic production in terms of either the yield or composition of compounds[24]. Production of antifungal metabolite has been known to be influenced by media components and cultural conditions, such as aeration, agitation, pH, temperature and glycerol concentration, which vary from organism to organism[25,26]. Also environmental factors like incubation temperature, pH and incubation time were found to have profound influence on antibiotic production[5,6,27].

McDaniel[28] and his colleagues have successfully attempted a statistical approach using a composite experimental design, with analysis and response surface plotting by computer, for optimizing nutrient parameters. The production of an antifungal antibiotic from *Thermomonospora* spMTCC3340 was reported for the first time using a full factorial method[10]. A study of antifungal antibiotic production by *Streptomyces chattanoogensis* MTCC 3423 using full factorial has been reported[12].

Growth and antibiotic production on synthetic media were found to be unsatisfactory[29]. Consequently we used a complex medium with organic nitrogen source, which supports both, growth and antifungal productivity. The effects of certain nutrients on antifungal production by *S. albovinaceus* strain no.10/2 in submerged batch culture were studied.

The growth medium plays a very important role in the production of microbial metabolites under different conditions. Culture medium is significantly modulates the production of antifungal metabolites[30,31]. The nutritional sources like carbon and nitrogen, as well as the environmental factors such as incubation period, pH and temperature are known to have a profound effect on antibiotic production by actinomycetes[32]. Optimization of culture conditions is essential to get high yields of the antimicrobial metabolites. Hence, the present study described the optimization of culture conditions for the production of antifungal metabolites by *S. albovinaceus* strain no.10/2. The inoculum medium as well as the production medium used in our study was the same (Fig.1 and 2). This gave maximum antifungal production. This may be due to decrease of lag time when we use the same inoculum as well as the production medium.

Generally a quickly metabolized substance like glucose is responsible for catabolism repression but in some cases it is also reported to enhance antifungal metabolite production[33,34]. Some of the nutrients, e.g., glucose, maltose, dextrin, starch, glycerol, amino acids and proteins, are consumed very readily, and in fact they are the best sources of carbon. Sucrose, xylose, raffinose, and certain other sugars, sugar alcohols, and sugar acids are utilized less readily, but more readily by some actinomycetes than by others[22,23]. Production of antibiotics occurs during a distinct idiophase of culture growth. Increase in biomass production was not necessarily correlated with the increase in antifungal / antibacterial activity, rather in several cases an increase in biomass production showed decreased antimicrobial activity[15]. It was shown that increased antifungal activity of the fungus in media containing a simple sugar, like glucose plus a slow releasing carbon source, like malt extract can be explained by the high production rate of secondary metabolites when their producing organisms grow in complex media[35]. Various mono-carbohydrates, di-saccharides and polysaccharides (all used in equal concentrations of 1%) were tested in growth experiments for their ability to support antifungal production by strain no.10/2 (*S. albovinaceus*). The results are given in (Fig. 3). The microorganism was able to grow in all the tested carbon sources. In case of disaccharides, the production of antifungal was less than mono-carbohydrates but more than polysaccharide. Among the wide variety of carbon sources tested, mannitol, followed by maltose and then fructose proved to be the most suitable for antifungal production. Mannitol is commonly formed via the hydrogenation of fructose which may be explaining the comparison result. The highest titers of antifungal compounds and high yields of biomass were obtained when mannitol was added to the production medium. The cultivation medium supplemented with mannitol was thus employed in all further experiments. Mohamed et al.[17], showed that mono - sugars supported the growth of microorganism and increased the cell growth to about 4 folds or more compared to control (medium without carbohydrate). On the other hand, all polysaccharide carbohydrates supported only cell growth. It was also shown that growth and antifungal activity were also good in media containing fructose, maltose, malt extract and molasses[36]. In contrast activity and growth of the fungus was very low when sucrose and starch were used as carbon source. These results are in agreement with our results. Several researchers showed that the best carbon source for maximum antifungal activity was glucose[10,12,37]. The antifungal activity was increased with incorporation of D+ xylose and L-hydroxyproline in the production medium[15].

Earlier experiment has shown that mannitol is the best carbon source for antifungal production. The effect of various concentrations of mannitol, varied from 0.25 % to 3.0 % on antifungal production was studied. The results are shown in (Fig. 4). The results showed that both volumetric and specific production of antifungal continues to increase

and reached a maximal value at 12.5 g/l, above this concentration, the antifungal production decreased. As mannitol concentration increased from 1.25 % to 30 % the antifungal production decreased. On the other hand, mannitol was completely consumed when used at a concentration of 1.25 % or lower. The increase in mannitol concentration above this level resulted in the accumulation of mannitol in the cultivation medium and the remained amount depended on the initial concentration. Therefore, mannitol in a concentration of 12.5 g/l was used in the subsequent experiments. The effect of carbon source on growth and antibiotic production is dependent upon several factors such as carbon concentration[38]. In this connection, Zhu et al.[39] studied the effects of glucose concentration on avilamycin biosynthesis in *S. viridochromogenes* and found that high concentrations of glucose led to the absence of the precursors for avilamycin biosynthesis and affected antibiotic synthesis. In some microorganisms, the inhibitory effect of glucose has been related to a decrease in pH[40]. Maltose at 1% (w/v) was more suitable for antibiotic production and activity than other concentration; this phenomenon was introduced by Inoue *et al*., [41]. Glucose at 1% (w/v) gave maximum antibiotic production[40,42]. It was showed that the antifungal activity was maximum at 2 %[10,15]. High concentration of glucose is generally considered as repressor of secondary metabolisms and maximum cell growth rates can inhibit antimicrobial agent production[43].

The activity of the antibiotic also varied with changes in nitrogen source[29,44]. Nitrogen source exhibited a significant effect on the antibiotic production[17,45]. Various nitrogen sources, organic and inorganic were tested to study their ability to support antifungal production. The results are shown in (Fig. 5). The microorganism was able to grow on all nitrogen sources tested. Among the wide variety of nitrogen sources tested, malt extract followed by meat extract proved to be the most suitable for antifungal production. The production of antifungal was less with inorganic nitrogen sources compared with organic sources. These results are in agreement with the results of other investigators[17,26,29,36]. In contrast, Sujatha[5] indicated that the highest antibiotic production was obtained in culture of isolate BT-408 containing ammonium nitrate as a nitrogen source, followed by cultures containing sodium nitrate, potassium nitrate and alanine. Also low activity was observed with malt extract, potassium nitrate, sodium nitrate, urea[45]. Lian-Xiang D[44] showed that the highest antibiotic production was achieved in the medium containing CSL. Mohamed et al[17] showed that the best nitrogen source for supporting antibiotic production of natamycin was beef extract. Soybean meal was found to be a best nitrogen source for supporting antifungal production[12,46]. It is well known that changes in the kind and concentration of nitrogen source influence greatly antibiotic production[45].The results also showed that the concentration of malt extract (Fig. 6) greatly influenced the production of the antifungal with maximum antifungal yield being obtained in cultures supplemented with 1% of malt extract. These results are in agreement with the results of other investigators[12,47]. Sujatha[5] showed that the best concentration of ammonium nitrate for maximum antibiotic yield being obtained in cultures supplemented with 2.5 g/l of ammonium nitrate.

The influence of medium composition, incubation temperatures, and initial pH on microbial growth and antibiotic production was also reported in various *Streptomyces* strains[48,49]. The production and activity of antimicrobial substance in LAB may vary at different pH levels. The culture pH and temperature affect the antifungal activity and growth of the fungus[36,50]. Changes in external pH affect many cellular processes such as the regulation of the biosynthesis of secondary metabolites. The change in pH of the culture medium induces production of new substances that affect antibiotic production. The importance of pH for antifungal production by *Streptomyces* was reported by several investigators who observed that the optimum pH for antibiotic production range between 7.0 and 7.5[15,37,50]. The yield of antifungal was maximum when pH was maintained at 7.2 (Fig. 7). This is in agreement with the results obtained by Sujatha et al.[5]. In contrast, it was showed that maximum antifungal antibiotics production was obtained at acidic pH[6,51,52]. It has been reported that maximum production of bacitracin and other antibiotics occurred at alkaline pH[53]. Increasing the medium pH led to an increase in the antifungal production up to a certain limit above which any increase in the pH value was accompanied by a decrease in the antifungal production and activity[54].

Data showed that there is a relation between antibiotic productivity and different inoculums sizes[55]. The inoculum age and density markedly influence the productivity and economics of bioprocesses[56]. The maximum yield of

antifungal by our isolate was obtained when inoculum level of 10 % was used (Table 1). Our result is in agreement with the results obtained by other investigators[51,54] .Chao-Min L et al. [54] indicated that candicidin production in SPC medium was dependent on the amount of mycelium inoculated into the production medium. The larger the inoculums (washed mycelia), the higher the yield after 5 days of fermentation. In contrast, it was shown that in preliminary experiments revealed an optimum inoculums size of 5.5 %[57]. It was obvious that higher spore content led to the production of more hyphae in the early stages of incubation. These hyphae entangled and prevented the formation of pellets. With low spore concentrations, pellet size increased and overall biomass production decreased. This led to a reduction in antibiotic production. This occurred in our experiment after 10 % level of inoculums.

As mentioned earlier the environmental factors like incubation temperature, pH and incubation period were also have profound influence on growth and antibiotic production as surveyed in *Streptomycetes* species[27]. Incubation period and temperature are essential factors that modulate LAB growth and significantly affect the amounts of antifungal metabolites produced. The condition of incubation influenced quantitatively the biosynthesis of antibiotics as well as biomass reported by Al Zahrani[58]. Studies carried out by some investigators revealed that maximal production of antifungal substances occurred after 96 h[5,50,52]. This is in agreement with our results (Fig. 8). In contrast, the highest biomass and antifungal activity was observed at an incubation time of 72 h by some other investigators[59]. In our case maximum antimicrobial metabolite production was took place at late log phase indicating that metabolite production was directly proportional to the growth rate. It is reported that antibiotic production usually occurs in stationary Phase[60]. Maximum production of metabolite was achieved in late log phase, indicating that the metabolite production was directly proportional to the growth rate which remained constant during stationery phase[31]. Nutrient deficiency is responsible for onset of antibiotic biosynthesis. Production of antibiotics occurs during a distinct idiophase of culture growth[15]. When carbon or nitrogen source is a limiting factor, growth is rapidly reduced and antibiotic biosynthesis takes place in the stationary phase. In other cases, antibiotic production is associated with the growth phase[29]. The maximum biosynthesis was achieved at the end of an incubation period of 5 days for the antifungal agent production by actinomycete culture[31,55]. The production of the antifungal agent was maximum on the 5th day then decreased gradually till the 10th day of incubation[61]. The antifungal production declined indicating its accumulation after a certain period of streptomycetal growth.

In industrially important antibiotic fermentations dissolved oxygen (DO) was found to be an important parameter[62]. Increased levels of dissolved oxygen have lead to enhanced antibiotics production[63]. Agitation affects aeration and mixing of the nutrients in the fermentation medium[31]. Adequate agitation was found to increase antibiotic metabolite production[64]. Chao-Min L et al, [54] showed that oxygen absorption rates (OAR) affected both mycelial growth and candicidin synthesis. Yegneswarant et al, [62] indicated that the most effective control strategy was to control DO only during active growth when the biosynthetic enzymes were probably synthesized. Also they showed that increase in the final cephamycin yield was observed when dissolved oxygen was controlled at saturation levels during the growth phase, compared to the experiments without dissolved oxygen control. Lian-Xiang D et al,[44] indicated that improvement of dissolved oxygen tension was favorable for antibiotic production and pellets formation. Several techniques have been used to control DO in fermentations, the most common being the use of agitation speed and the aeration rate to the fermentor[62]. König B et al, [65] studied the complicated interrelations between the stirrer speed, the stirrer type, the shear stress, the morphology of the mycelium and broth viscosity as well as the effect of the oxygen transfer behavior on antibiotic productivity[65]. The main variable investigated was the stirrer speed. At low stirrer speeds, gas dispersion is inadequate and the insufficient oxygen transfer rate is a limiting factor. At higher stirrer speeds, the oxygen supply of pulpy mycelia is improved and more cell mass is formed. The DO concentration in combination with air flow rate affected the pattern of the antibiotics produced. At high DO levels, an additional macrolide antibiotic, macrocin, was synthesized to more than one-third the amount of tylosin at high aeration rate[66]. Shiru J et al.[46], showed that, soybean oil can significantly enhance oxygen transfer coefficient which lead to increase in the tetracycline production. Malcolm et al.,[67] indicated that the improved oxygen availability affected antibiotic

production both by increasing the rate of specific antibiotic biosynthesis and by maintaining this higher rate throughout the production period. This is in agreement with our results. A 25 ml production medium in 250 ml flask gave maximum inhibition zone for the antifungal activity (Table 2). Also fermentation when carried out in 250 ml bottom indented flask gave maximum antifungal activity (Table 3).

Variation in fermentation environment like cultural characteristic and media composition often resulted in change in antibiotic production in terms of either the yield or composition of compounds[24]. Optimization of medium composition allowed a significant increase in antibiotic production[17]. The production of an antifungal antibiotic from *S. albovinaceus* strain no.10/2 is being reported for the first time. Our results revealed that optimization of medium composition allowed a significant increase in antifungal production by *S. albovinaceus* strain no.10/2. The highest concentration of antifungal activity was produced under nutritional conditions when mannitol was used as a carbon source at 1.25 % and supplemented the medium with malt extract at 1 %. Also, initial pH of 7.2, 10 % level of inoculum, incubation period of 96 h, and agitation in 250 ml bottom indented flask and medium to flask ratio of 1:10 gave maximum antifungal production. In conclusion, it is suggested that the improved medium gave enhancement of antifungal activity when compared with that of the basal fermentation medium (Fig. 9).

Further works on isolate no (10/2) like anticancer activity, purification of the active principle, toxicology and commercial viability study are under progress.

5. REFERENCES

(1) Fernando Pela´ ez. 2006. The historical delivery of antibiotics from microbial natural products - Can history repeat? *Biochemical Pharmacology*. 71, 1981 – 990.

(2) Shadomy S. 1987. Preclinical evaluation of antifungal agent. In: R.A. Fromtling (Ed.). Recent Trends in the Discovery, Developments and Evaluation of Antifungal Agents. Prous Science, New Jersey. pp. 8-14.

(3) Shiburaj S. 2003. Screening, isolation and characterization of an antibiotic producing Actinomycete *Streptomyces setonii* 19NRA1 (Ph.D thesis), University of Kerala.

(4) Pamela Wiener. 2000. Antibiotic production in a spatially structured environment. *Ecology Letters*. 3, 122-130.

(5) Sujatha P et al. 2005. Studies on a new marine streptomycete BT-408 producing polyketide antibiotic SBR-22 effective against methicillin resistant *Staphylococcus aureus. Microbiological Research.* 160, 119 - 126.

(6) Venkata Dasu V and Panda T. 1999. Studies on production of griseofulvin. *Bioprocess Engineering*. 21, 489 – 495.

(7) Bharti A et al. 2010. Antifungal Activity of Actinomycetes isolated from Garhwal Region. *Sci Eng Technol Manag*. 2(2), 3-9.

(8) Atta HM. 2009. An Antifungal Agent Produced by *Streptomyces olivaceiscleroticus*, AZ-SH514. *World Appl Sci*. 6(11), 1495-1505.

(9) Waksman SA. 1959. The Actinomycetes: Nature, Occurrence and Activities (Isolation, identification, cultivation and preservation.). Williams & Wilkins Co., Baltimore, Vol. 1. pp 17 - 28.

(10) Gupte MD and Kulkarni PR. 2003. A study of antifungal antibiotic production by *Thermomonospora* sp MTCC 3340 using full factorial design. *J Chem Technol Biotechnol.* 78, 605 - 610.

(11) Khan ZK and Gyanchandani A.1998. Trends in antifungals: past, present and future, in *Antifungal Agents Past, Present and Future Prospect*, ed by Varma RS, Khan ZK and Singh AP. National Academy of Chemistry and Biology, Lucknow, pp 55–128.

(12) Gupte MD and Kulkarni PR. 2002. A study of antifungal antibiotic production by *Streptomyces chattanoogensis*MTCC 3423 using full factorial design. *Lett Appl Microbiol.* 35, 22–26.

(13) Thaer Abdelghani et al. 2009. Antibacterial activity of bacterial isolates of soil bacteria collected from Palestine. *Current Trends in Biotechnology and Pharmacy*. 3 (2), 85-91.

(14) Thaer Abdelghani. 2011. Production of antibacterial metabolites by strain no.10/2 (*S.albovinaceus*) and media optimization studies for the maximum metabolite production. *IJPI'S Journal of Biotechnology and Biotherapeutics.* 1(5), 1- 11.
(15) Tripathi CKM et al. 2004. Production of antibacterial and antifungal metabolites by Streptomyces violaceusniger and media optimization studies for the maximum metabolite production. *Medicinal Chemistry Research*. 13(8/9), 790 – 799.
(16) Casida LE. 1987. Fermentation media, in *Industrial Microbiology*, ed by Casida LE. John Wiley and Sons, Inc, New York, USA, pp 117–135.
(17) Mohamed AF et al. 2000. Optimization of the cultivation medium for natamycin production by Streptomyces natalensis. *Journal of Basic Microbiology*. 40 (3), 157 – 166.
(18) Gresham R and Inamine E. 1986. Nutritional improvement of processes, in *Manual of Industrial Microbiology and Biotechnology*, ed by Demain AL and Solomon NA. American Society for Microbiology, Washington, USA, pp 41–48.
(19) Holmalahti J et al.1998. Production of dihydroabikoviromycin by Streptomyces anulatus: production parameters and chemical characterization of genotoxicity. *J Appl Microbiol*. 85, 61–68.
(20) Vastrad BM and Neelagund SE. 2011. Production and optimization of tetracycline by various strains of *Streptomyces* under solid state fermentation using pineapple peel as a novel substrate. *Recent Research in Science and Technology*. 3(2),1-8.
(21) Berdy J. 2005. Bioactive microbial metabolites. A personal view. *Journal of Antibiotic*. 58,1-26.
(22) Fuji N et al. 1997. UCE6, a new antitumor antibiotic with topoisomerase I-mediated DNA cleavage activity produced by actinomycetes: producing organism, fermentation, isolation and biological activity. *Journal of Antibiotics.* 50, 490 – 495.
(23) Lowe SE et al. 1997. The effect of carbon source, temperature and aeration on the production of Ascosteroside, a novel antifungal agent, by Ascotricha amphitricha. *Journal of Antibiotics*. 50, 412 – 417.
(24) Didomenico B. 1999. Novel antifungal drugs. Microbiol. 2, 509- 515.
(25) Iwai Y and Omura S. 1982. Culture conditions for screening of new antibiotics. *J Antibiot. 35*, 123-41.
(26) Yu J et al. 2008. Effect of liquid culture requirements on antifungal antibiotic production by *Streptomyces rimosus* MY02. *Bioresour Technol*. 99, 2087-2091.
(27) Srinivasan MC et al. 1991. Physiology and nutritional aspects of actinomycetes: an overview. *World J Microbiol Biotechnol*. 7, 171–184.
(28) McDaniel LE et al. 1976. Application of response surface optimization techniques to polyene macrolide fermentation studies in shake flask. *Dev Ind Microbiol.*17, 91–98.
(29) Gesheva V et al. 2005. Effects of nutrients on the production of AK- 111-81 macrolide antibiotic by *Streptomyces hygroscopicus*. *Microbiological Research*. 160, 243-248.
(30) Batish VK et al. 1990. Effect of nutritional factors on the production of antifungal substance by *Lactococcus lactis* biovar diacetylactis. Australian *Journal of Dairy Technology*. 45, 74-76.
(31) Augustine SK et al. 2005. Production of a growth dependent metabolite active against dermatophytes by *Streptomyces rochei* AK 39. *Indian J Med Res.* 121, 164-170.
(32) Himabindu M and Jetty A. 2006. Optimization of nutritional requirements for gentamicin production by *Micromonospora echinospora*. *Indian J Exp Biol*. 44, 842-848.
(33) Fukuda T et al. 2005. Phenatic acids A and B, new Potentiators of antifungal miconazole activity produced by *Streptomyces* sp. K03–0132. *J Antibiot*. 58, 252- 259.
(34) Reichenbach H et al.1988. Myxobacteria: A source of new antibiotics. *Trends Biotechnol*. 6, 115-21.
(35) Martin JF and Demain AL. 1980. Control of antibiotic biosynthesis. *Microbiol Rev*. 44 (2), 230-251.

(36) Vahidi H et al. 2004. Effect of cultivation conditions on growth and antifungal activity of *Mycena leptocephala*. *African Journal of Biotechnology*. 3 (11), 606-609.
(37) Kumar S and Kannabiran K. 2010. Diversity and Optimization of process parameters for the growth of *Streptomyces* VITSVK9 spp. isolated from Bay of Bengal, India. *Journal of Natural & Environmental Sciences*. 1(2), 56-65.
(38) Chen et al. 2008. Natamycin production by *Streptomyces gilvosporeus* based on statistical optimization. *J Agric Food Chem*. 56(13), 5057-5061.
(39) Zhu et al. 2007. Regulation of avilamycin biosynthesis in *Streptomyces viridochromogenes*: effects of glucose, ammonium ion, and inorganic phosphate. *Appl Microbial Biotech*.73, 1031-1038.
(40) Espeso EA et al. 1993. PH regulation is a major determinant in expression of a fungal penicillin biosynthetic gene. *EMBO J*. 12, 3947-3956.
(41) Inoue S et al. 1982. Effect of phosphate and asparagin, on streptomycin formation by *Streptomyces griseus*. *J Ferment Technol*. 60(2), 105-110.
(42) Marwick JD et al. 1999. Bioprocess intensification for production of novel marine bacterial antibiotics through bioreactor operation and design. *Mar Biotechnol*.1, 495-507.
(43) Gallo M and Katz E. 1972. Regulation of secondary metabolite biosynthesis: Catabolite repression of phenoxazinone synthase and actinomycin formation by glucose. *J Bacteriol*. 109, 659-67.
(44) Lian-Xiang D et al. 2003. Morphological changes of *Rhizopus chinesis* 12 in submerged culture and its relationship with antibiotic production. *Process Biochemistry*. 38, 1643 -/1646.
(45) Neha S and Vibhuti R. 2012. Optimization of cultural parameters for antifungal and antibacterial metabolite from microbial isolate: Streptomyces rimosus MTCC 10792 from soil of Chhattisgarh. *International Journal of Pharmacy and Pharmaceutical Sciences*. 4 (4), 94-10.
(46) Shiru J et al. 1999. Effect of Soybean Oil on Oxygen Transfer in the Production of Tetracycline with an Airlift Bioreactor. *Journal of Bioscience and Bioengineering*. 87 (6), 825-827.
(47) Chan L et al. 2002. Optimization of a cultural medium for bacteriocin production by *Lactococcus lactis* using response surface methodology. *Journal of Biotechnology*. 93, 27–34.
(48) Banga, J et al. 2008. Studies on medium optimization for the production of antifungal and antibacterial antibiotics from a bioactive soil actinomycete. *Med Chem Res*. 17, 425–436.
(49) Sallam LAR et al. 2010. Some Physiological Factors Affecting Rapamycin Production by *Streptomyces hygroscopicus* ATCC 29253. *J American Sci*. 6, 188-194.
(50) Mustafa O.2009. Antifungal and antibacterial compounds from *Streptomyces* strains. *African Journal of Biotechnology*. 8 (13), 3007-3017.
(51) Jicheng Y et al. 2008. Effect of liquid culture requirements on antifungal antibiotic production by *Streptomyces rimosus* MY02. *Bioresource Technology*. 99 (6), 2087-2091.
(52) Reddy N S & Ranganathan B. 1985. Effect of time, temperature and pH on the growth and production of antimicrobial substance by Streptococcus lactis ssp diacetylactis Sl-67-C *Milchwissenshaft*.40, 346-348.
(53) Basilio A et al. 2003. Patterns of antimicrobial activities from soil actinomycetes isolated under different conditions of pH and salinity. *J Appl Microbiol*. 95, 814-23.
(54) Chao-Min L et al. 1975. Factors Affecting the Production of Candicidin. *Antimicrobial Agents Chemotherapy*. 7(2), 196-202.
(55) Houssam M et al. 2011. Studies on Isolation, Classification and Phylogenetic Characterization of antifungal substance produced by *Streptomyces albidoflavus*-143. *New York Science Journal*. 4(3), 40 -53.
(56) Ramkrishna S and Swaminathan T. 2004. Response surface modeling and optimization to elucidate and analyze the effects of inoculums age and size on surfactin production. *Biochemical Engineering Journal*. 21 (2), 141-148.

(57) Jia SJ et al. 2001. Study on the Fermentation Condition of the Antibiotic Substance Produced by Rhizopus Chinesis Sp. Yaowu Shengwu Jishu *Med Biotechnol*. 8(4), 213-/6.
(58) Al-Zahrani SHM. 2007. Studies on the antimicrobial activity of *Streptomyces* sp. isolated from Jazan. JKAU. 19, 127-138.
(59) Srinivasulu et al. 2002.Neomycin production with free and immobilized cells of *Streptomyces marinensis* in an airlift reactor. *Process Biochem*. 38, 593-598.
(60) Sahin N and Ugar A. 2003. Investigation of antimicrobial activity of some *Streptomyces* isolations. *Turk J Biol*. 27, 73-78.
(61) Amira A et al. 2011. Production, Purification and Characterization of the Antifungal Agent of *Streptomyces finlayi*. *Australian Journal of Basic and Applied Sciences*. 5(9), 549-558.
(62) Yegneswaran PK et al. 1991. Effect of Dissolved Oxygen Control on Growth and Antibiotic Production in Streptomyces clavuligerus Fermentations. *Biotechnology Progress*. 7, 246 – 250.
(63) Hilgendorf P et al.1987. Constant dissolved oxygen concentrations in cephalosporin C fermentation: Applicability of different controllers and effect on fermentation parameters. *Applied Microbiology and Biotechnology*. 27(3), 247 - 251.
(64) Stevens CM et al. 1962. Incorporation of molecular oxygen at C-17 of cephalosporin C during its biosynthesis. *Persp Biol Med. 5*, 432-435.
(65) König B et al. 1981. Process engineering investigations of penicillin production. *Applied Microbiology and Biotechnology*. 12 (4), 205-211.
(66) Chen HC and Wilde F.1990. The effect of dissolved oxygen and aeration rate on antibiotic production of *Streptomyces fradiae. Biotechnology and Bioengineering*. 37(6), 591 – 595.
(67) Malcolm JR et al. 1988. Effect of aeration on antibiotic production by *Streptomyces clavuligerus. Journal of Industrial Microbiology and Biotechnology*. 3(6), 357– 364.

****