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Studies on optimization of growth parameters for enhanced production of antibiotic activity by isolated Marineactinomycete.

M. V. K. Narsingarao*, T. Samuel David, N. Nagaraju, G. Ravisankar and T. Saroja

Department of Biology, Dr. V. S. Krishna Govt College, Visakhapatnam, India.

****Corresponding Author's Email: maddalanarresh@gmail.com***

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ABSTRACT

The aim of the present study is to optimize the growth conditions for improved production of antimicrobial activity by promising marine actinomycetes isolated from marine sediments. The indigenous isolate KRBT-401was then investigated for the effect of different nutrients and cultural conditions for antibiotic production. Pridham and Gottlieb's inorganic salts medium was used as the production medium base. The carbon sources employed are arabinose, fructose, galactose, glucose, glycerol, lactose, maltose, mannitol, meso-inositol, starch, sucrose and xylose at 1% (w/v). Among the various carbon sources studied, glucose produced more antibiotic yield followed by starch and glycerol. Similarly the effect of various organic nitrogen sources, inorganic nitrogen sources and aminoacids was studied on the production of antibiotic. Ammonium nitrate at a concent ration of 3.0g/L was found to be the suitable concentration for maximum antibiotic yield. By performing studies on various physiological parameters, nutrients and trace metal ions that affect antibiotic yield, a synthetic medium was formulated for the maximum production of antibiotic under shake flask culture by KRBT-401. The antibiotic productivity employing optimized fermentation conditions (285µg/ml) was 260% higher than the antibiotic yield using initial fermentation conditions (115µg/ml).

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Introduction:

Actinomycetes are widely distributed in nature and have biotechnological potential in bioremediation, for cleaning up the environment (Alvarez et. al., 2017). Terrestrial actinomycetes produce resistant spores, that are known to be transported from land to sea, where they can remaind or mant for many years (Bullet.al, 2000). It has been frequently assumed that actinomycetes isolated from marine samples are merely are sult of these dormant spores of terrestrial origin.

The supporting evidence of the existence of marine actinomycetes came from the description of Rhodococcusmarinonascene, the first marine actinomycetes pecies to be characterized and reported (HelmkeandWeyland,1984). Actinomycetes have a profound role in the marine environment apart from antibiotic production. The degradation and turnover of various materials are a continuous process mediated by the action of a variety of microorganisms.

The natural products are the most abundant sources for compounds having antagonistic activity against pathogenic organisms. As the matter of fact, most of the using drugs are of natural origin, derived or chemically synthesized from natural products or synthesized products based on natural products structures (Demainet.al.,2009). These natural products obtained from plants, microorganisms and animals. Among these micro organisms have great potential as sources of biologically active compounds (Demainet. al.,2009). They also consider the most economic commercial sources for these compounds. About 23,000 bioactive secondary metabolites produced from microorganisms, around45% from these compounds produced by actinomycetes.

The nature of the produced secondary metabolites was characteristic for the actinomycetes strain and greatly influenced by nutrition and fermentation conditions. So the bioactive metabolites can increase several folds by optimization studies of all the factors affecting their production. (Rameshet.al., 2012; Manivasaganet. al., 2014; Geshevaet.al., 2005;Ushaet.al.,2014.)

This research aims at finding the most potent actinomycete chose for further optimization studies includes: carbon and nitrogen sources, inoculums type, incubation periods and pH of the fermentation medium.

Materials and Methods:

Optimization of Media for the Production of Antifungal Compound:

A detailed optimization study was carried out on the production of antimicrobial compound employing the indigenous isolate KRBT-201(Isolate taken from previous studies). The effects of following parameters on growth and antimicrobial compound production were undertaken.

Effect of Various Carbon Sources:

Effect of various carbon sources on growth and antimicrobial compound production was studied by incorporating them at 1% (w/v) level into the Pridham and Gottlieb's inorganic salts medium. The carbon sources employed are arabinose, fructose, galactose, glucose, glycerol, lactose, maltose, mannitol, meso-inositol, starch, sucrose and xylose. To optimize the concentration of glucose for maximum growth and antibiotic production, it was incorporated into the production medium at different concentrations of 2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 17.5and 20.0g/L.

Effect of Various Nitrogen Sources:

The influence of various nitrogen sources on growth and antimicrobial compound production was studied by adding inorganic nitrogen sources and organic nitrogen sources at 0.2% (w/v) level into the Pridhamand Gottlieb's inorganic salts medium. Glucose at 1% (w/v) level was employed as carbon source. The inorganic compounds employed are ammonium citrate, ammonium nitrate, ammonium sulphate, potassium nitrate and sodium nitrate. The organic nitrogen compounds employed are soyabean meal, peptone, beef extract, yeast extract, tryptone, and amino acids-alanine, arginine, asparagine, asparticacid, glutamicacid, leucine, histidine, methionine, phenylalanine, serine, threonine, tryptophan and tyrosine.

The concentrations of ammonium nitrate used to determine the optimum concentration for growth and antifungal compound production are (g/L) 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 and 4.5. Each concentration of ammonium nitrate was incorporated into the Pridhamand Gottlieb's inorganic salts medium.

Effect of Different Concentrations of K_2HPO_4:

To optimize the concentration of K_2HPO_4 for antimicrobial compound production, it was incorporated into the production medium at different concentrations of 0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8, 3.2, 3.6 and 4.0g/L.

Effect of Different Concentrations of $MgSO_4$:

To determine the optimum concentration of $MgSO_4$ for antimicrobial compound production, $MgSO_47H_2O$ were in corporate into the Pridham and Gottlieb's inorganic salts medium at 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75 and 2.0g/L.

Effect of Incubation Temperature:

To determine the optimum incubation temperature for antimicrobial compound production, production medium was inoculated and incubated at different temperatures of 15, 20, 25, 30, 35, 40, 45 and 50°C for 96hrs.

Effect of Initial pH:

To study the effect of initial pH of the medium on growth and antimicrobial compound production, the production media were adjusted to different initial pH values of 6.0, 6.4, 6.8, 7.2, 7.6 and 8.0.

Effect of Incubation Period:

To study the optimal incubation period for maximum antimicrobial compound production, the flasks with the medium were inoculated and incubated at 30°C. Samples were withdrawn periodically at every 24h for 5days and assayed for antibiotic activity as described earlier.

Production of Antimicrobial Compound with Optimized Parameters:

Antifungal compound production was also investigated by employing all the optimized nutritional and cultural parameters.

Measurement of Growth:

The growth of the organism was measured as dry weight of the mycelium. The contents of the culture flask were filtered through a previously weighed dry Whatmann No.1 filter paper, washed twice with distilled water and then the filter paper along with the mycelia mass was dried in a hot air oven at 80˚C for 18-24 hrs and then the filter paper was weighed.

Antimicrobial Compound Assay:

Culture samples (1ml) were taken at specified time intervals, centrifuged at 3000rpm for 15min at 4˚C and then separated into culture filtrate and mycelium. The antimicrobial compound activities of mycelia free culture supernatant against test organisms were measured by standard cup plate method (GroveandRandall,1955) as described earlier for Initial and optimized media parameters.

Results and Discussion:

Effect of Carbon Source:

Microorganisms have a metabolic capacity to utilize a variety of carbon sources and to adapt to changes in osmotic strength, stress conditions, oxygen, and nutrients limitations (Brücknerand Titgemeyer, 2002). The presence of a rapidly metabolizable carbon source, especially glucose, results inacoordinated change of metabolic functions in many bacteria (Saierand Crasnier, 1996). As a component of culture media, glucose provides carbon atoms for biomass and product generation. The effect of various carbon sources on the growth and production of antimicrobial compounds by KRBT-401 was shown in Table1. The isolate grew well on all media supplemented with different carbon sources. However, maximum production of antimicrobial compound was obtained with media supplemented with glucose followed by fructose and glycerol. They ield obtained with glucose is180µg/ml and with starch and glycerol are 80µg/ml and 120µg/ml respectively, while low levels of antimicrobial compound was produced with arabinose, lactose, rhamnose and xylose. Moderate levels of antimicrobial compound were produced when maltose, galactose and starch were used.

The growth of the isolate with various carbon sources was studied in terms of dry weight of the mycelium. The higher biomass production was observed with glucose (3.0mg/ml). Poor biomass production was observed with mannitol (0.4mg/ml) xylose (0.8 mg/ml) and lactose (0.9mg/ml).

The effect of different concentrations of glucose on growth and antimicrobial compound production was investigated and the results are presented in Figure1. At 10g/L concentration of glucose, the antimicrobial compound titer was190µg/ml with a biomass of 3.2mg/ml. While at 12.5g/L glucose, 180µg/ml was the antimicrobial compound titer with a biomass of 3.0mg/ml and at 15g/L glucose concentration, the antimicrobial compound titer was 180µg/ml with a biomass of 3.0mg/ml.

Higher concentration of glucose in the medium was observed to decrease the growth and also the antimicrobial compound production. The data suggested that12.5g/Lg glucose concentration was optimum for antimicrobial compound production. The results also showed that an increase of glucose level in the culture medium from10g/L to12.5g/L led to1.2-fold increase in antimicrobial compound production. The molecular nature of the glucose up take system of *Streptomyces coelicolor* (van Wezelet.al., 2005) has recently revealed that the glucose permease gene could be found twice in the genome, (named *glc*P1 and *glc*P2). This specifies the uptake of glucose at higher concentrations than other carbon sources by the genus *Streptomyces*. However the high concentration of glucose in the medium was observed to decrease the growth and also the antimicrobial compound production. Soliveriet.al., (1988) reported the production of the macrolide polyene antibiotics PA-5 and PA-7, produced by *Streptoverticillium* sp. 43/16. The production of these compounds increases with increase in concentrations of glucose up to 40mM, but is inhibited at higher concentrations.

Table1: Effect of Different Carbon Sourceson Growth and Antimicrobial Compound Production by KRBT-401.

Carbon source1% (w/v)	Growth dry wt (mg/ml)	Antimicrobial compound Yield (µg/ml)
Arabinose	2.0	25
Fructose	2.9	135
Galactose	1.5	90
Glucose	3.0	180
Glycerol	2.5	120
Lactose	0.9	40
Maltose	2.8	115
Mannitol	0.4	50
Mannose	1.6	55
Meso-inositol	1.8	65
Rhamnose	2.9	40
Starch	1.3	80
Sucrose	1.8	45
Xylose	0.8	30

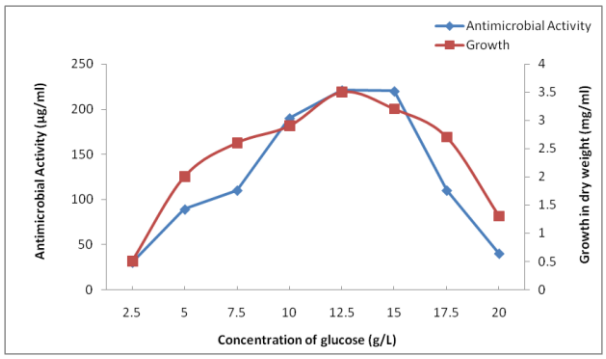

Figure: 1. Effect of Glucose Concentration on Growth and Production of Antimicrobial Compound by KRBT-401.

Effect of Nitrogen Source:

Nitrogen source along with the carbon source plays an important role in the antimicrobial compound production. The effect of various organic and inorganic nitrogen sources on the growth and antimicrobial compound production is shown in Table 2. Among the various inorganic nitrogen sources, the maximum antimicrobial compound production was achieved with ammonium nitrate (230µg/ml) followed by sodium nitrate (150µg/ml) and potassium nitrate (145µg/ml) and alanine (140µg/ml).

The results suggest that the level of antimicrobial compound production may be greatly influenced by the nature and type of nitrogen source supplied in the culture medium. Antimicrobial compound accumulation begins to increase in many cases only after the nitrogen source in the culture broth has been almost entirely consumed. Addition of ammonium as

nitrogen source to the fermentation medium markedly increased the antibiotic production of AK-111-81 by *Streptomyces hygroscopicus* 111-81 (Gesheva et. al., 2005).

The effect of different concentrations of ammonium nitrate on growth and antimicrobial compound yield was studied and the results are presented in Figure 2. The results showed that the concentration of ammonium nitrate greatly influenced the production of the antimicrobial compound with maximum antibiotic yield (225µg/ml) being obtained in cultures supplemented with 2.5g/L of ammonium nitrate. The results also showed that ammonium nitrate at a concentration of 2.5g/L induced maximum biomass (3.4mg/ml) production. However, the yield decreased with both lower and higher concentrations of ammonium nitrate. In a similar study by Potvin and Péringer (1994) reported that the higher initial concentrations of ammonium salt reduced the erythromycin production yield with increasing the biomass growth by a mutant strain of *Saccharopolyspora erythraea.*

Table: 2. Effect of different inorganic Nitrogen sources on growth and Production of antimicrobial compound by KRBT-401.

Nitrogen source	Growth dry wt. (mg/ml)	Antibiotic yield (µg/ml)
Inorganic source 1 % (w/v)		
Ammonium citrate	0.9	50
Ammonium nitrate	3.4	230
Ammonium sulphate	0.5	55
Monosodium Glutamate	0.8	90
Potassium nitrate	2.9	145
Sodium nitrate	3.2	150
Amino acids 0.05 % (w/v)		
Alanine	2.4	130
Arginine	2.3	85
Asparagine	2.7	80
Aspartic acid	2.4	90
Glutamic acid	3.0	110
Hydroxyproline	2.8	105
Leucine	2.2	90
Methionine	3.1	80
Phenylalanine	2.4	55
Serine	2.3	110
Threonine	2.8	115
Tryptophan	1.9	60
Tyrosine	1.8	95

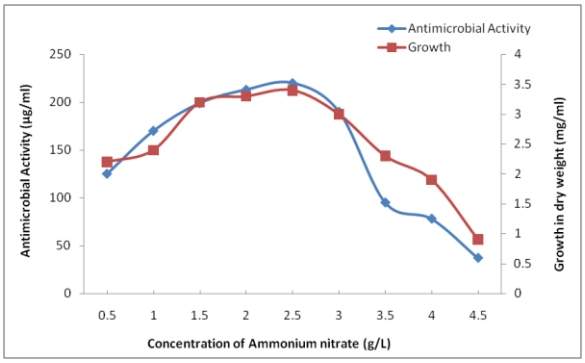

Figure: 2. Effect of Ammonium nitrate concentration on Growth and Production of Antimicrobial Compound by KRBT-201

Effect of K_2HPO_4:

Addition of a large amount of inorganic phosphate induces the consumption of carbon and nitrogen sources and respiration is accelerated resulting in good growth, but the production of antibiotics is usually reduced. The effect of different concentrations of K_2HPO_4 on growth and antimicrobial compound production was studied and the results are presented in Figure 3. At 0.8 g/L concentration of K_2HPO_4, the antimicrobial compound titer was 150µg/ml with a biomass of 2.5 mg/ml. With 1.2 g/L K_2HPO_4, 255 µg/ml of antimicrobial compound titer was obtained with a biomass of 3.2mg/ml. At 1.6g/L K_2HPO_4 in production medium, 170µg/ml antimicrobial compound production was recorded, while the biomass in the flask was about 3.4 mg/ml. It is evident from the results depicted in Figure 3 that a concentration of 1.2 g/L gave maximum yield of antimicrobial compound.

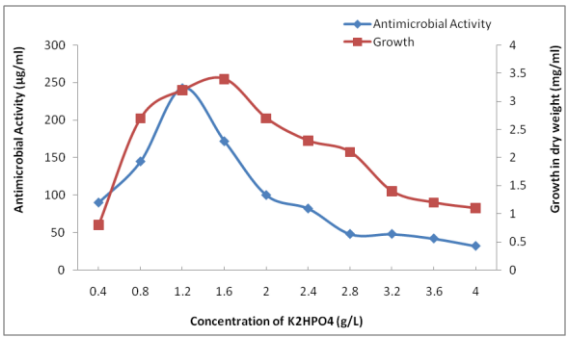

Figure: 3. Effect of K_2HPO_4 Concentrations on Growth and Production of antimicrobial Compound by KRBT-201

Effect of $MgSO_4$:

The effect of different concentrations of $MgSO_4$ on growth and antimicrobial compound yield was investigated and the results are presented in Figure 4. The results indicate that the concentration of $MgSO_4$ greatly influenced the production of the antimicrobial compound. Maximum antimicrobial compound yield was obtained in cultures supplemented with 0.5 g/L of $MgSO_4$ (260µg/ml). The results also suggest that $MgSO_4$ at a concentration of 0.5 g/L induced maximum biomass (3.6mg/ml) production. Further, it is clear from

the data obtained that below and above the critical concentration of 0.5 g/L of $MgSO_4$, both growth and antimicrobial compound yield was significantly low.

The effect of $MgSO_4$ and various other metal salts on production of the antibiotics was investigated and has been reported by several authors (Young et. al., 1985; Williams and Edward, 1977; Abbas & Edwards, 1990).

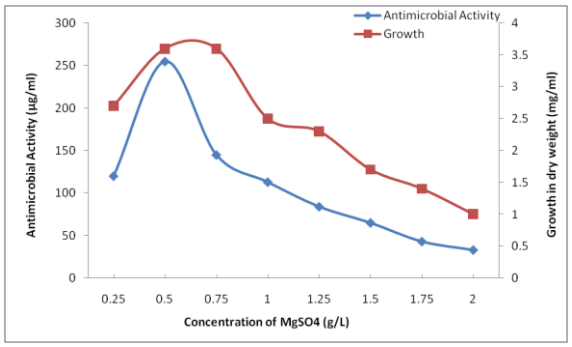

Figure: 4. Effect of MgSO4 Concentrations on Growth and Production of Antimicrobial Compound by KRBT-201

Effect of Incubation Temperature:

The effect of temperature on growth and antimicrobial compound production is shown in Figure 5. The optimum temperature for the higher production of antimicrobial compound is 30°C, where maximum titer of 260µg/ml was obtained. The titers of antimicrobial compound decreased to 40µg/ml when the temperature was increased to 35°C. Decrease in temperature i.e., to 25°C resulted in low titers of antimicrobial compound (125 µg/ml). The data presented in Figure 3.10 shows that 30°C temperature is optimum for maximum antimicrobial compound production. The effect of temperature on the physiology of growth and on antimicrobial compound production was studied by James and Edwards (1989).

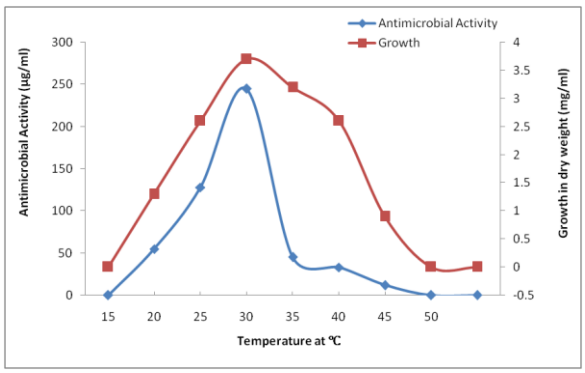

Figure: 5. Effect of Temperature on Growth and Production of Antibiotic by KRBT-201

Effect of Initial pH:

The effect of pH on the growth and antimicrobial compound production is shown in Figure 6. Present investigation indicated that initial medium pH influences antimicrobial compound production by KRBT-201. The result showed that maximum biomass (3.6mg/ml) and antimicrobial compound production (280µg/ml) were at pH 7.2. The antimicrobial compound production dropped significantly in acidic pH of 6.0 (35µg/ml) and in alkaline pH of 8.4 (40µg/ml) and 8.8 (20µg/ml). There was no growth and antimicrobial compound production at highly alkaline pH of 9.2. Several reports were published on the impact of pH on the production of antibiotics. (Bhuyan, 1962; Basak and Majumdar, 1973).

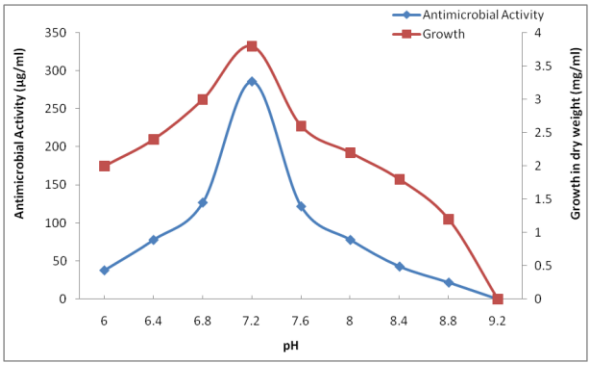

Figure: 6. Effect of pH on Growth and Production of Antibiotic by KRBT-401 Effect of Incubation Period

The growth of KRBT-401 and the production of antimicrobial compound were monitored over a period of 5 days. To study the optimal incubation time for production, the fermentation samples were withdrawn periodically and assayed. The effect of different incubation periods on antimicrobial compound yield indicated that the production of antimicrobial compound (230µg/ml) increased up to 96hrs of incubation and later showed a decline (Figure 7). This corroborates the finding of Higashide (1984) who pointed out that production of macrolide antifungal compound tended to decrease on prolonged incubation. The highest biomass production (3.7mg/ml) was also obtained after 96hrs of incubation.

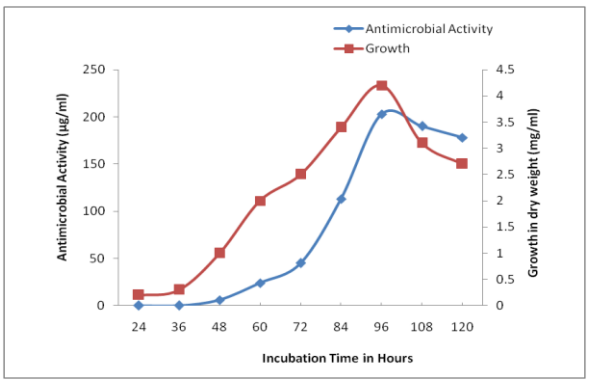

Figure: 7. Effect of incubation period on growth and production of antibiotic by KRBT-401

The synthetic medium formulated in the present investigation for the maximum production of antimicrobial compound by KRBT-401 has the following composition: 12.5 g/L glucose; 2.5g/L ammonium nitrate; 1.2g/L K_2HPO_4; 0.5 g/L $MgSO_4.7H_2O$; trace salt solution 1.0 ml {($CuSO_4.5H_2O$

(0.64g/L); $FeSO_4.7H_2O$ (0.11g/L); $MnCl_2.4H_2O$ (0.79g/L); $ZnSO_4.7H_2O$ (0.15g/L)}. A 10% (v/v) of 48hrs aged inoculum was transferred to the modified production medium and incubated at 30˚C for 96 hrs.

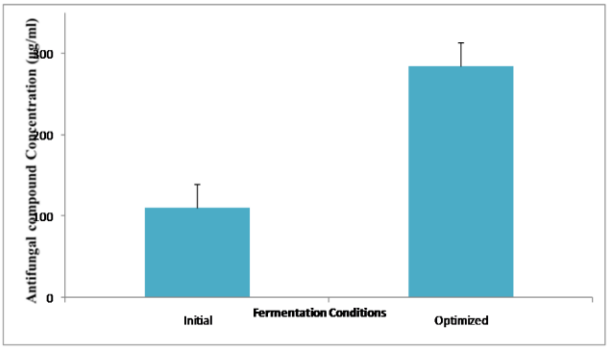

Figure: 8. Antimicrobial Compound Production under Optimized Fermentation Conditions

The extent of antimicrobial compound yield was studied by employing the improved medium and by maintaining the optimized cultural conditions. Simultaneously antimicrobial compound production employing initial fermentation conditions was also conducted. The results are shown in Figure 9. It is evident from the result that the antimicrobial compound productivity employing optimized fermentation conditions (285µg/ml) was more than 2 fold than the antimicrobial compound yield using initial fermentation conditions (115µg/ml) (Figure 9 &10).

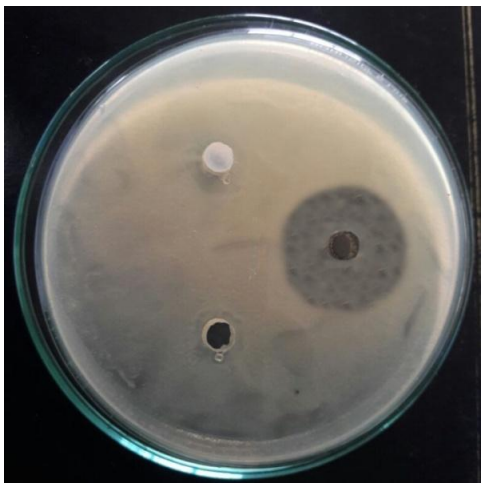

Figure: 9. Antibacterial activity with optimized parameters

The antimicrobial compound production was enhanced by the formulated medium containing carbon and nitrogen source, the macro elements - potassium, magnesium, phosphate, and sulfate and the microelements - iron and zinc were found essential for antimicrobial compound production, in as much as they were essential for growth. Overall data indicates that increase of yield is achieved by using optimized production medium and cultural conditions.

Literature survey indicated that the secondary metabolite yield not only depends on the nature of the strain, but also on the composition of carbon and nitrogen sources (Aharonowitz & Demain, 1978; Gallo & Katz, 1972), inorganic phosphorous and other metal ions (Zygmunt, 1964; Acker & Lechevalier, 1954), cell density (Sanchez & Brana, 1996) and aeration and agitation (Sunesson et. al., 1997; Yagneswaran et. al., 1991) conditions.

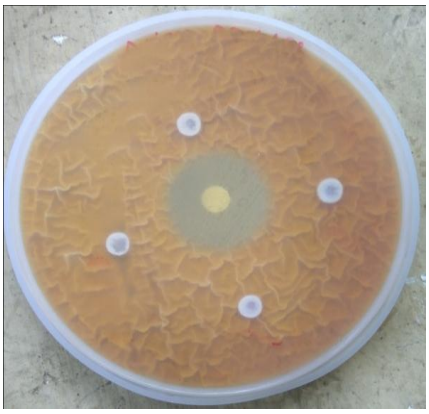

Figure: 9. Antifungal activity with optimized parameters

References:

1. Abbas, AS., Edwards, C. (1990). Effects of metals on *Streptomyces coelicolor* growth and actinorhodin production. *Appl. Environ. Microbiol*. 56 (3): 675-680.
2. Acker, RF., Lechevalier, H. (1954). Some nutritional requirements of *Streptontyces griseus* 3570 for Growth and Candicidin Production. *Appl. Microbiol.* 2(3):152-157.
3. Aharonowitz, Y., Demain, AL. (1978). Carbon Catabolite Regulation of Cephalosporin Production in *Streptomyces clavuligerus. Antimicrob Agents Chemother*. 14(2):159-164.
4. Alvarez A, Saez J M, Costa J S D, Colin V L, Fuentes M S, Cuozzo S A, Benimeli C S, Polti M A and Amoroso M J. Actinobacteria: Current research and perspectives for bioremediation of pesticides and heavy metals. Chemosphere, 166:41-62, 2017.
5. Basak, K., Majumdar, SK. (1973). Utilization of carbon and nitrogen sources by *Streptomyces kanamyceticus* for kanamycin production. *Antimicrob Agents Chemother*. 4(1):6-10.
6. Bhuyan, BK. (1962). Pactamycin Production by *Streptomyces pactum*. *Appl. Microbio.* 10(4): 302 -304.
7. Brückner, R., Titgemeyer, F. (2002). Carbon catabolite repression in bacteria: choice of the carbon source and autoregulatory limitation of sugar utilization. *FEMS Microbiol Lett*. 209: 141-148.
8. Bull A.T., Ward A.C. and Goodfellow, M. 2000. Search and discovery strategies for biotechnology: the paradigm shift. *Microbiol. Mol. Biol. Rev*., 64: 573-606.
9. Demain A L and Sanchez S. Microbial drug discovery: 80 years of progress. The Journal of Antibiotics, 62:5-16, 2009.
10. Gallo, M., Katz, E. (1972). Regulation of secondary metabolite biosynthesis: catabolite repression of phenoxazinone synthase and actinomycin formation by glucose. *J. Bacteriol.* 109(2): 659-667.
11. Gesheva V, Ivanova V and Gesheva R. Effect of nutrients on the production of AK-111-81 macrolide

antbiotic by *Streptomyces hygroscopicus*. Microbiological Research, 160:243-248, 2005.
12. Gesheva, V., Ivanova, V., Gesheva, R. (2005). Effects of nutrients on the production of AK- 111-81 macrolide antibiotic by *Streptomyces hygroscopicus*. *Microbiol. Res*. 160: 243– 248.
13. Helmke E. and Weyland, H., 1984. Rhodococcus marinonascens sp.nov., an actinomycete from the sea.Int. J. Syst. Bacteriol. 34 : 127- 138
14. Higashide, E. (1984). In: Biotechnology of Industrial antibiotics. The macrolides: properties, biosynthesis and fermentation. (ed.: Vandamme E.J.), New York.Marcel Dekker. pp. 451-509.
15. James, PDA., Edwards, C. (1989). The effects of temperature on growth and production of the antibiotic Granaticin by a thermotolerant Streptomycete. *J Gen Microbiol.* 135: 1997-2003.
16. Manivasagan P, Venkatesan J, Sivakumar K and Kim Se-K. Marine actinobacterial metabolites: current status and future perspectives. Microbiological Research, 168:311-332, 2013.
17. Potvin, J., Péringer, P. (1994). Ammonium regulation in*Saccharopolyspora erythraea*. Part I: Growth and antibiotic production. *Biotechnology Letters*. 16(1): 63-68.
18. Ramesh S and William A. Marine actinomycetes: An ongoing source of novel bioactive metabolites. Microbiological Research, 167:571-580, 2012.
19. Saier, MH. Jr., Crasnier, M. (1996). Inducer exclusion and the regulation of sugar transport. *Res Microbiol.* 147: 482-489.
20. Sanchez, L., Brana, AF. (1996). Cell density influences antibiotic biosynthesis in *Streptomyces clavuligerus*. *Microbiology*. 142: 1209-1220.
21. Soliveri, J., Mendoza, A., Maria-Enriqueta Arias. (1988). Effect of different nutrients on the production of polyene antibiotics PA-5 and PA-7 by *Streptoverticillium sp* 43/16 in chemically defined media. *Applied Microbiology and Biotechnology*. 28: 254-257.
22. Sunesson, AL., Nilsson, CA., Carlson, R., Blomquist, G., Andersson, B. (1997). Production of volatile metabolites from *Streptomyces albidoflavus* cultivated on gypsum board and tryptone glucose extract agar—influence of temperature, oxygen and carbon dioxide levels. *Ann. occup Hyg*. 41(4): 393- 413.
23. Usha K M, Vijayalakshmi M, Sudhakar P and Dayanand A. Optimization of process parameters for improved production of bioactive metabolites by *Streptomyces Tritolerans* DAS165T. British Microbiology Research Journal, 4(4):428-442, 2014.
24. Van Wezel, GP., Mahr, K., Konig, M., Traag, BA., Pimentel-Schmitt, EF., Willimek, A., Titgemeyer, F. (2005). GlcP constitutes the major glucose uptake system of *Streptomyces coelicolor* A3(2). *Mol Microbiol.* 55: 624-636.
25. Williams, WK., Edward, K. (1977). Development of a Chemically Defined Medium for the Synthesis of Actinomycin D by *Streptomyces parvulus*. *Antimicrob Agents Chemother*. 11(2): 281-290.
26. Yagneswaran, PK., Gray, MR., Thompson, BG. (1991). Effect of dissolved oxygen control on growth and antibiotic production in *Streptomyces clavuligerus* fermentations. *Biotechnol.Prog*. 7: 246-250.
27. Young, MD., Kempe, LL., Badert, FG. (1985). Effects of phosphate, glucose, and ammonium on cell growth and lincomycin production by Streptomyces lincolnensis in chemically defined Media. Biotechnology and Bioengineering. 27: 327-333.
28. Zygmunt, WA. (1964). Influence of inorganic phosphorus on oxytetracycline formation by *Streptomyces rimosus*. *Appl. Microbiol.* 12(3): 195-196.