American International Journal of Biology
June 2017, Vol. 5, No. 1, pp. 1-24
ISSN: 2334-2323 (Print), 2334-2331 (Online)

Published by American Research Institute for Policy Development
DOI: 10.15640/aijb.v5n1a1
URL: https://doi.org/10.15640/aijb.v5n1a1

Production of Antibacterial and Antifungal Metabolites by (S. albovinaceus) Strain no. 10/2 and Media Optimization

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Abstract

A new strain of Actinomycete (*S. albovinaceus*) no.10/2 with a broad antifungal and antibacterial activitywas isolated from soil sample of Palestine.The medium composition for antibioticproduction fromstrain *S.albovinaceus* was optimized using shake-flask methodology.The parameters that resulted in maximum antibiotic production are: 1.25 % mannitol and 1 % malt extract were found to be best carbon and nitrogen sources respectively for growth and antifungal and antibacterial production. Similarly initial pH of 7.2, 10 % level of inoculum, incubation period of 96 hrs, 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 inantifungaland antibacterial yield.This is one of the first reports for strain no.10/2 (*S. albovinaceus*) reported from Palestine soil with antibacterial and antifungal activities.

Keywords:Optimization,Streptomyces, S. albovinaceus, antifungal, antibacterial

1. Introduction

The discovery and use of naturally occurring products for the treatment of human diseases is prevalent throughout the human history.This involves the screening of microorganism and plant extracts (Shadomy, 1987). Microbial production of antibiotics is one ofthe rapidly expanding branches of industrial microbiology.

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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 emergingphenomenon of antibiotic resistance (Shiburaj, 2003).Antibiotic production is predominantly a feature of soil fungi and bacteria. Filamentous soil bacteria belonging to the genusStreptomyces are widely recognized as industrially important microorganisms because of their ability to produce many kinds of novel secondary metabolitesincluding antibiotics (Pamela, 2000).About 75 % of the known commercially and medically useful antibiotics are produced by Streptomyces (Sujatha*et al.,* 2005). Waksman,(1959)has recognized some natural substrates as ideal sources for the isolation of Actinomycetes, of which soil was found to be the richest source.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 (Gupte and Kulkarni, 2003).Influence of particular nutrients on the antibiotic biosynthesis is determined by the chemical structures of antibiotic substances.

The screening programs for new Actinomycetesand for their antibiotics are still proceeding at a very rapid pace.So far, few reports appeared in literature about production of antibioticsfrom 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 antimicrobial (antifungal and antibacterial) production.This paper deals with the effect of medium composition on antifungal and antibacterialproductionby strain *S. albovinaceus*(10/2) in batch cultures.

2. Materials and Methods

2.1 Microorganism and growth conditions

Producer strain was isolated from soil sample of Palestine. The strain was identified as a strain no.10/2 (*S. albovinaceus*) with broad antifungal and antibacterial activities (Thaer *et al.,* 2009). 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 (Maniatis*et al.,* 1989).

2.2 Media and cultural conditionsoptimization

Seed culture was prepared by inoculating one full slant culture into 50 ml of inoculum medium and incubatedon rotary shaker (220 rpm) at 28 °C for 48 hrs. The fermentation medium was inoculated with 10 % level of 48 hrs inoculum and incubated on rotary shaker (220 rpm) at 28 ºC for 96 hrs.The inoculation as well as theproduction media were the same and selected from different media and contained % (w/v): meat extract 0.3,tryptone 0.5, yeast extract 0.5, dextrose 0.1, potato starch 2.4, calcium carbonate 0.2, pH was adjusted to 7.0 beforesterilization.

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 (Yarbrough, 1993). Nutritional manipulation enhances the positive regulatory mechanisms of the production strains during cultivation (Tripathi*et al.,* 2004). As such it is decided to investigate the optimum cultural conditions for optimum antibioticproduction. Ourproduction medium was used as the base to determine the optimal nutritional and culturalconditions for growth and antibiotic production.

The following parameters were investigated for the optimum antibiotic production by our isolate *S*. albovinaceusno. 10/2: selection of a suitable inoculum medium, selection of a suitable production medium, selection of best carbon sourceand its optimum concentration, selection of best nitrogen source and its optimum concentration, influence of initial pH on antibiotic production, influence of inoculum level on antibiotic production, effect of incubation period, influence of aeration, and influence of agitationon antibiotic production. Finally production of antibiotics with the optimum conditions (using modified production medium and cultural conditions formulated based on the above observations) were studied.

2.3 Antimicrobial activity

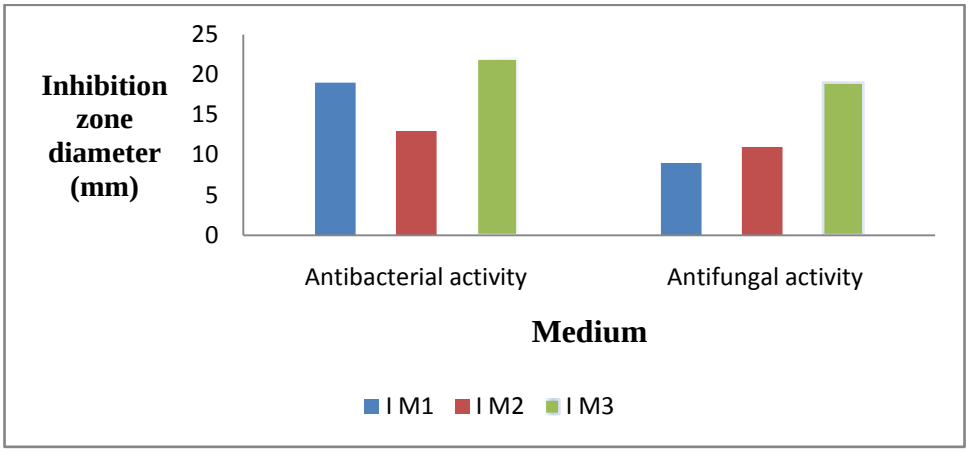
It was performed by withdrawing samples at the end of the fermentation cycle (96 hrs), centrifuged, and the supernatant was assayed for antimicrobial activity by standard cup plate methodusing *Bacillus pumilus* for antibacterial activity and against *Candida albicans*for antifungal activity.

3. Results

Selection of a suitable inoculum medium for antibiotic 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. In order to select the best inoculum medium for maximum antibiotic production, three different inoculummedia IM1, IM2 and IM3 were tried.The results are shownin Figure 1. Inoculum medium (IM3) gave maximum antimicrobial yield; hence it was used in further studies as the inoculum medium.

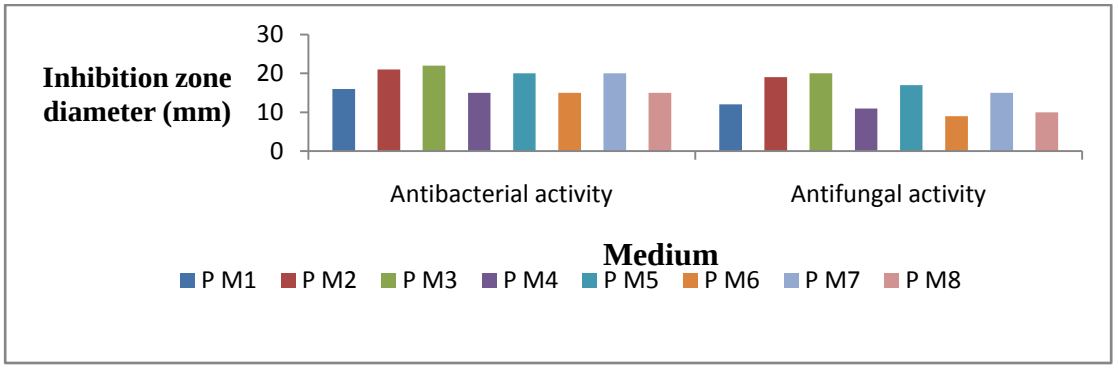
Figure 1:Selection of a suitable inoculum medium for antibiotic production



Selection of a suitable production medium for antibiotic production

In order to select the best production medium for maximum antibiotic production 8 different media PM1 toPM8 were tried. The results are shown in Figure 2. Production medium (PM3) gave maximum yield of antibiotic. Hence, it was selected for further optimization studies for antibiotic production. It was shown that the inoculation as well as the production media used the same and the composition is as given before in materials and methods.

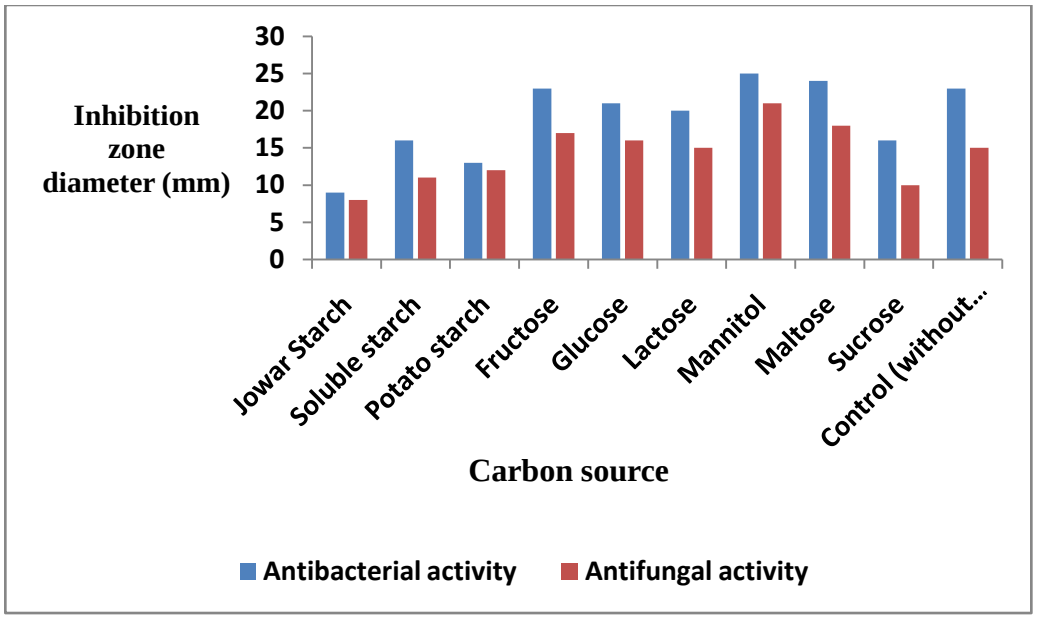
Figure 2:Selection of a suitable production medium for antibiotic production



Selection of best carbon source for antibiotic production

To select the best carbon source for maximum antibiotic production, differentcarbon sources were supplemented to the basal production mediumPM$_3$ in place of potato starch at a concentration of 1 % to study their effect on antibiotic production. The results are shown in Figure 3. Mannitol was found to be the best carbon source for maximum antibiotic production followed by maltose. So, mannitol was selected for further optimization studies for antibiotic production.The cultivation medium supplemented with mannitol was thus employed in all further experiments.

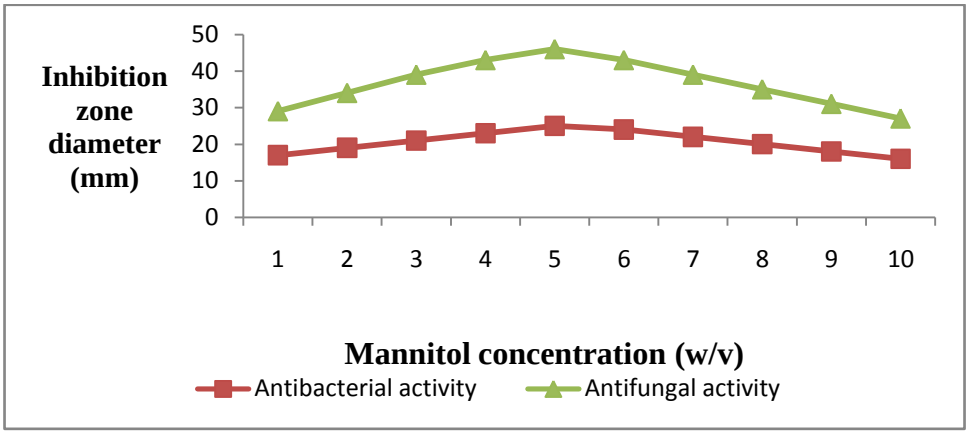
Figure 3: Selection of best carbon source for antibiotic production



Effect of different concentrations of mannitol on antibiotic production

Different concentrations of mannitol varied from 0.25 to 3 % (w/v)were tried to study their effect on antibiotic production.The results are shown in Figure 4. Mannitol at a concentration of 1.25 % (w/v) gave maximum antibiotic yield. Therefore, mannitol at a concentration of 1.25 % (w/v) was thus employed in all further experiments.

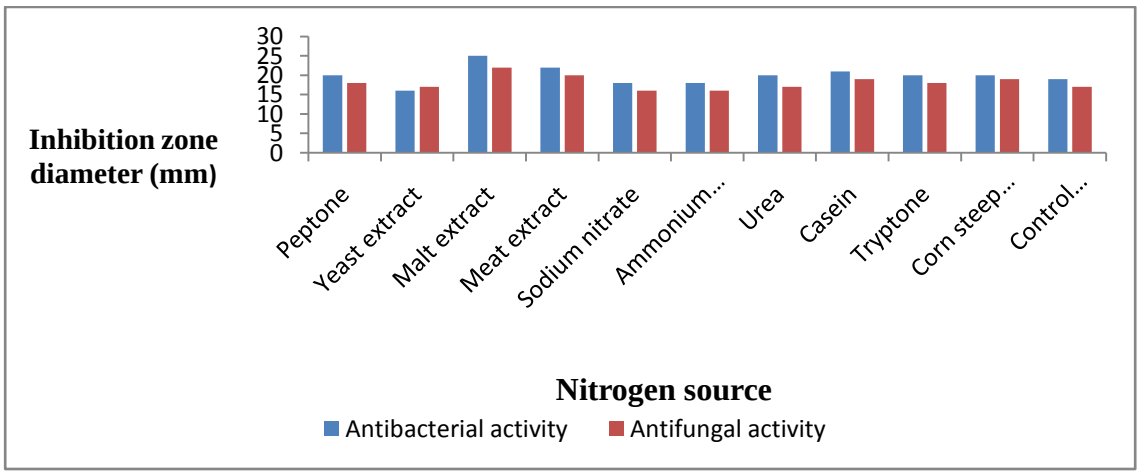
Figure 3: Selection of best carbon source for antibiotic production



Selection of best nitrogen source for antibiotic production

To select the best nitrogen source for maximum antibiotic production, different nitrogen 'sat a concentration of 1 %, organic were tested. The results are presented in Figure 5.Nitrogen source exhibited a significant effect on the antibiotic production. The best nitrogen source for supporting antibiotic production was malt extract. So, malt extract was selected for further optimization studies for antibiotic production.

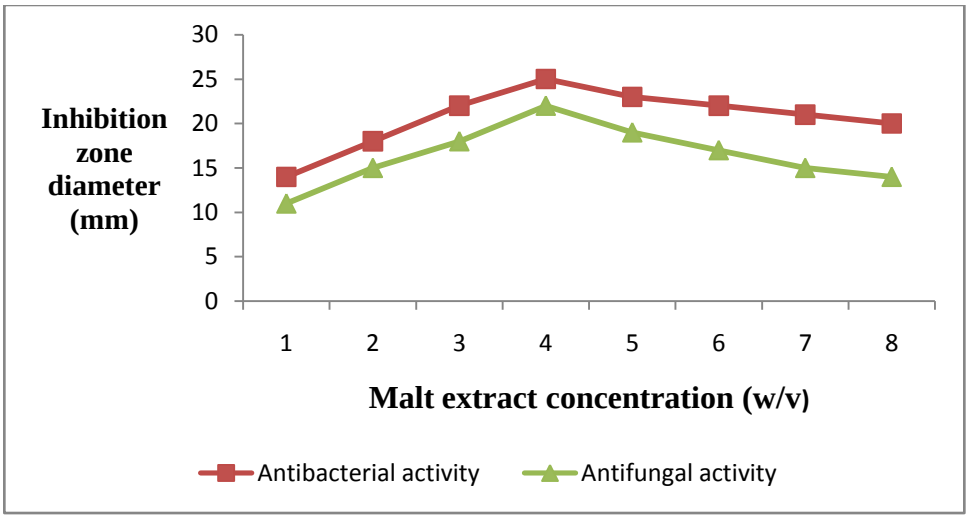
Figure 5: Selection of best nitrogen source for antibiotic production



Effect of different concentrations of malt extracton antibiotic production

From the earlier experiment it was shown that malt extract is the best nitrogen source for antibiotic production.It was thought, therefore, to test whether antibiotic 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 Figure6. Malt extract at a concentration of 1.0 % (w/v) gave maximum antibiotic yield. Therefore, malt extract at a concentration of 1.0 % (w/v) was used in the subsequent experiments.

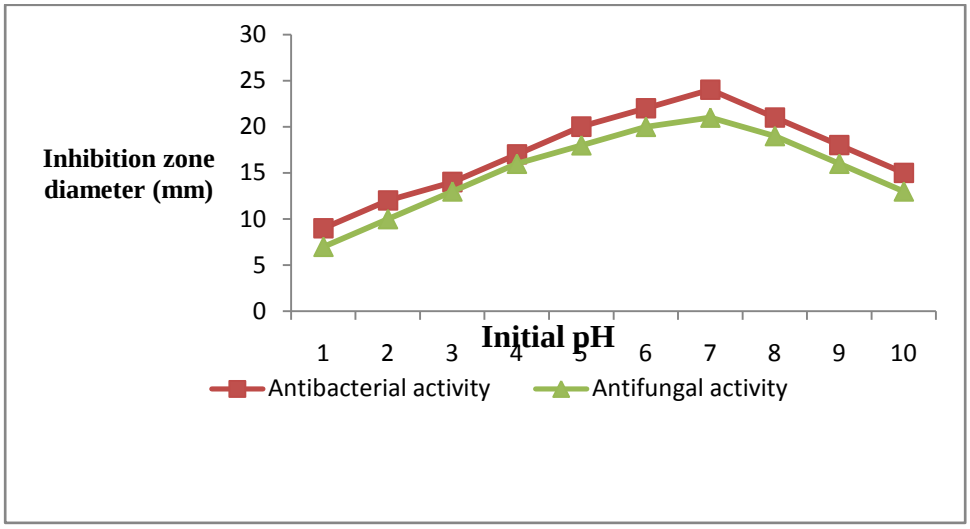
Figure 6: Effect of different concentrations of malt extract on antibiotic production



Influence of initial pH on antibiotic production

Production media with different initial pH varied from 5.5 to 8.5 were tried to study their effect on antibiotic production. The results are shown in Figure 7. The yield of antibiotic was maximum when production medium with an initial pH 7.2 was used.

Figure 7:Influence of initial pH on antibiotic production



Influence of inoculums level on antibiotic production

To study the effect of level of inoculums on antibiotic production, different levels of inoculavaried from 2.5 to 15 % (v/v) were tried. The results are presented in Table 1. Inoculums level of 10 % yielded maximum antibiotic production.

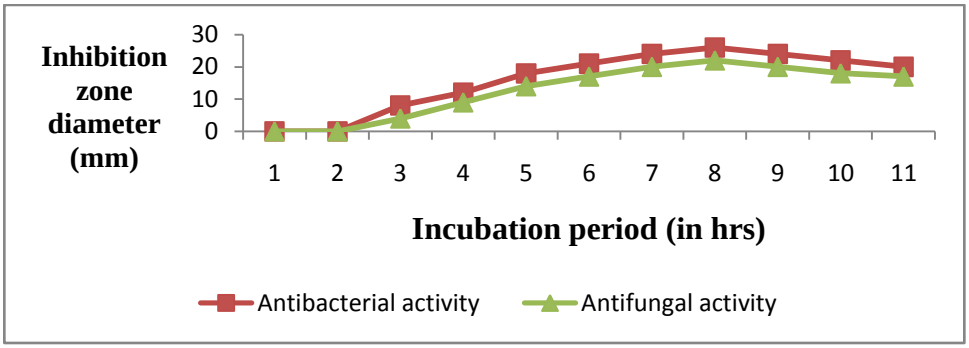
Table 1: Effect of level of inoculum on antibiotic production

Level of inoculum in % (v/v)	**Inhibition zone diameter (mm)**	
	Antibacterial activity	**Antifungal activity**
2.5%	23	13
5.0%	24	16
10.0%	**26**	**22**
15.0%	25	17

Effect of incubation period on antibiotic production

The influence of incubation period on antibiotic production was studied. Different incubation periods varied from 12 to 132 hrs were studied. The results are shown in Figure 8. The yield of antibiotic was maximum when an incubation period of 96 hrs was used.

Figure 8:Effect of incubation period on antibiotic production



Influence of aeration on antibiotic production

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

Table 2: Influence of aeration on antibiotic production

Volume of medium (ml)	Inhibition zone diameter (mm)	
	Antibacterial activity	Antifungal activity
25	**28**	**22**
50	25	19
75	21	16
100	19	13

Influence of agitation on antibiotic production

In order to study the effect of agitation on antibiotic production, different types of flasks with different capacities were used. The results are shown in Table 3. When fermentation was carried out in 250 ml bottom indented flask it gave maximum antibiotic production.

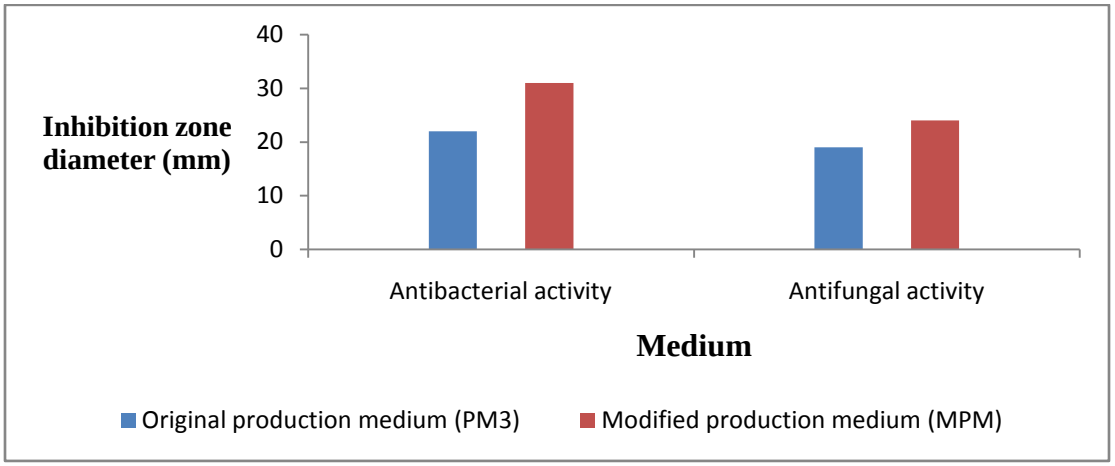
Table 3: Influence of agitation on antibiotic production

Type of flask	**Inhibition zone diameter (mm)**	
	Antibacterial activity	**Antifungal activity**
Un-indented		
250 ml	25	19
500 ml	21	13
Side indented		
250 ml	29	20
500 ml	23	15
Bottom indented		
250 ml	**30**	**23**
500 ml	24	16

Production of antibiotic with optimum conditions

Based on the results obtained with all optimum parameters, an attempt was made to evaluate the extent of improvement in the modified formulated production medium. The results are shown in Figure 9. Significant improvement in the antibiotic titre was observed with the modified production medium for antibacterial activity.

Figure 9:Production of antibiotic with optimum condition



4. Discussion

Qualitative and quantitative aspects of antibiotic production by the microorganisms are dependent on theselective environmental pressure prevailing at its source of isolation and manipulation of growth and nutritional conditions during fermentation exerts substantial influence on the level of metabolite production (Yarbrough, 1993). 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 (Casida, 1987). The biosynthesis of antibiotics is regulated by the type and concentration of different medium components such as carbon, nitrogen, phosphate, metal ions and other medium ingredients (Martin and McDaniel, 1977). Many factors influence the extent of growth of microbial cells, chemical composition, and the nature and concentration of specific metabolic products produced3. There is considerable selectivity in the utilization of these substances by different kinds of Actinomycetes (Fuji *et al.,* 1997; Lowe et al., 1997; Himabindu and Jetty, 2006). Wild strains are very rich in chemical diversity and novel lead drug molecules(Sanchez and Demain, 2002).

Actinomycetes perform significant biogeochemical roles in nature and are highly valued for their unparalleled ability to produce wide variety of biologically active secondary metabolites(Berdy, 2005; Vastrad and Neelagund, 2011). Actinomycetes are able to utilize a great variety of organic compounds as sources of energy (Fuji *et al.,* 1997; Lowe *et al.,* 1997). 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 (Mohamed *et al.,* 2000).

Production of antibiotic 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 (Iwai and Omura, 1982; Yu *et al.,* 2008). Also environmental factors like incubation temperature, pH and incubation time were found to have profound influence on antibiotic production (Srinivasan *et al.,* 1991;VenkataDasu and Panda, 1999; Sujatha*et al.,* 2005).

Growth and antibiotic production on synthetic media were found to be unsatisfactory (Gesheva*et al.,* 2005). Consequently we used a complex medium with organic nitrogen source, which supports both, growth and antifungal productivity. The effects of certain nutrients on antimicrobial production by *S. albovinaceus*strain no.10/2 in submerged batch culture were studied. 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 antibiotic by *S. albovinaceus*strain no.10/2. The inoculum medium as well as the production medium used in our study were the same (Figure 1 and 2).

This gave maximum antibiotic production. This may be due to decrease of lag time when we use the same inoculum as well as the production medium. Sanchez and Demain(2002), have listed several carbon sources which interfere with the antibiotic production. Sugar can affect the metabolism directly by decreasing the time for switching over to stationary phase(Stanbury*et al.,* 1997). Generally a quickly metabolized substance like glucose is responsible for catabolism repression but in some cases it is also reported to enhance antibiotic production (Reichenbach*et al.,* 1988; Fukuda *et al.,* 2005). 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 (Fuji *et al.,* 1997; Lowe *et al.,* 1997).

It was shown that increased antimicrobial activity produced 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 (Martin and Demain, 1980). Various mono-carbohydrates, di-saccharides and polysaccharides (all used in equal concentrations of 1%) were tested in growth experiments for their ability to support antibiotic production by *S. albovinaceus*strain no.10/2. The results are given in (Figure 3). The microorganism was able to grow in all the tested carbon sources. In case of disaccharides, the production of antibiotic 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 antibiotic production. Mannitol is commonly formed via the hydrogenation of fructose which may be explaining the comparison result. The highest titers of antibiotic 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.,*(2000), 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 (Vahidi*et al.,* 2004). 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.

The effect of various concentrations of mannitol, varied from 0.25 % to 3.0 % on antibiotic production was studied. The results are shown in (Figure 4). The results showed that both volumetric and specific production of antibiotic continues to increase and reached a maximal value at 12.5 g/l, above this concentration, the antibiotic production decreased. As mannitol concentration increased from 1.25 % to 30 % the antibiotic 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 (Chen *et al.,* 2008). In this connection (Zhu *et al.,* 2007), 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 (Espeso*et al.,* 1993). High concentration of glucose is generally considered as repressor of secondary metabolisms and maximum cell growth rates can inhibit antimicrobial agent production (Gallo and Katz, 1972). The level of antibiotic production may be greatly influenced by the nature; type and concentration of the nitrogen source supplied in the culture medium. Depending on the biosynthetic pathways involved, nitrogen sources may affect antibiotic formation(Geshev*aet al.,* 2005). Mohamed *et al.,* (2000) 20 showed that nitrogen source exhibited a significant effect on the natamycin production. The activity of the antibiotic also varied with changes in nitrogen source (Lian-Xiang *et al.,* 2003; Geshev*aet al.,* 2005).

Nitrogen source exhibited a significant effect on the antibiotic production (Mohamed *et al.,* 2000; Neha and Vibhuti, 2012).Various nitrogen sources, organic and inorganic were tested to study their ability to support antibiotic production. The results are shown in (Figure 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 antibiotic production. The production of antibiotic was less with inorganic nitrogen sources compared with organic sources. These results are in agreement with the results of other investigators (Mohamed *et al.,* 2000; Vahidi*et al.,* 2004; Geshev*aet al.,* 2005; Yu *et al.,* 2008).

In contrast, (Sujatha, 2005)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, and urea. It is well known that changes in the kind and concentration of nitrogen source influence greatly antibiotic production (Neha and Vibhuti, 2012).

The results also showed that the concentration of malt extract (Figure 6) greatly influenced the production of the antibiotic with maximum yield being obtained in cultures supplemented with 1% of malt extract. These results are in agreement with the results of other investigators (Chan *et al.,* 2002; Gupte and Kulkarni, 2002). Sujatha, (2005) 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. Also the environmental factors like pH, level of inoculum, and incubation period were also found to have profound influence on antibiotic production(VenkataDasu and Panda, 1999; Sujatha*et al.,* 2005). The environmental requirements and tolerance for growth and antibiotic production has been studied in detail. The maximum antibiotic activity was obtained at a pH of 7.2 (Figure 7) suggesting its inclusion in the neutrophilic Actinomycetes group. This is in agreement with the results obtained by (Sujatha*et al.,* 2005). Borenstajn& Wolf (1955), reported that for oxytetracycline production a pH value of 7.0 was suitable. Antibiotic production was significantly reduced when the Streptomycetewas grown under conditions such that the pH of the medium rose above pH 8(Perlman *et al.,*1955).

The culture pH and temperature affect the antimicrobial activity and growth of the microbes (Vahidi*et al.,* 2004; Mustafa, 2009). 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 antibiotic production by *Streptomyces* was reported by several investigators who observed that the optimum pH for antibiotic production range between 7.0 and 7.5 (Tripathi*et al.,* 2004; Mustafa, 2009; Kumar and Kannabiran, 2010). The yield of antibiotic was maximum when pH was maintained at 7.2 (Figure 7). This is in agreement with the results obtained by (Sujatha*et al.,* 2005).

In contrast, it was showed that maximum antibiotics production was obtained at acidic pH (Reddy &Ranganathan, 1985; VenkataDasu and Panda, 1999; Jicheng*et al.,* 2008). It has been reported that maximum production of bacitracin and other antibiotics occurred at alkaline pH (Basilio*et al.,* 2003). Increasing the medium pH led to an increase in the antibiotic production up to a certain limit above which any increase in the pH value was accompanied by a decrease in the antibiotic production and activity (Chao-Min *et al.,* 1975).

The effect of inoculum level on the production of penicillin and griseofulvin was studied. It was reported that the biochemical factors such as the levels of the enzyme activity and efficiency were at least as important as morphology in determining the yield of the antibiotic (Smith and Calam, 1980). Data showed that there is a relation between antibiotic productivity and different inoculums sizes (Houssam M *et al.,* 2011). The inoculum age and density markedly influence the productivity and economics of bioprocesses (Ramkrishna and Swaminathan, 2004). The maximum yield of antibiotic 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 (Chao-Min *et al.,* 1975; Jicheng Y *et al.,* 2008).

In contrast, it was shown that in preliminary experiments revealed an optimum inoculums size of 5.5 % (Jia*et al.,* 2001). 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 (Srinivasan *et al.,* 1991).

Incubation period and temperature areessential factors that modulate lab growth and significantly affect the amounts of antimicrobial metabolites produced. The condition of incubation influenced quantitatively the biosynthesis of antibiotics as well as biomass reported by (Al Zahrani, 2007). Studies carried out by some investigators revealed that maximal production of antibiotic substances occurred after 96 hrs (Reddy &Ranganathan, 1985; Sujatha*et al.,* 2005; Mustafa, 2009).This is in agreement with our results (Figure 8). In contrast, the highest biomass and antibiotic activity was observed at an incubation time of 72 hrs by some other investigators (Srinivasulu*et al.,* 2002). 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 (Sahin and Ugar, 2003). Nutrient deficiency is responsible for onset of antibiotic biosynthesis. Production of antibiotics occurs during a distinct idiophase of culture growth (Tripathi*et al.,* 2004). 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 (Geshev*aet al.,* 2005). The antibiotic production declined indicating its accumulation after a certain period of streptomycetal growth.

Dissolved oxygen (DO) is known to be an important parameter in industrially important antibiotic fermentations (Yegneswaran*et al.,* 1991). Flickinger and Perlman (1980), found a 2-3-fold increase in neomycin production by *Streptomyces fradiae* when DO was maintained above 5 kPa, by oxygen enrichment. 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(Yegneswaran*et al.,* 1991).

Agitation affects aeration and mixing of the nutrients in the fermentation medium (Augustine *et al.,* 2005). Adequate agitation was found to increase antibiotic metabolite production (Stevens *et al.,* 1975; Hersbach*et al.,* 1984; Hilgendorf*et al.,* 1987; Lian-Xiang *et al.,* 2003). Chao-Min *et al.,* (1975)showed that oxygen absorption rates (OAR) affected both mycelial growth and candicidin synthesis. Yegneswarant*et al.,* (1991)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. Malcolm *et al.,* (1988) 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 5 antibiotic activity (Table 3).

From the present investigations, it is clear that a novel strain of *S. albovinaceus* (10/2) with excellent antibiotic production has been identified, and the medium and cultural conditions for maximum antibiotic production have been optimized. The optimization studies resulted in the development of a modified production medium with enhanced yield (Figure 9). The production of 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 antibiotic production by *S. albovinaceus*strain no.10/2.

The highest concentration of antibiotic 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 hrs, and agitation in 250 ml bottom indented flask and medium to flask ratio of 1:10 gave maximum antibiotic production.

In conclusion, it is suggested that the improved medium gave enhancement of antibiotic activity when compared with that of the basal fermentation medium (Figure 9.) The antibiotic producing microorganism isolated and investigated in this study has shown broad spectrum antimicrobial activity. Preliminary chemical characterization (data not shown) of the isolated compounds has demonstrated that the organism produced antibacterial and antifungal compounds which is very advantageous as this organism produces two broad spectrum antimicrobial compounds simultaneously and their isolation is easy. Full chemical characterization of the compounds isolated, strain improvement, use of different inducers to improve the yield of the antibiotic, and whole cell immobilization techniques will be carried out.

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