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Optimization of the medium constituents for maximizing antibiotic production by marine actinomycete exhibiting promising antibacterial activity.

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ABSTRACT

In order to improve the productivity of activities substance of actinomycetes SLB-8, the effects of the medium components, fermentation time, and initial P^H on the biological activity of SLB-8 were investigated by detecting the mycelium growth inhibition rate of SLB-8. The results show that the inhibition rate is the highest under the optimum medium composition (g/L) containing sucrose 15.0, Malt extract 10.0, K_2HPO_4 0.5, $MgSO_4$ 0.5, NaCl 0.5 and $FeSO_4$ 0.01 $Caco_3$ 0.5. KNO_3 1.0, Distilled water 1.0L The maximum antibiotic activity is obtained at the inoculation volume 10%, medium volume in 250 ml flask 50 ml, rotary speed of 180 r/min, fermentation time 4 days, temperature 28°C and initial pH 8.0. The inhibition rate has been increased by 6.71% under the optimized condition.

Keywords: actinomycetes, fermentation, optimization.

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INTRODUCTION

Marine bacteria are being developed for the discovery of bioactive substances with new types of structure, with growing intensive interest. The achievements have been well reviewed[1], where many novel antibiotics were obtained from actinomycetes[2]. Almost 80% of the world antibiotics are known to come from actinomycetes, mostly from the genera *Streptomyces* and *Micromonospora*. Actinomycetes are widespread in nature and can be found in most ecological niche[3.]The nutritional source like carbon, nitrogen and minerals and environmental factors like time, temperature and PH are found to have profound influence on antibiotic production by actinomycetes[5]. Marine actinomycetes are efficient producers of new secondary metabolites that show a range of biological activities including antibacterial, antifungal, anticancer, insecticidal, and enzyme inhibition. Bioactive compounds from marine actinomycetes possess distinct chemical structures that may form the basis for synthesis of new drugs that could be used to combat resistant pathogens[6]. Thus actinomycetes associated with marine plants and animals are expected to be a potential source for new natural bioactive agents.

Among the genus of Actinomycetes group, *Streptomyces* is the major and more than 500species of this genus have been reported by Euzeby[7.] Almost two third of the naturally occurring antibiotics are produced by *Streptomyces's*. The *Streptomyces* species isolated from mangrove environment showed divergent in their phylogenetic analysis and possessed good antibacterial and antifungal activities[8]. Antiparasitic, antitumor, insecticide, herbicide, alkaloid, enzyme inhibitor, immuno active peptide, antithrombotic agent[8,9] Therefore the present work was focused on Optimization of the medium constituents for maximizing antibiotic production by marine actinomycetes exhibiting promising antibacterial activity.

MATERIALS AND METHOD

Selection of microorganism:

Actinomycete SLB-8 which was earlier isolated in the Department of Biotechnology, Vagdevi College of pharmacy, Gurazala, Guntur, AP, India was selected for the optimization studies.

Maintenance of the cultures:

This culture SLB-08 was maintained by sub culturing on to Yeast Extract Malt Extract slants and they were stored at 4°C until required.

Composition of Yeast Extract Malt Extract medium

Yeast Extract	0.4%
Malt Extract	1.0%
Dextrose	0.4%

Agar	2.0%
Distilled water	100ml
pH	7.2

Inoculum Medium

Inoculum Media-1(IM-1)		Inoculum Media-2(IM-2)	
Soyabeen meal	1.0%	Soya bean meal	1.5%
Corn steep liquor	1.0%	Glycerol	0.5%
Glucose	1.0%	Sodium chloride	0.5%
Calcium carbonate	0.5%	Calcium carbonate	0.1%
Distilled water	100 ml	Distilled water	100 ml
pH	7.0	PH	7.0-7.2

Production Medium

Production Medium-01 (PM-01)		Production Medium-02 (PM-02)	
Glucose	20.0g	Soluble starch	5.0 g
Soybean meal	20.0g	Glucose	3.0g
Peptone	6 5g	Soybean meal	5.0g
CaCO3	4g	Peptone	4.0g
Distilled water	1.0 L	Yeast paste	4.0g
pH	7.2-7.4	KNO3	1.0g
		NaCl	0.5g
		CaC03	0.5g
		KH2PO4	0.5g
		MgSO4	0.5g
		FeSO4	0.01g
		Distilled water	1.0 L
		pH	7.2-7.4
Production Medium-03 (PM-03)		**Production Medium-04 (PM-04)**	
Yeast Extract	0.5g	glucose	1.0g
Dextrose	1.0g	starch	1.0g
Starch	2.0g	peptone	0.75g
Casein hydrolyze	0.5g	meat extract	0.75g
Calcium carbonate	0.4g	sodium chloride	0.3g
Distilled water	1.0 L	Distilled water	1.0 L
PH	7.2	PH	6.5
Production Medium-05 (PM-05)		**Production Medium-06 (PM-06)**	
Corn steep liquor	1.0g	Soya bean meal	1.5g
Soluble starch	2.5g	Glucose	1.5g
Ammonium sulphate	0.5g	Glycerol	0.25g
Calcium carbonate	0.5g	sodium chloride	0.5g
Distilled water	1.0 L	Calcium carbonate	0.1g
pH	6.8	Distilled water	1.0 L
		pH-	7.0

Indication*- g-Grams L-Liter

Study of antimicrobial activity:-

5ml of sterile water was added to each Yeast Extract Malt Extract slant contain SLB-08 and organism was scrapped with a sterile loop and transferred in to 50ml of production medium (PM-01, 02, 03, 04, 05, 06), then the flasks were incubated at 28°C for 120 hours on rotary shaker. After shake flask fermentation fermented product was collected in a sterile container and centrifuged. The resulting supernatant was tested for extra cellular anti-microbial activity following Cup Plate Assay. The antibiotic activity was assessed using Nutrient agar for bacteria.

Cup Plate Assay

Four wells were prepared in the plates with the help of a cork-borer (0.6 cm). 200 µl of each sample was introduced into well.. The plates were incubated overnight at 37° C. Microbial growth was determined by measuring the diameter of zone of inhibition. For each bacterial strain, Controls were maintained.

The following test organisms were used for antibacterial activity

a)Gram Positive- Bacillus *subtilis*

b) Gram negative - E.coli

Selection of suitable inoculum medium for the antibiotic production:

To minimize the time lag in fermentation process **two** different Inoculum media similar to the selected production medium were used. Spore suspension from young 7 days old slant culture was transferred to the above mentioned Inoculum media and incubated on rotary shaker (120 rpm) at 28°C for 48 hrs. After 48 hrs. 10% level of inoculum was transferred to the selected production medium and incubated on rotary shaker (120 rpm) at 28°C for 5 days. Samples were taken at the end of the fermentation cycles, centrifuged and assayed for antimicrobial activity by cup plate method against *Bacillus subtilis* and E.coli

Selection of a suitable production medium for antibiotic production:

Antibiotic production of the isolates was carried out in six different production media. 10% of the inoculum was added to the above media and incubated on a rotary shaker (120 rpm) at 28°C for 5 days. Samples were withdrawn at the end of the fermentation cycle, i.e., 120 hrs. Centrifuged and assayed for antibacterial

Effect of various carbon sources on antibiotic production:

Various carbon sources were selected to the study their effect on antibiotic production. These are soluble starch, sucrose, fructose, glucose, xylose, maltose, lactose and control (without glucose). Each of the carbon sources was incorporated at 1% level into the basal medium PM_2in place of Glucose. All the flasks were incubated at 28°C on a rotary shaker (120 rpm) for 5 days. At the end of the fermentation, the broth was centrifuged and samples were evaluated for their antibiotic activity by cup plate method against *Bacillus subtilis & E.coli*

Determination of optimum concentration of sucrose on antibiotic production:

The following concentrations of sucrose were evaluated to determine its optimum concentration for maximum antibiotic production. i.e., 0.5%, 1.0%, 1.5%, 2.0%, 2.5%. Each of the above concentrations were incorporated into the basal medium, inoculated with 10% inoculum and incubated at 28°C for five days on a rotary shaker at 120 rpm. The antimicrobial activity was determined as per the general procedure.

Effect of various nitrogen sources on antibiotic production:

Yeast extract, casein, tryptone, urea, malt extract, meat extract, sodium nitrate, ammonium sulphate, corn steep liquor, peptone, glutamic acid and control (without nitrogen) source were tried to study their effect on antibiotic production. Each of the nitrogen sources were incorporated at 1% concentration into the basal medium in place of nitrogen sources in the original production medium. The fermentation and assay of their antibacterial and antifungal activities were carried out as before.

Effect of initial pH of the medium on antibiotic production:

The effect of initial pH of the medium was studied with pH values 5.0, 6.0, 7.0, 8.0, 9.0 and 10 The fermentations were carried out and the antibiotic produced was assayed as before.

Effect of incubation period on antibiotic production:

To determine the effect of incubation period on antibiotic production, the flasks with the medium were inoculated and incubated at 28°C. Samples were withdrawn at different times and assayed for antibiotic content. The time intervals were 24, 48, 72, 96 and 120 hrs. The fermentations were carried out and antibiotic content were assayed as before.[22, 23]

RESULTS AND DISCUSSION

Selection of suitable inoculum medium for antibiotic production

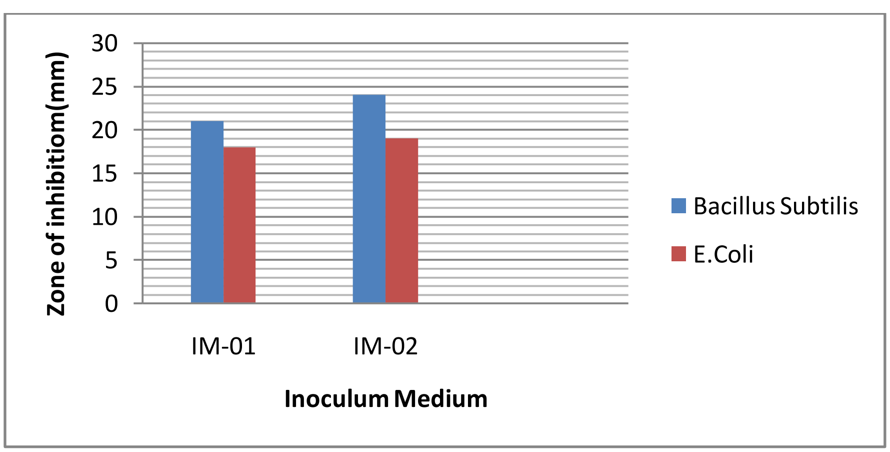

Figure 1: Selection of suitable inoculum medium for antibiotic production

To select the best inoculum medium for antibiotic production, two different inoculum medium IM_1, IM_2 were evaluated. The results indicate that inoculum medium IM2 supported maximum antibiotic yield (figure 1).

Selection of suitable production medium for antibiotic production

In this study the effect of fermentation medium for actinomycetes producing anti-bacterial activity on the Bacillus substilis, E.Coli are presented in Figure 2.Production medium-02(PM-02) shows better anti-bacterial producing properties than other production mediums.

The inoculum medium IM2 and production medium PM-02 were selected and used for all our further studies using isolate SLB-08

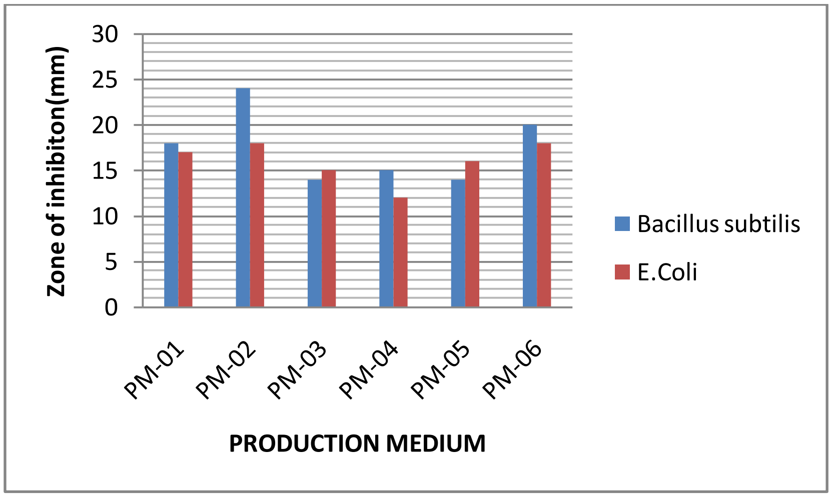

Figure2: Selection of suitable production medium for antibiotic production

Effect of various carbon sources on antibiotic production:

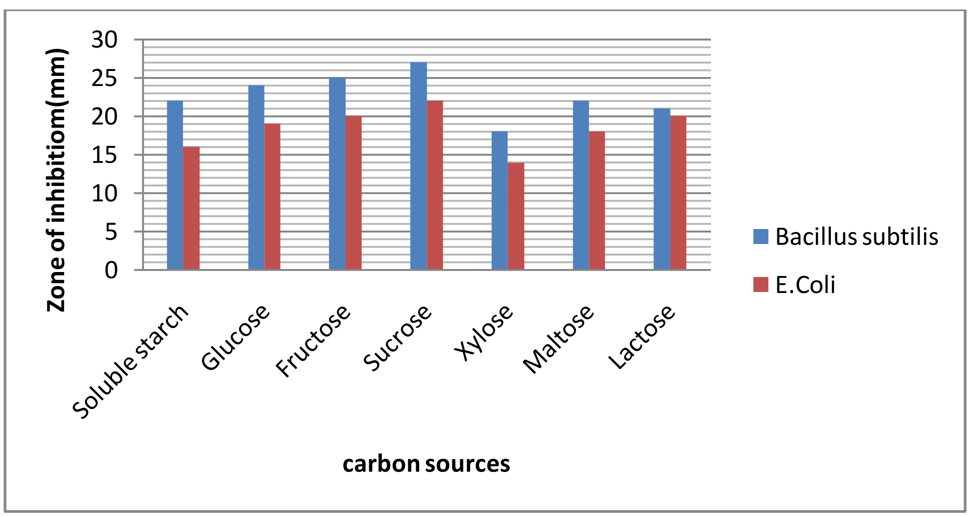

Figure 3 Effect of various carbon sources on antibiotic production:

The exogenous addition of various carbon sources to media may improve cell growth and antibiotic production [12].The results indicate that SLB-08 showed highest antibacterial activity, when Sucrose was supplemented in the medium. Addition of other sources to the medium also favored the antibacterial production but the activity was less when compared with Sucrose(Figure 3). The result was similar with the reported literature where glucose was found to be the suitable carbon source for growth of microorganism as well as for the production of bioactive metabolite .[13, 22]

Determination of optimum concentration of Sucrose source

The results indicate that supplementation of 1.5 %w/v Sucrose into the production medium was found to be the optimum concentration for maximum antibacterial activity and variation of this source concentration on either sides showed reduced antibacterial activity (Figure 4). Similar observation was reported by Syed et al[14]. that 2.0 %w/v D-glucose was found to be the optimum concentration for maximum antibiotic production. High concentration of glucose is generally considered as repressor of secondary metabolisms and maximum cell growth rates can inhibit antimicrobial agent production [17]

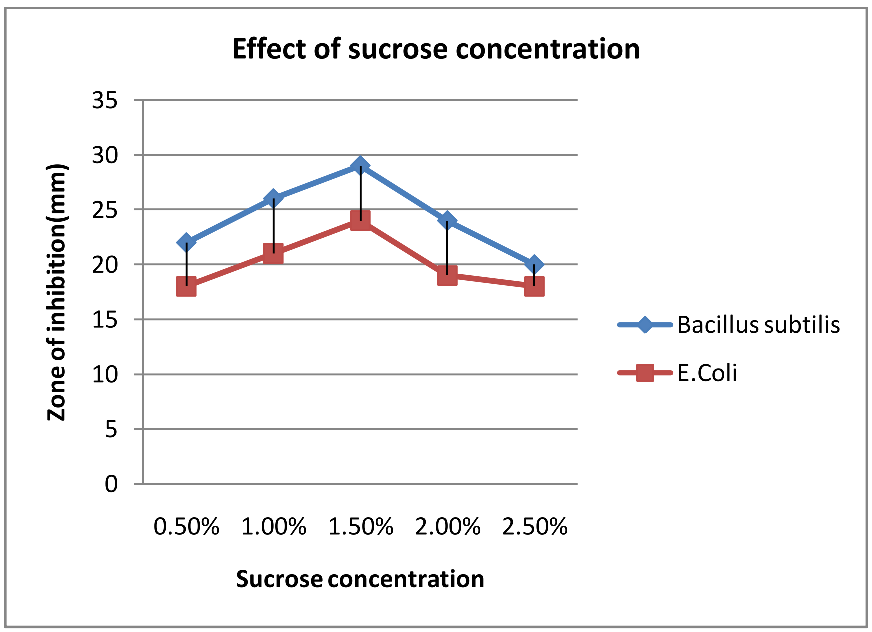

Figure 4 Determination of optimum concentration of Sucrose source

Effect of various nitrogen sources on antibiotic production

Nitrogen source was an important factor for the biosynthesis of antibiotics. The results indicate that maximum antibacterial activity was observed with malt extract and all the other sources tested are poor nitrogen sources for antibacterial production (Figure 5). Similar result was reported by albovinaceus.[15] that malt extract as the best nitrogen source for the production of antibacterial metabolite by S. albovinaceus strain no. 10/2.

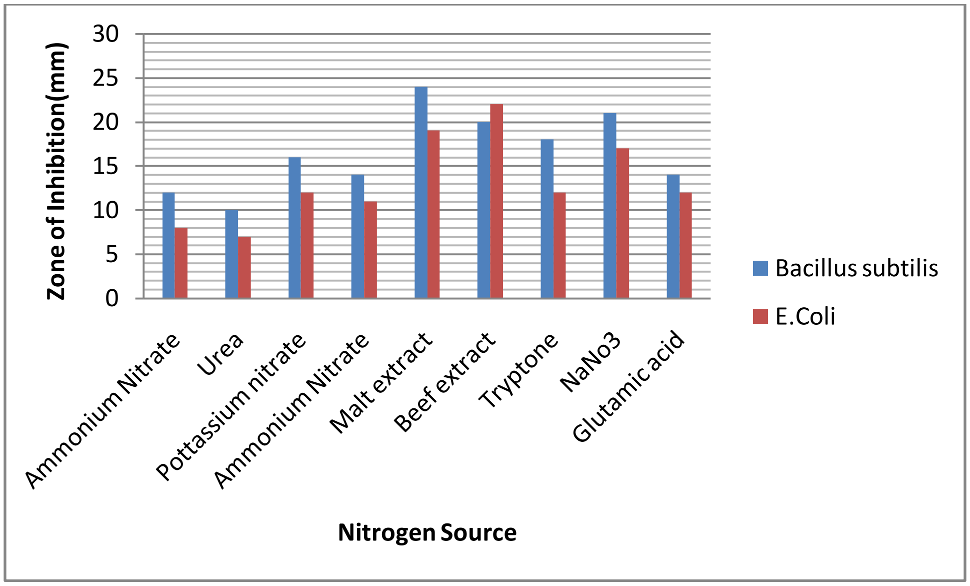

Figure 5: Effect of various nitrogen sources on antibiotic production

Effect of initial pH of the medium on antibiotic production

The effect of initial pH of the medium was studied varying the pH range of 5.0 to 10.0.The results indicate that the antibacterial activity increased and attained maximum with increase in the initial pH of the medium from 5.0 to 8.0; and further increase in pH reduced the antibacterial activity (Figure 6). Similar result of 6.0 as optimum pH was reported by Jain and Pundir and prakasm et al[18, 19]for the production of antimicrobial metabolite by A. terreus. The effect of initial pH of the production medium on antibacterial activity was found to be very important parameter since the variation in activity was observed with the manipulation of initial pH of the medium

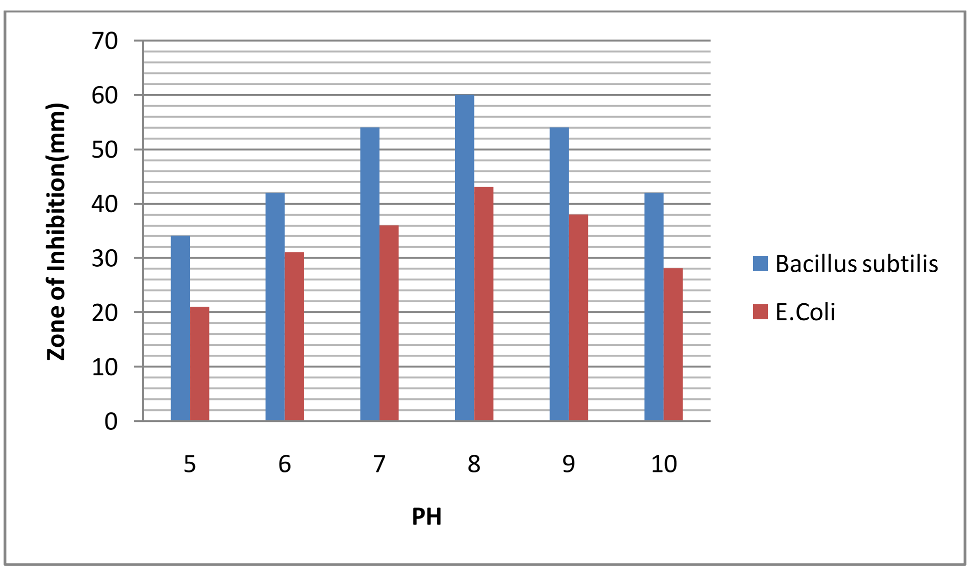

Figure:6 Effect of initial pH of the medium on antibiotic production

Effect of incubation period on antibiotic production:

The results indicate that a gradual increase in antibacterial activity was observed with increase in the incubation time from day 2 to day 4 and further increase in incubation time resulted in gradual decrease of antibacterial activity (Figure 7). The reduction in activity after an optimum incubation might be due to a reduced growth rate resulting from rapid depletion of nutrients available to the organism. In other terms, as nutrients are exhausted and toxic products accumulate, the growth rate of the cells deviates from the maximum and eventually growth ceases as the culture enters the stationary phase. After a period of time, the culture enters the death phase and the numbers of viable cells decrease resulting in the decrease in production Stan burry et al. Similar result was reported by Abdelghani and Sujatha et al[15, 21]. that 4 days of incubation as optimum incubation time for the production of antibacterial metabolite.

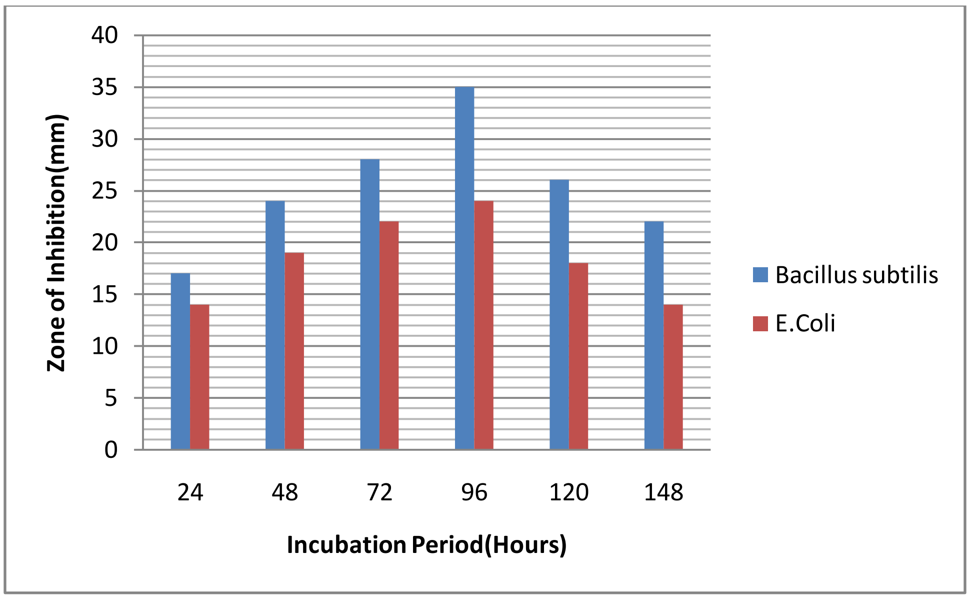

Figure 7: Effect of incubation period on antibiotic production

CONCLUSION

In the present study an attempt was made to optimize the medium constituents for maximizing antibiotic production by marine actinomycete isolateSLB-08. The result indicate that sucrose at 1.5% concentration was found to be best carbon source when compared to other carbon sources such as glucose, fructose, maltose etc. Malt extract at 1% concentration was found to be best Nitrogen sources compared to Nitrogen sources Maximum antibiotic activities for SLB-08 was recorded when the initial pH of the medium is 8., Incubation period 96hrs.The antibiotic research which includes optimization of the media and cultural conditions are imperative for any stain improvement program.

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